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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
UNIVERSIDADE DO PORTO

DISSERTAÇÃO – ARTIGO DE REVISÃO BIBLIOGRÁFICA

Mestrado Integrado em Medicina

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Porto, 2017

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Abbreviations List

EOS - Early Onset Sepsis
LOS - Late Onset Sepsis
VLBW – Very Low Birth Weight
GBS - Group B streptococcus
IAP - Intrapartum Antimicrobial Prophylaxis
CD - Custer of differentiation
TLR - Toll-Like receptors
Treg - Regulatory T cell
SIRS – Systemic Inflammatory Response Syndrome
GAPDH - Glyceraldehyde 3-Phosphate Dehydrogenase
qPCR – Quantitative real-time amplification system
ESBL - Extended Spectrum Beta-Lactamase
pPROM - Preterm Premature Rupture of Membranes
NAAT - Nucleic Acid Amplification Test.

ACKNOWLEDGEMENTS

À Dra. Paula Maria das Neves Ferreira da Silva, pela disponibilidade e generosidade em aceitar a orientação científica deste trabalho e pelo apoio na sua realização.

ABSTRACT

In the early 1970s, *Streptococcus agalactiae*, or Group B Streptococcus, emerged as a major pathogen for newborns. Since then, in most industrialized countries, this bacterium has been considered the leading cause of severe neonatal diseases, namely pneumonia, sepsis and meningitis. Neonatal Group B Streptococcus infections are prevented by intrapartum administration of antibiotic prophylaxis to mothers of children at risk of developing early diseases. This strategy has diminished the prevalence of early onset neonatal sepsis. However, there are concerns about the potential generation of antibiotic resistance, drug-induced adverse reactions and the negative impact on the gut microbiota, inducing dysbiosis. Moreover, mortality remains high, particularly in premature and very low birth weight infants and 50% of the neonates that survive to infection present neurological sequels.

Recently studies have been offering a better understanding of the ontogeny of the human immune system, which is essential for the development of efficient and safe interventions aiming at providing protection to neonates against pathogens. Also, several researches have been done for the identification of new markers for neonatal sepsis diagnosis and prevention. This search for an ideal sepsis biomarker (with high sensitivity and specificity) it is important for the diagnosis to be made accurately and mainly at the earliest possible, which allow for the use a more selective antibiotic therapy. Moreover, there is a need for the standardization of pediatric sepsis definition that is essential for a better awareness and management of this disease. Therefore, in this article, a literature review of Group B Streptococcus neonatal sepsis is presented and discussed by using the published studies from the last ten years by search in PubMed- MEDLINE database, focusing on the issues mentioned above and others relevant to the topic.

This review show that although there has been a considerable increase in knowledge of neonatal sepsis induced by Group B Streptococcus, further researches are needed to develop a new preventive approaches and new diagnosis markers, to improve the outcomes of this devastating disease.

KEY-WORDS

Neonatal sepsis; Group B Streptococcus; *Streptococcus agalactiae*, infection; neonatology; intrapartum antibiotic prophylaxis; diagnosis; prevention; management.

RESUMO

No início da década de 70 do século passado, o *Streptococcus agalactiae*, também designado de Estreptococos do grupo B, emergiu como um importante agente patogénico nos recém-nascidos. Na maioria dos países industrializados, esta bactéria tem vindo a ser considerada a principal causa de doenças neonatais graves, como pneumonia, sépsis e meningite. As infeções neonatais por Estreptococos do grupo B são atualmente prevenidas pela administração antibiótica intraparto. Esta profilaxia diminuiu consideravelmente a prevalência de sépsis neonatal. Contudo, vários problemas têm vindo a ser apontados com o uso de antibióticos como por exemplo, o desenvolvimento de resistências a agentes antimicrobianos, reações adversas induzidas pela administração, o impacto negativo sobre a flora intestinal ocasionando alteração do microbiota (disbiose). Além disso, a mortalidade infantil causada pela infecção por esta bactéria permanece alta, particularmente em recém-nascidos prematuros e de muito baixo peso. Mais ainda, cerca de 50% dos neonatos que sobrevivem à infeção ficam com sequelas neurológicas permanentes.

Estudos recentes têm trazido mais conhecimento sobre a ontogenia do sistema imunológico humano. Este conhecimento é essencial para o desenvolvimento de intervenções eficazes e seguras nos recém-nascidos de modo a torna-los protegidos contra os agentes patogénicos. Além disso, várias pesquisas foram realizadas com o objetivo de identificar novos marcadores para o diagnóstico e prevenção da sépsis neonatal. A procura de um biomarcador de sépsis com alta sensibilidade e especificidade é essencial para que o diagnóstico seja feito com maior precisão e o mais cedo possível, o que permitirá o uso de uma antibioterapia mais seletiva. Além disso, há uma necessidade de padronização da definição de sépsis pediátrica de modo a que haja um melhor reconhecimento e gestão desta patologia. Neste artigo, estes e outros aspectos importantes da sépsis neonatal por Estreptococos do grupo B serão apresentados e discutidos, através de uma revisão da literatura, usando o motor de busca de livre acesso à base de dados MEDLINE, o PubMed, para escolher os artigos publicados nestes últimos 10 anos sobre este tema.

Esta revisão mostra que embora nos últimos 10 anos tenha havido um aumento considerável no conhecimento acerca da sépsis neonatal induzida pelo Estreptococos do grupo B, é ainda necessário mais investigação sobre este tema, de modo a desenvolver-se novas abordagens preventivas e novos marcadores de diagnóstico contra esta doença que ainda é tão devastadora.

PALAVRAS-CHAVES

Sépsis neonatal; Estreptococos do grupo B; *Streptococcus agalactiae*; infeção; neonatologia; profilaxia antibiótica intraparto; diagnóstico; prevenção; tratamento.

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1. Introduction

Neonatal sepsis is one of the leading causes of neonatal mortality and morbidity worldwide, representing a serious public health problem associated with low survival rates and potential long-term sequelae [1]. Management is far from being optimal due to a challenging diagnostic and therapeutic limitation and therefore efforts are mainly focused on prevention. Group B streptococcus (GBS) is the main agent responsible for neonatal sepsis and meningitis [2], which led to many developed countries to adopt GBS maternal screening and intrapartum antibiotic prophylaxis (IAP). Despite a significant decrease in neonatal sepsis rates in the last decades, IAP efficacy is not absolute, and it has been pointed out several limitations and disadvantages [3-6]. To improve the outcomes of this devastating disease in future, it is important to review and discuss all the knowledge gathered until now. Therefore, the aim of this study is to present a literature review about the subject: neonatal Group B streptococcal sepsis. Topics like epidemiology, etiology, pathophysiology, IAP, diagnosis and treatment will be presented and discussed.

2. Methods

Research Strategy and Selection Criteria: a systematic literature search in PubMed-MEDLINE was done using the studies published from January 2007 to May 2017. Keywords used were: GBS, *Streptococcus agalactiae*, sepsis, neonates, intrapartum antibiotic prophylaxis, fetal immune system and neonatal sepsis treatment. Medical text books such as Nelson's Textbook of Pediatrics and Harrison's Principles of Internal Medicine, and some articles published before 2007 but considered relevant to this subject, were also included. In total, 92 articles were used on this review. We excluded case reports, low quality studies and conference meeting abstracts.

3. Epidemiology and Etiology

Neonatal sepsis refers to a systemic infectious condition in neonates, associated with hemodynamic changes and other clinical manifestation with a high morbidity and mortality impact [7]. It is estimated that 36% of all deaths in the neonatal period (≤ 28 days of life) are due to invasive infections [8]. If term infants were considered separately, neonatal sepsis accounts for 15,6% of all causes of neonatal death (annex 1), while if only preterm infants are evaluated, sepsis is responsible for approximately 20% of all deaths [9]. Infection rates vary from 1-5 to 49 per 1000 live births [10], depending on both maternal and infant geographical associated risk factors, and medical resources distribution.

According to symptom's presentation timing, it is possible to distinguish between early onset sepsis (EOS) and late onset sepsis (LOS). EOS is defined as the onset of sepsis within 72 h of postnatal life, while LOS has been defined as the onset of sepsis between 3-7 days of life [7]. This distinction is relevant because EOS and LOS reflect different etiologies and pathophysiology processes. EOS is caused by bacterial pathogens transmitted vertically, while LOS is considered to be transmitted by both vertically and horizontally [7].

3.1 Early Onset Neonatal Sepsis

Nowadays, EOS has an estimated prevalence of 0,7-1 per 1000 live-births in developed countries that adopted guidelines for intrapartum antibiotic prophylaxis (IAP), such as the United States [8]. Despite a significant decrease on prevalence of this disease after IAP introduction [14], it still remains a serious post-partum period complication, with mortality rates averaging 11% [8]. Moreover, both incidence and mortality are notably higher in very low birth weight (VLBW) preterm infants [11].

EOS pathogens are typically maternal vaginal flora´s commensals, who colonize the newborn during its passage through the birth canal or, in a minor case, by ascending to amniotic fluid or placenta, causing in-utero infection [12]. The organisms most frequently involved are GBS and *Escherichia coli,* and account for more than 70% of all cases [11]. Other bacterial pathogens like *Staphylococus aureus*, *Enterococus* spp., other streptocci, *Haemophilus influenza, Listeria monocytogenes,* and fungal pathogens such as *Candida spp,* are also involved [43]. VLBW infants are more likely to have sepsis from Gram-negative organisms [13]. Viral pathogens are not typically associated with an EOS presentation, and must be distinguished from bacterial sepsis [11].

Half of the newborns born from GBS colonized mothers will also be colonized at time of birth, however the large majority of them are asymptomatic, and only 2% have evidence of sepsis [8]. After IAP implementation in the United States, GBS EOS incidence reduced substantially [14].

GBS still remains the most common EOS etiologic agents, especially in term infants. However, the decrease in GBS sepsis rates due to IAP has shifted the focus to *Escherichia coli* [3]. This bacterium is more lethal than GBS [11]. Some epidemiological studies, show that *E. coli* EOS incidence rates remained similar in spite of IAP guidelines [15], while others report increased incidence of *E. coli* EOS, particularly among VLBW infants [16]. Despite these different results, it is consensual that there is an increase in EOS due to ampicillin-resistant *E. coli* [8]. Since ampicillin is one of the first line agents for neonatal sepsis´s empirical treatment, higher resistance rates are worrisome. Moreover, *E. coli* is the most common EOS etiologic agent in preterm and VLBW infants [9]. Preterm infants with EOS were shown to have a higher mortality rate than preterm infants with LOS, with *E. coli* causing the highest mortality rate [13].

3.2 Late Onset Neonatal Sepsis

The incidence of neonatal LOS is inversely related to neonatal gestational age and birth weight, and varies geographically from 0.61% to 14.2% among hospitalized newborns [7, 17]. LOS is a common complication of the preterm newborn with prolonged hospitalization, due to the use of invasive procedures and devices such as mechanical ventilation and intravascular catheters, which allow for invasive disease caused by nosocomial acquired microorganisms. The advances in neonatal intensive care have improved the survival of neonates but led to an increased rate of LOS [17].

Gram-positive bacteria are responsible for most of LOS cases (~70%) [8, 10]. Other possible pathogens are Gram-negative bacteria (18-25%), and fungi (5-12%) [8, 10]. Regarding Gram-positive bacteria, the most common etiologic agent is the coagulase negative staphylococci. GBS accounts for only 5% of all cases [18]. *Staphylococcus aureus* is more common in neonates with vascular-access catheters [7]. Deaths attributed to LOS increase with postnatal age, 36% in newborns aged 8–14 days, and 52% in those aged 15–28 days [19].

4. Pathophysiology

4.1 Neonatal Sepsis Risk Factors

Risk factors for EOS can be grouped into maternal and infant risk factors. Maternal risk factors include invasive procedures applied during pregnancy such as amniocentesis, as well as impaired maternal immunological function and poor maternal nutrition [11]. Labor risk factors are also considered. Maternal fever and prolonged rupture of membranes (more than 18 hours prior to delivery), increases EOS risk to 1% [20]. Chorioamnionitis leads to a 1-4% EOS incidence, due to swallowing of infected amniotic fluid by the fetus. Longer length of labor, amniotic membrane rupture, and internal placement of fetal or uterine devices are also associated with a higher chorioamnionitis risk [21]. Infant related EOS risk factors consist of prematurity, low birth weight, APGAR scores ≤6 in the first five minutes [11], and male sex, since in the presence of a male fetus the trophoblast has the potential to generate a more pro-inflammatory environment [22]. Preterm low birth weight infants have a 3 to10 higher incidence of infection than full-term normal birth weight infants [7]. Premature infants usually present low maternal IgG levels and diminished skin and mucous membrane barrier function, which can be further exploited in ill infants with invasive procedure needs.

Main LOS risk factors were already described. Moreover, extended stay in the healthcare environment and formula feeding can disturb the normal gut colonization process, which could facilitate bacterial translocation into the bloodstream [23]. However, despite theoretically promising, meta-analysis studies showed that probiotics not significantly reduce sepsis incidence [24]. It has been demonstrated that human milk feeding is associated with a threefold reduction of LOS risk [25].

Regarding specific risk factors for GBS EOS, the most important is maternal intrapartum GBS colonization. 10-30% of pregnant women are estimated to be colonized with GBS, and if IAP is not implemented, around 1-2% of infants born from these mothers will develop EOS [14]. The gastrointestinal tract serves as the primary reservoir for GBS and is the likely source of vaginal colonization. Additional risk factors are GBS bacteriuria, a history of a previous child born with GBS infection and low maternal serum IgG antibodies tiers against specific GBS capsular polysaccharides [14].

4.2. Newborn´s immune system

4.2.1. Neonatal immune system development

It is crucial to understand the immunological events behind neonatal sepsis in order to improve diagnostic tools and therapeutics approaches that will lead to a better outcome. Multi-parameter of flow cytometry combined with mass spectrometry and other technologies have been used to study the ontogeny of the immune system [26].

Newborns are usually considerably immunocompromised because the immune system is immature with broad deficits across both innate and adaptive immune functions [26]. However, it is not advisable to presume that the immune response in fetuses, newborn and infants is simply hypofunctional or underdeveloped [26]. It is better to consider the immune system of neonates as functionally distinct of the adult counterpart, and is important to remember that the survival of the allogenic fetus in the uterine microenvironment depends on the maternal and fetal immune tolerance [27].

Regarding the gestational development of specific cell populations, neutrophils are an important effector cell in newborn protection against pathogens as the bacterium GBS. However, neutrophils numbers are relatively low until 32 gestational weeks, and preterm newborns lack functional neutrophil extracellular traps and effective generation of potent reactive oxygen species [26]. In this regard, cases of early-onset GBS sepsis are usually characterized by a low number of neutrophils, and impaired neutrophil recruitment, with reduced adhesion capabilities [28]. Thus, qualitative and quantitative neutrophils deficiencies may contribute for the increased susceptibility of neonates to sepsis.

Complement system is also impaired in neonates born prematurely [29], as well as the ability to produce high amounts of antibodies against T-independent antigens, which impairs response against encapsulated bacteria, such as GBS [30].

T-cell function is more deviated to favor a Th2 response than Th1´s, leading to decreased $CD8^+$ T-cell cytotoxic activity and an increased T-regulatory (Treg) cell population [31]. Lack of immunological memory and exclusive reliance on maternal antibodies also contribute for the high susceptibility of neonates to microbes.

4.2.2. Fetal Immune Tolerance

One of the unique characteristics of the developing fetal immune system is its ability to maintain a tolerant state to prevent a response against maternal and self-antigens. This is accomplished by an increased Treg cells frequency in fetal tissues, composing 15-20% of all $CD4^+$ cells in fetal lymphoid tissues [32]. While essential for fetal development, this predisposition of fetal cells to differentiate into Treg cells is accompanied by an overall tolerant state that leads to an increased susceptibility to microbes. Despite this tolerant state, preterm infants show an enhanced production of pro-inflammatory cytokines compared to adults when invaded by a pathogenic microorganism, which often leads to a systemic inflammatory response syndrome (SIRS) [33].

IL-10 is one of the most important immunosuppressive and anti-inflammatory molecules in pregnancy [27]. It has been showed that neonates are committed to produce IL-10 and this could be used by GBS to survive in the host [30]. GBS produces an extracellular virulence factor, called glyceraldehyde 3-phosphate dehydrogenase (GAPDH) that induces a rapid IL-10 production by the host [34]. High levels of IL-10 impair neutrophil recruitment into infected organs, preventing bacterial clearance. Therefore, IL-10 production very early after GBG infection allows bacterial immune evasion. This is supported by results showing that IL-10 blocking trough anti-IL-10R monoclonal antibodies (mAB) administration confers protection of neonates to bacterial challenge [30].

4.3 GBS virulence factors

GBS encodes virulence factors for adherence, invasion and colonization of host tissues. Examples include a polysaccharide sialic acid rich capsule and pore-forming toxins [35]. GBS colonizes maternal genital and lower gastrointestinal tract as a commensal microorganism, but also acts as an invasive pathogen in other tissues. Therefore, this bacterium is able to adapt to different hosts environments by regulating its virulence factors. Understanding how GBS regulates virulence factors expression could be useful in the development of new selective therapeutic targets to replace the IAP administration. Pore-forming toxins promote pathogen entry into host´s cells, allowing for intracellular survival and systemic dissemination [35].

A sialic acid-rich capsule prevents the host´s immune system to recognize GBS as nonself, restraining C3 complement factor deposition and phagocytosis [36]. Studies have also demonstrated that this polysaccharide capsule encoded by GBS can be modified by O-acetylation, changing the host´s antibody response recognition against GBS´s capsular antigen [35]. This bacterium also encodes a superoxide dismutase, and a C5a peptidase that impairs neutrophil recruitment [36]. Serine proteases produced by GBS cleave extracellular matrix components and allows for host´s immunity system evasion. Strains cold shock protein (CspA) defective have a diminished virulence [37]. Pili mediate resistance to cationic antimicrobial peptides and allows attachment to host cells. GBS strains lacking PilB demonstrate decreased virulence [38].

4.4 Neonatal Sepsis

4.4.1 Systemic Inflammatory Response Syndrome

Systemic inflammatory response syndrome (SIRS) can be broadly defined as the systemic manifestations that result from an uncontrollable, dysregulated immune response, being sepsis a SIRS response in the context of a proven microbiological infection. While there were numerous attempts to give an objective definition to SIRS, sepsis, severe sepsis and septic shock in pediatric population, this has been proven difficult, especially for preterm newborns. *Goldstein, Giroir et al.* reported listed criteria for pediatric sepsis in both newborns and neonates but, preterm infants were excluded from this definition [39]. Key developmental differences modulate neonatal sepsis pathophysiology, and so age is a variable that strongly affects the underlying immune status and response to therapy. Standardization for sepsis criteria to all newborn is difficult, leading ultimately to a high reliance on the physician´s clinical suspicion, which has a significant positive predictive value, superior than 70% [40].

The major difference in pediatrics SIRS criteria, when compared to adult´s is that pediatric SIRS require temperature or leukocytes alterations to be present [39].

Table 1 - Definitions of systemic inflammatory response syndrome, sepsis, severe sepsis and septic shock in pediatrics*

SIRS Presence of at least two of the following four criteria, one of which must be abnormal temperature or leucocyte count: • Core Temperature of > 38,5ºC or <36ºC • Heart Rate < 10th percentile for age (children <1 year only) or > 2 SD • Mean Respiratory Rate > 2 SD or mechanical ventilation for an acute process • Leukocyte count elevated or depressed for age or > 10% immature neutrophils
Sepsis SIRS in the presence of or as a result of suspected or proven infection
Severe Sepsis Sepsis associated with cardiovascular dysfunction, acute respiratory distress syndrome or two or more organ dysfunctions
Septic Shock Sepsis and cardiovascular organ dysfunction

**Adapted from Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric S. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics, 2005*

Sepsis´s pathophysiology is not yet completely understood. An inflammatory stimulus, such as a bacterial toxin, triggers production of proinflammatory mediators, including TNF-α and IL-1. These cytokines cause neutrophil–endothelial cell adhesion, clotting activation, and microthrombi formation. They also lead to other molecular mediators release, including leukotrienes, lipoxygenase, histamine, bradykinin, serotonin and IL-1 [41]. There is an overactivation of the innate immune system. Although some patients die during the initial, hyperinflammatory phase of sepsis, most patients perish later, in association with an immunosuppressive state [31]. Immune response to pathogens also induces an anti-inflammatory endogenous response, mediated by both cytokine antagonists and cytokines with anti-inflammatory proprieties (annex II). This anti-inflammatory compensatory response may play a greater role in septic shock´s pathophysiology in children, when compared to adults [31].

4.4.2 Neonatal Septic Shock

Regarding septic shock's pathophysiological features, initially arteries and arterioles dilate, decreasing peripheral arterial resistance and cardiac output typically increases. This stage has been referred to warm shock. Later, catecholamine production and release becomes insufficient to maintaining cardiovascular system´s homeostasis, due to apoptosis of adrenal medullary cells [42]. Cardiac output may decrease, BP falls (with or without an increase in peripheral resistance), and typical features of shock appear. Bradycardia may be a sign of SIRS in the newborn age group but not in older children, in whom it is a terminal event [39]. There are several developmental differences in hemodynamics, coagulation cascade, and inflammatory response to sepsis, alongside the already mentioned immune response in pediatric population that differentiates pediatric sepsis from adult sepsis.

Hypovolemia, either absolute or relative, is the most common cause of shock in children. Abnormalities in peripheral overregulation and/or myocardial dysfunction likely play a greater role in the hemodynamic derangements associated with pediatric septic shock in neonates and young infants [31]. Myocardial depression is a common pathophysiological feature in pediatric septic shock, due to the developmental differences in infant's myocardial structure, compared to adults. Infants have a limited capacity to increase stroke volume during stress, and so neonates and young infants are more dependent on an increase in heart rate to generate increased cardiac output, which is difficult, due to newborns' relatively higher baseline heart rate [31].

Coagulopathy may develop because of intravascular coagulation with consumption of major clotting factors and excessive fibrinolysis. Microvascular thrombosis contributes, alongside vascular tone dysfunction, to end-organ dysfunction. The physiologic homeostatic balance of anticoagulants versus procoagulant factors is biased towards procoagulation with defective anticoagulation, favoring microvascular thrombosis, and also bleeding diathesis. There is also depletion of natural anticoagulants [42].

Table 2- Cardiovascular dysfunction criteria*

Despite administration of isotonic intravenous fluid > 40 ml/Kg in 1 hour, any of the following:
Need for vasoactive drug to maintain BP in normal range
Decrease in BP < 5th percentile for age or systolic BP <2 SD below normal for age
Two of the following: • Unexplained metabolic acidosis • Increased arterial lactate • Oliguria: Urine output < 0,5 ml/Kg/hr • Prolonged capillary refill: >5 sec • Core to peripheral temperature gap > 3ºC

**Adapted from Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric S. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics, 2005*

5. Neonatal Sepsis GBS prophylaxis

5.1 Intrapartum Antibiotic Prophylaxis

To this date, receiving parenterally antibiotics during labor is the only proven strategy to protect a newborn from early-onset group B strep disease [14]. Previous to guidelines implantation, series reported 1,8/1000 live births with early-onset GBS sepsis, while nowadays rates vary around 0,35-0,41/1000 live births in most developed countries [43].

Introduction of generalized use of IAP was met with concern that sepsis presentation in the newborn could be masked. However, further studies showed that there was no difference in sepsis clinical presentation between neonates exposed to intrapartum antibiotics and those who not [44]. Despite high compliance rates for prenatal screening and intrapartum antibiotics in western countries, there are still missed opportunities, and most early-onset group B streptococcal disease in recent years occurs in newborns of prenatally GBS-negative mothers [45]. Therefore, it is necessary additional measures to prevent neonatal GBS infection. This gap between maternal colonization status and newborn´s sepsis at the onset of labor, seems to be responsible for most of the disease burden in countries with a high screening compliance rate. Early-onset neonatal GBS disease has remained stable in the last decade, without further decrease [46].

5.2 New Prevention Strategies

Despite the success of prenatal screening test in decreasing GBS infection in neonates, it has a lower predictive positive value compared to GBS screening during labor. This could be prevented by performing the microbiological cultures closer to the delivery date, but even then, positive predictive value will not probably be as high as when GBS screening is done during labor [45]. To address these problems, nowadays investigation is focused in rapid testing methods to quickly evaluate the need for IAP during delivery, as well as in primary prevention with the use of a maternal vaccine against most common GBS serotypes [11]. The most promising method for rapid intrapartum screening is RT- PCR, based on GBS DNA. It can yield results in 45 minutes, much faster than the results obtained with microbiological cultures [47]. However, it is not used in routine clinical practice, due to the cost, availability and contamination potential. Other molecular assays do not have sufficient sensitivity to replace microbial cultures, but are useful as adjunctive tests [65].

Currently there is no maternal vaccine that protects newborns from GBS invasive disease. Research continues, since vaccination has several advantages over IAP. Decreasing antibiotic use will lead to lower risk of resistant GBS strains and also to a decrease in Gram-negative infections rising prevalence. Since GBS serotypes differ dramatically around the world [11], it would be more beneficial to use a vaccine against an antigen not specifically associated with a serotype. Vaccination would theoretically prevent a small but significant percentage of preterm births and more than half (60-70%) of neonatal GBS infection [48]. Despite all this potential of new prevention strategies, the importance of clean delivery practices and handwashing during delivery cannot be omitted. There is strong evidence that this approach decrease neonatal sepsis in both home and health facility settings [49].

5.3 Timing of screening and Intrapartum Antibiotic Prophylaxis recommendations

GBS colonization status is not definitive and changes during pregnancy. Therefore, it is advisable to assess bacterial colonization in late pregnancy in all pregnant women. Ideally screening should be done between 35^{th} - 37^{th} gestational weeks, allowing late third trimester colonization status to be used as a surrogate marker for intrapartum colonization [14]. An exception can be made in women with either asymptomatic or symptomatic GBS bacteriuria at any time during pregnancy, or in women who had a previous infant with invasive GBS disease.

In these situations, late trimesters screening is not needed and pregnant women should receive intrapartum antibiotic prophylaxis to prevent early-onset GBS disease. Colonization early in pregnancy is not predictive of early-onset GBS disease [14].

A culture-based screening approach was proven to more efficient in decreasing the burden of neonatal disease compared with previous used risk based IAP [11]. Risk-based method identified suitable candidates for intrapartum chemoprophylaxis accordingly to the presence of intrapartum risk factors. Specimen collection should be done with swabbing of both the lower vagina and rectum in order to increase culture yield. Antimicrobial susceptibility testing of GBS isolates could be important, especially in penicillin-allergic woman, which alternative is clindamycin, an antibiotic associated with increasing resistance among GBS isolates [14].

The timing of prenatal GBS screening may have the largest effect on its Positive Predictive Value [11]. Woman who were not screened during pregnancy, who deliver shortly after GBS screening, or who culture results are not available at the time of delivery, should be managed according to the presence of intrapartum risk factors. IAP is recommended for unknown GBS status at the onset of labor and any of the following signs: delivery at <37 weeks' gestation; amniotic membrane rupture ≥18 h; intrapartum temperature ≥38.0°C; intrapartum NAAT positive for GBS [12]. Management for GBS prophylaxis in women with preterm rupture of membranes can be consulted in annex III. Table 3 shows the theoretical reduction in GBS EOS among different prevention strategies.

Table 3 - Prevention Strategies for Group B Streptococcus Early Neonatal Sepsis*

Prevention Strategy	Method	Theoretical reduction in EOGBS Disease
Bacteriological screening only	All pregnant women at 35-37 weeks gestation are swabbed for GBS. All women who screen positive are treated with intrapartum antibiotics, regardless of the presence of risk factors for neonatal GBS Disease.	65 – 86%
Risk-factor only	No swab for GBS is performed at 35-37 weeks gestation. Women with ≥1 risk factor for GBS Disease are treated with intrapartum antibiotics.	50 – 62%
Combined screening & risk factor strategy	All pregnant women at 35-37 weeks gestation are swabbed for GBS. Only women who screen positive *and* have ≥1 risk factor for neonatal GBS Disease are treated with intrapartum antibiotics.	51 – 75%

**Adapted from Royal College of Obstetricians and Gynecologists (2003). Prevention of early onset neonatal group B streptococcal disease. Clinical Practice Guideline No. 36.*

5.4 Antibiotic selection for Intrapartum Antibiotic Prophylaxis

Penicillin is the first line agent used for GBS chemoprophylaxis therapy, both in mothers with positive prenatal GBS screening tests, and mothers with unidentified GBS status [14]. Ampicillin is an acceptable alternative. There is a rare possibility for severe allergic reaction that will require an emergency approach, and woman with history of anaphylaxis or angioedema, after receiving penicillin or cephalosporin should receive vancomycin or clindamycin for IAP [14]. If penicilin-alergic, but without a history of this serious reactions, cefazolin is the preferred agent, because its activity is similar to first-line agents, with the advantage of not having documented resistances [36]. When clindamycin for IAP is indicated, susceptibility antimicrobial testing should be performed before on prenatal GBS isolates. Whether this is not possible, vancomycin is the agent of choice in allergic penicillin mothers with history of serious allergic reactions [14]. Antibiotics should be administered parentally for IAP since oral administration has no beneficial effect. Administration should occur during labor, and not before, since there is no complete bacteria eradication, and multiplication can occur quickly.

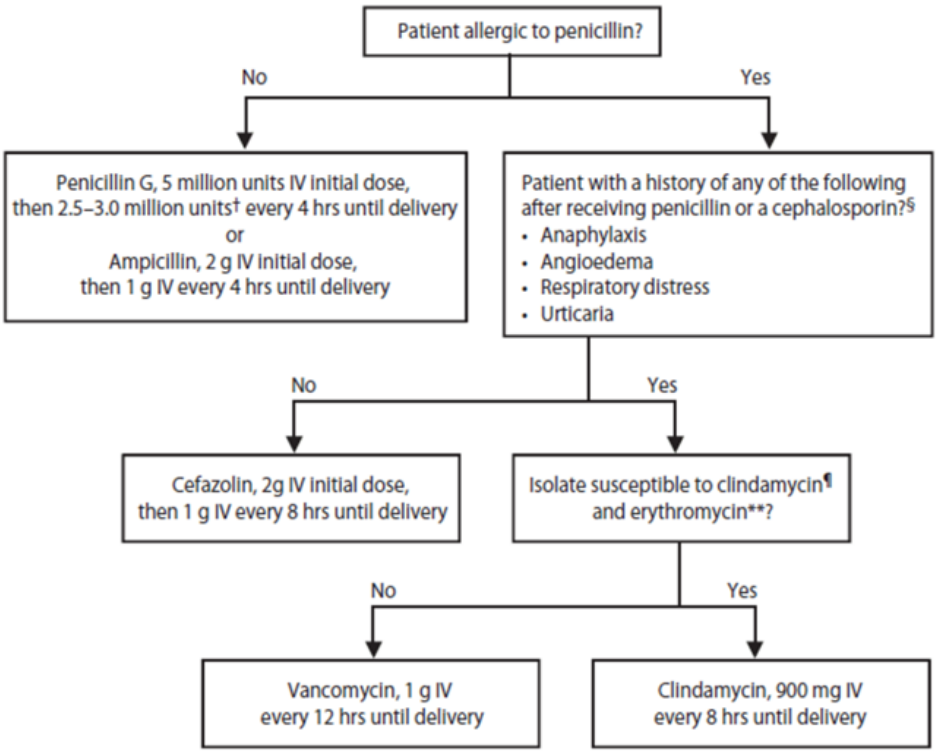

Figure 1 - CDC-recommended regimens for intrapartum antibiotic prophylaxis for prevention of early-onset GBS disease

Adapted from Prevention of perinatal group B streptococcal disease—revised guidelines from CDC, 2010.

5.5 Intrapartum prophylaxis´s potential adverse effects

After IAP introduction an increase in relative neonatal sepsis incidence due to non-GBS microorganisms, as well as an increase in the incidence of various pathogens resistant to antibiotics, specifically to ampicillin have been observed [3]. In VLWB infants, studies identified an increasing number of *Candida spp* infections and Gram-negative infections. Among Gram-negative infection, one study documented increase in the ampicillin resistance of *E. coli* strains [4].

Use of intrapartum ampicillin or penicillin can be considered an independent risk factor for ampicillin/penicillin resistance among late-onset infections, doubling the risk of antibiotic resistance [4]. Despite IAP use, LOS incidence has not decreased and IAP has led to increase in erythromycin and clindamycin resistant GBS isolates [4]. Another potential threat of IAP is intestinal disbiosis. Epidemiological studies found an association between antibiotic usage in early infancy and occurrence of various autoimmune diseases, such as diabetes, and asthma, as well as obesity and allergic and atopic diseases [5]. This could be linked to the short term and long term effects of antibiotics in the diversity and composition of the gut flora, which can have implications in the immune system development. According to a prospective study in infant´s gut microbiota characterization in children born from mother´s who received IAP, at 3 months of age present significant alterations in the overall microbiota with reduction in microbiota richness and diversity [6].

6. Clinical Presentation

Both EOS and LOS clinically present as an ill-defined, highly variable, unspecific group of symptoms. Severity is also diverse. Clinical diagnosis is difficult, particularly in preterm and low birth weight infants due to their undeveloped immune system that leads to a misleading clinical picture [11]. General nonspecific symptoms include alterations in feeding behavior, which occurs early in the disease course, lethargy, poor cry and pale skin [10, 50]. Fever, hypothermia or a normal body temperature can all be present, however it is more common for a septic infant to be hypothermic [10, 11]. Fever is usually associated with low birth weight babies or babies born to a febrile mother [3, 45]. Nonspecific signs that may be present are anuria, metabolic acidosis, hypoglycemia or hyperglycemia [3, 45]. Signs such cyanosis, apnea, tachycardia, bradycardia and hypotension are associated with hemodynamic instability and may precede a hypotensive shock [50].

The physician must be aware that all organs and systems can be affected , being the respiratory system one of the most commonly involved, as pneumonia is often the presenting infection [11]. Respiratory distress, a common finding in this setting can masquerade congenital disorders such as congenital heart disease or congenital diaphragmatic hernia. Also, any noninfectious inflammatory syndrome can mimic the signs and symptoms of neonatal sepsis [51]. Clinical suspicion is of utterly importance for diagnosis.

Table 4 - Initial Signs and Symptoms of Infection in Newborn Infants*

Cardiovascular System	**Respiratory System**	**Renal System**
Pallor; cold skin; sweaty skin	Apnea, dyspnea	Oliguria
Tachycardia	Tachypnea	
Hypotension	Retractions	
Bradycardia	Cyanosis	
Gastrointestinal System	**Central Nervous System**	**Hematologic System**
Abdominal Distension	Irritability, lethargy	Jaundice
Vomiting	Tremors, seizures	Splenomegaly
Diarrhea	Hyporeflexia, hypotonia	Pallor
Hepatomegaly	Abnormal Moro reflex	Petechia, purpura
	Full fontanel	Bleeding

**Adapted from Enrione M, Powel K. Sepsis, Septic Shock, and Systemic Inflammatory Response Syndrome. Kliegman R. Nelson's Textbook of Pediatrics (20th Ed.). Saunders, 2016)*

Unfortunately, etiologic suspicion based solely on clinical findings and signs, is not possible. A few signs and symptoms, and their association, are more frequently associated with certain pathogens, such as a cutaneous erythematous rash associated with *Candida spp,* or Gram-negative other than *E. coli* [7].

Regarding long term complications, EOS is associated with an increased risk of bronchopulmonary dysplasia in preterm infants as well as neurodevelopment delay and other neurologic complications. However, these associations are very often confounded by multiple factors, such as the prematurity degree. Term infants who are affected by GBS infection also have a high complication rate. A significant percentage will suffer neurologic sequela, such as seizures, blindness and deafness and cognitive delays [11].

7. Diagnosis and Laboratory Markers

7.1 Initial Workup

It is not advisable to wait for the results of the blood cultures to initiate the treatment when neonatal sepsis is suspected, since it takes 24-48 hours. Bacterial isolation from blood is the gold standard for sepsis diagnosis [1]. It is recommended to perform at least two blood cultures, one percutaneous and one for each vascular access. Earlier therapeutic intervention is of the most importance to warrant lower mortality and morbidity associated rates. Laboratory results should be obtained as soon as possible to guide initial management.

Optimal results require culturing of 6 mL of blood, which is not feasible [1]. A minimum of 0,5-1 mL is advisable [7]. Blood cultures are not sensible enough to exclude sepsis and false positive cases can occur due to asymptomatic bacteremia or contamination. Positive cultures range from 8-73% in the diagnosis of potential neonatal sepsis [50], thus there is a high need for the use of non-culture dependent methods and other supplemental tests (annex IV). Laboratory tests are helpful in guiding antibiotic treatment, avoiding unnecessary treatment of non-infected neonates and thus unnecessary emergence of multidrug resistant organisms. Typical sepsis workup consists of obtaining a complete white blood count, with differential leucocyte count analysis, urine cultures, and a lumbar puncture for cell count and culture. Acute phase reactants, such as C-reactive protein (CRP), and procalcitonin (PCT) also assist in the diagnosis. Image testing, such as thoracic x-ray, is considered when respiratory signs and symptoms are present. Tracheal aspirate culture is also potentially indicated in intubated newborns with suspected sepsis [11]. Despite meningitis incidence in bacteremia infants being up to 23% [51], the need of lumbar puncture to rule out meningitis in infants with suspected sepsis is controversial. In the high-risk, healthy-appearing infant, data suggests that the likelihood of meningitis is extremely low. Lumbar puncture should be performed in presence of a negative blood culture in infants whose clinical course or laboratory data strongly suggest bacterial sepsis, or infants whom initial antimicrobial therapy didn´t improve their clinical state [51].

Urine testing is not needed in neonates with suspected sepsis in their first few days of life because most urinary tract infections in this age group are secondary to hematogenous dissemination to the kidney by bacteremia, and so urine analysis wouldn’t add more useful information that the one provided by blood cultures [51]. However, urinalyses and urine culture should be considered for subsequent workups in neonates who remain symptomatic, especially in LOS [52].

7.2 Complete blood count

Total and differential white blood cell count, absolute neutrophil count, and immature to total neutrophil ratio are generally used for neonatal sepsis screening [7]. On their own, none of these tests is sensible nor specific enough to accurately identify or exclude neonatal sepsis in the majority of newborns. Laboratory tests are more useful for excluding infection, rather than for identifying it. They have a high negative predicative value, but the positive predictive value is too low to allow for clinical decision guidance [11, 53].

A total leucocyte count of <5000 to 7500/ mm3 can be used to infer the diagnosis of neonatal sepsis [53], however this test has a low predictive value and low sensibility (29%) [11]. Gestational age affects the leukocyte count, and so lower leukocyte number cut-offs are used in younger infants. This cut-off is applied only in newborns immediately after birth, since absolute neutrophil counts rise after birth, requiring higher cut-offs values [54]. Neutrophil's absolute peak values are reached within 6-12 hours after birth, so it could be more reliable to obtain total leucocyte counts at this time for sepsis screening [54]. Leucopenia has shown to have low sensitivity and high specificity for neonatal sepsis [53], and it shouldn´t been forgotten that leucocyte counts may be normal in the early course of neonatal sepsis [1]. Immature to total neutrophil ratio ≥ 0,2 is associated with increased odds of infection, but conditions such perinatal asphyxia, and labor stress can also change the ratio [51]. Neutropenia is more predictive of neonatal sepsis than neutrophilia [53].

Platelets counts have no role in neonate's sepsis diagnosis or response to treatment [11]. Lactate levels are predictors of death in septic shock, but it is necessary to confirm its predictive value in pediatric patients [55].

7.3 Acute Phase Reactants

Procalcitonin (PCT) and C-reactive protein (CRP) are acute phase reactants, which levels increase due to bacterial infection or due to a range of noninfectious conditions such as meconium aspiration syndrome, traumatic or ischemic tissue injury or in infants with hemodynamic instability [1]. Viral infections are not usually associated with a raised CRP or PCT level [11]. Since it takes 10-12 hours for CRP levels to substantially raise after the onset of symptoms, peaking at 24h, it is advisable to measure CRP levels between 24-48h, preferably trough serial determinations to increase sensibility [56]. Due to this late increase,

CRP is considered a late marker of sepsis, with low sensibility when measured during the earlier phases [51].

PCT serum concentrations rise more rapidly than CRP, peaking at 6 to 8 hours, and is considered a mid-phase of sepsis marker. Therefore, PCT is a more sensitive marker for early sepsis detection, with its levels remaining elevated for 24 hours [1].

Preterm infants have lower CRP baseline values and a lower rise in response to infection, making this laboratory marker even less sensible [57]. The normal accepted cut off for significant level of CRP is >6 mg/l [53]. Highly sensitive CRP is more sensitive than conventional CRP, having increased sensitivity for neonatal infection, but it´s role in a routine clinical setting still needs to be evaluated [53]. Serial normal CRP determinations have been shown to have a high negative predicative value, and thus repeated normal values are a strong sign against bacterial sepsis [56], being an indication to interrupt antibiotic therapy [7, 58, 59].

PCT serum concentration is not affected by gestational age, and its serum concentration remains high when compared to other biomarkers, making it more useful in predicting the severity of infection and the response to treatment [53]. However, PCT concentrations are affected by maternal GBS colonization and prolonged rupture of membranes [1]. There is a physiological increase within the first 24h of birth. The normal values for neonates >72 hours of age is <0,1ng/ml [57]. PCT levels decline rapidly with appropriate therapy and can be used to assess response to treatment, as well as in predicting severity of infection and outcomes [60]. Despite CRP being the most studied laboratory test in neonatal sepsis diagnosis, a few studies showed that PCT has better sensitivity and specificity than CRP [61, 62].

7.4 Other biomarkers and diagnostic techniques

Cytokines levels such as interleukin 6 (IL-6), IL-8, tumor necrosis factor α (TNF-α) and γ interferon (IFN-y) rise very quickly in the setting of neonatal sepsis, even before the development of sepsis´s signs and symptoms and acute phase reactants [1]. They all generally have very similar sensitivities and specificities [11].

IL-6 levels peak up to 48 prior to the onset of clinical sepsis and can be considered as an early and sensitive marker of neonatal infection [11]. Its levels fall quickly in response to

treatment due to IL-6´s short half-life, and therefore it has a narrow window of opportunity [1, 53]. TNF-α has a kinetic profile very similar to the IL-6 [63], and its levels are not affected by gestational or postnatal age [64]. IL-8 not only plays a role as a marker of early sepsis, but its levels are also associated with infection severity [65].

Interpretation of the combined levels of these laboratory markers, together with acute phase reactants is superior to the use of each one individually for the early detection of neonatal sepsis. This was proven for the combined use of IL-8 and CRP values [64], IL-6 and PCR [11] and combined IL-6, PCR and PCT levels [11]. All are highly predictive for the diagnosis of early onset sepsis.

Cell surface markers such as CD11β, Scd 163 and CD64 have their expression increased minutes following exposure to bacterial products, and can be measured with flow cytometric technology, being reliable markers of early sepsis. Both sensitivity and specificity are very high[53]. Inflammatory biomarkers can also be searched in the amniotic fluid or in the cord blood for earlier sepsis detection. There is known that the presence of infection and inflammation in the intrauterine environment predispose the neonatal to sepsis, but amniotic fluid collection is not routinely practiced for EOS detection, due to being associated with inherent risks [65]. Studies suggest that among cord blood biomarkers, IL-6 and IL-8 seem to have the best discriminatory value to early neonatal sepsis diagnosis [65]. Operational difficulties in cytokines detection and cell surface markers, as well as cytokines elevation in nonspecific settings, limit their use in clinical practice [53].

Quantitative real-time amplification systems (qPCR) and DNA microarray-based methods are another possible adjunctive diagnostic approach. Despite their high sensibility, and rapid results, they don´t provide information about antibiotic resistance, and they don´t allow distinction between true positive cases and asymptomatic colonization [65,66]. Mass spectrometry can aid with early identification of organisms from blood microbial cultures, allowing for directed antibiotic therapy, and more recently multiplex PCR can identify quickly common pathogens, as well as antimicrobial resistance genes [66].

Novel biomarkers in current investigation are haptoglobin, serum amyloid A, hepcidin, interα inhibitory proteins, angiopoietin 2 and soluble receptor uroquinase [53, 65]

8. Treatment

Neonatal sepsis´s treatment should aim for the responsible pathogen elimination, maintenance of the child´s hemostasis using supporting measures, and avoidance of an exaggerated prejudicial inflammatory response to infection [67]. Therefore, treatment should offer an adequate antibiotherapy, as well as a suitable management for severe sepsis and septic shock that allows for preservation of vital signs and bodily functions. Consideration of EOS or LOS presentation affects antimicrobial choice [7].

8.1 Antibiotic selection

Empiric antibiotic treatment is initiated when sepsis is suspected, based on the presenting clinical signs and symptoms, or, in some cases, in asymptomatic children, as is in the case of children born to a mother with chorioamnionitis [68].

The choice of the best antibiotic regimen for treatment is empirical, based on the age of presentation, likely etiologic agents, and local antibiotic susceptibility patterns. This last parameter has an increasing significance in the last few years, due to the rising prevalence and resistance of Gram-negative microorganisms to antibiotherapy. Empirical coverage for EOS should focus on the most probable etiologic agents, such as GBS and *E. coli* [68].

Antibiotic classes used in neonatal sepsis´s treatment include beta-lactams (e.g.: penicillin, ampicillin), the glycopeptide vancomycin, aminoglycosides, such as gentamicin and carbapenems. Ampicillin plus gentamicin regimen is the hallmark for early neonatal sepsis treatment [69]. Despite the widespread use of IAP, which caused concerns about the development of resistance to these antibiotics, ampicillin and gentamicin are still efficient for most common pathogen's treatment [51]. The strains of GBS are still susceptible to penicillin, ampicillin and first generation cephalosporins [14]. However, there are reports of a few isolated cases of GBS colonies with a higher than usual minimum inhibitory concentration to beta-lactams [70, 71].

Despite the apparently uniform GBS susceptibility to beta-lactam antibiotics, the same is not true for aminoglycosides, since GBS is usually resistant to this drug [69]. Nonetheless it´s an indispensable component in the initial therapy due to its synergic activity against GBS and *Listeria monocytogenes* when combined to ampicillin, as suggested by earlier studies [72]. Aminoglycoside usage, usually gentamicin, allows for inhibition of protein synthesis. Their use requires monitoring to achieve the correct dose and limit potential side effects [73].

There is an increasing prevalence in acquired community of extended-spectrum βlactamase-producing (ESBL) Gram-negative neonatal infection across the globe, and most of them are resistance to aminoglycosides as well [74]. The ongoing emergence of community ESBL-producing organisms calls for vigilance in monitoring local patterns for gentamicin´s susceptibility [11]. ESBL Gram-negative microorganisms are treated with carbapenems, such as meropenem [7]. Regarding the choice between ampicillin and penicillin, ampicillin seems the best choice in the absence of a etiologic diagnosis, due to its coverage to both Gram negative organisms, as well as the Gram positive organisms covered by penicillin [75]. Once GBS is identified it seems prudent to use penicillin, due to being a narrower spectrum agent, lowering the chances to increase other microorganisms' antibiotic resistances [69].

Third generation cephalosporins are a reasonable alternative to aminoglycosides, and a combination of ampicillin plus cefotaxime as an alternative therapy for empirical treatment has been proposed [51, 69]. However earlier studies have showed an increase in resistance development when cefotaxime is used routinely for neonatal sepsis treatment, especially in Gram negative pathogens, when compared to aminoglycosides. An increase in invasive candidiasis incidence has been also reported [76, 77]. If there is suspicion of meningitis while waiting for definitive laboratory results, cefotaxime may be added as an empirical agent [11]. Third and fourth generation cephalosporin drugs should be reserved for suspected Gram negative meningitides [7], with exception of ceftriaxone, which is not recommended when there is meningitis suspicion [78].

Regarding GBS infection specific therapy, if treatment with ampicillin and gentamicin was started empirically, gentamicin may be discontinued once laboratory results confirm GBS infection. Thereafter treatment may be completed with ampicillin alone or with penicillin G. However, prior to narrowing it is advisable to continue to administrate ampicillin and gentamicin until documentation of clearance of bacteremia and CNS infection [11].In GBS´s neonatal meningitis cefotaxime can be administrated daily [69].

In developing countries a ampicillin and gentamicin regimen may not be the best empirical antibiotic regimen of choice, due to differences in etiologic epidemiology, and should focus even more on individualized epidemiologic data from each hospital or region, when compared to developed countries [79]. LOS is empirically treated with vancomycin plus an aminoglycoside [12]. Doses of commonly used antibiotics in neonatal sepsis can be viewed on annex V.

8.2 Antibiotic Therapy Duration

Antibiotic therapy´s duration for microbial cultures proven early neonatal sepsis depends upon the pathogen. Usually, when Gram-positive microorganisms such as GBS are found, therapy is administered for 14 days, while in sepsis due to Gram-negative microorganisms 10 days of antibiotic therapy is adequate [68]. Complicated infections need an extended treatment, around 21-28 days [7]. In most cases symptomatic patients with proven sepsis improve clinically within 48-72 hours [68]. Antibiotics should be continued for those who remain symptomatic and/or with positive cultures. LOS´s duration of treatment depends on the pathogen and site [7].

There is a wide variation between centers regarding the appropriate duration of empiric antibiotherapy for suspected early neonatal sepsis with negative blood cultures, because actual recommendations in this setting are not based on strong evidence due to a lack of well-designed trials [79]. When considering the therapy duration in infants with negative blood cultures, despite presence of signs and symptoms suggestive of neonatal sepsis, decision should take in account clinical presentation and symptoms severity. Preterm infants are considered to be at high risk of sepsis, and so the usual practice is to initiate antibiotic therapy immediately, regardless of blood culture results if there is any degree of suspicion of neonatal sepsis [79]. In a retrospective study, the average duration of treatment in 695 infants (< 1000 g) with negative blood cultures was 5 ± 3 days [80].

It should be taken in account that false negative results are a possibility in pregnant woman who received GBS prophylaxis therapy to prevent onset of GBS infection. Decision to initiate therapy in culture negative sepsis (screen positive and clinical course consistent with sepsis) is a real challenge. The standard practice is to discontinue antibiotics as soon as blood cultures are confirmed negative (48–72 hours), alongside absence of clinical or hematologic signs of infection. In this clinical situation sepsis probability is low, and prolonged empirical treatment of preterm infants for periods longer than five days is associated with higher risk of late onset sepsis, necrotizing enterocolitis, and mortality [81]. Meningitis due to GBS infection or other bacteria not associated with complications, should be treated for 14 days minimum. GBS infections with a defined focus, such as osteomyelitis or endocarditis, should be treated for a longer time, usually for 3,4 or more weeks [51].

Antibiotic monitoring should be done to ensure that trough levels are adequate, and repeat blood cultures should be obtained, usually within 24 h of presumed effective therapy, to

document clearance. Persistent positive cultures could mean failure of antimicrobial therapy or evidence of intravascular site infection [11].

8.3 Septic shock management

While consensus has been reached about septic shock´s management in children and term neonates, there are not definitive guidelines for preterm neonate´s septic shock treatment [82].

Septic shock´s management begins with airway, breathing and circulation assessment. Only after vital signs stabilization, should antibiotic therapy be considered. Many septic shock neonates present with severe respiratory distress, and may require intubation. Initially it is essential to have a quickly available venous access to allow for volume resuscitation and vasopressor therapy whether needed, as well as for antibiotic administration. 2 venous accesses are recommended. When venous accesses are not immediately possible, intraosseous infusion or umbilical vein catheterization should be considered. Continuing assessment for cardiovascular dysfunction is critical [82].

Therapeutic endpoints are not well-defined, but it is advisable to keep a capillary refill less than two seconds, warm extremities, low blood lactates, urine output more than 1ml/kg/h and mixed venous saturation superior to 70% [83].

If hypotension is present, the first step is to give a fluid bolus, usually a crystalloid. However in preterm neonates there is lack of evidence regarding early volume expansion treatment [84], and there may be an increased risk of associated intracranial hemorrhage [82]. In hypotensive preterm neonates it is recommended to only give a single bolus of saline, and if not successful, begin therapy with vasoactive drugs as a second line approach [85]. Dopamine is the first choice, and if arterial tension doesn´t recover, additional therapy should include glucocorticoids, other catecholamine, inotropes or vasodilators [82]. Another treatment option for septic shock could be vasoconstrictor arginine-vasopressin, but further studies are needed [86]. In many clinical practices, hydrocortisone is the third-line agent in treatment of neonatal shock after volume resuscitation and dopamine, due to its suppression proprieties in the intensity of the systemic inflammatory response. Hydrocortisone has been shown to decrease heart rate, and decrease vasoactive medication requirements in both preterm and term neonates [87].

8. 4. New therapeutic strategies

There has been increasing research about new therapeutic approaches aiming to boost the neonate´s immune system, which can improve outcomes of antibiotic based therapy. A number of adjuvant strategies has been purposed and researched, but so far none of them has been successful, and are still labeled as experimental [11].

Examples include recombinant granulocyte colony-stimulating factor (rG-CSF) and recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF) administration, allowing for increased phagocytosis and cell-mediated pathogen killing. This functional benefit in neutrophil function was found in vitro studies, however when applied to a septic newborn, there wasn´t a significantly increased sepsis free survival [88]. No toxicity was observed [45]. Use of these agents has also been studied in a prophylactic setting, but there is also lack of evidence to support its application [89]. Some physicians have forgone attempts to treat neonatal sepsis by stimulating proliferation and function of existing neonatal neutrophil precursors and have instead administered adult neutrophils to at-risk neonates. A few limited trials have found a significant reduction on mortality [90]. More research is needed in order to validate this approach.

Intravenous non-specific IVIG therapy has also been studied as an adjuvant therapy in neonatal sepsis, which could be especially useful in premature infants due to their profound IgG deficiencies. Despite favorable results in earlier smaller trials [91, 92], a recent study concluded that intravenous immune globulin therapy had no effect on the outcomes of suspected or proven neonatal sepsis [93].

Pentoxifylline, a drug approved for certain adult population vascular disorders, suppresses pro-inflammatory cytokines. It has been proposed that when combined with antibiotic therapy, it attenuates the inflammatory response associated with bacterial lysis, induced by antibiotherapy. According to a metanalysis regarding pentoxifylline use in neonatal sepsis, there is low-quality evidence that it is associated with a lower sepsis mortality [94]. More research is necessary to validate this approach.

9. Conclusion

Despite all the advances made in neonatal sepsis caused by GBS in the last decades, that led to a decrease in neonatal sepsis incidence, and despite the reduced mortality due to advancements in the neonatal critical care, the incidence of GBS neonatal sepsis is stabilizing without further decrease and this review demonstrated the limitations associated with current forms of prevention and treatment that are slowly rising.

An increased EOS incidence by microorganisms other than GBS, the emergence of antibiotics-resistant microorganism, an increased LOS incidence due to higher survival rates in preterm newborns and an increasing number of studies linking neonatal use of antibiotics to the development of autoimmune diseases, show that the status quo should not be accepted in regard to the current management of neonatal sepsis. Also, despite it was observed a high number of studies related to etiology, diagnostic and treatment of adult sepsis, the same can´t be said in the pediatric population, especially regarding preterm infants, which are one of the groups most susceptible to neonatal sepsis.

This review showed that new research studies are continually being published, with focus on new prevention methods and more sensitive and specific laboratory markers for neonatal sepsis, which can have a substantial impact in the near future by leading to better outcomes and to less adverse effects associates with antibiotic use. Knowledge about the pathophysiologic events behind neonatal sepsis, and the immune system ontogeny are also being actively researched. The use of non-culture based diagnostics and sepsis scores to predict and diagnose septic neonates are areas of active investigation. Assessment of the cost-benefit relation in these new approaches is clearly needed, due to the lack of their use in routine clinical practice. Concepts and attitudes standardizations about neonatal sepsis could lead to a more efficacious management.

10. References

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11. ANNEXS

Annex I - Global cause-specific numbers of neonatal deaths, proportions and risks in 2013 *

	Early neonatal period		Late neonatal period		Total		
Cause	No. of deaths[a] (uncertainty range)	Proportion, %	No. of deaths[a] (uncertainty range)	Proportion, %	No. of deaths[a] (uncertainty range)	Proportion, %	Risk[b]
Preterm birth	834.8 (608.1–1083.5)	40.8	152.1 (91.0–229.0)	21.2	986.9 (699.1–1312.5)	35.7	7.2
Intrapartum complications	552.7 (407.6–711.4)	27.0	92.1 (54.8–133.4)	12.9	644.8 (462.4–844.7)	23.4	4.7
Congenital disorders	217.0 (140.9–325.9)	10.6	72.8 (42.5–124.5)	10.2	289.8 (183.3–450.4)	10.5	2.1
Sepsis	163.7 (62.4–271.6)	8.0	266.7 (156.5–393.2)	37.2	430.4 (218.9–664.8)	15.6	3.1
Pneumonia	98.9 (48.8–200.3)	4.8	37.6 (21.5–58.7)	5.2	136.4 (70.3–259.0)	4.9	1.0
Diarrhoea[c]	6.7 (0–57.4)	0.3	10.0 (3.2–25.6)	1.4	16.6 (3.2–83.0)	0.6	0.1
Tetanus[c]	21.1 (7.4–53.2)	1.0	27.1 (8.1–67.2)	3.8	48.2 (15.5–120.4)	1.7	0.3
Other[d]	149.9 (72.7–250.3)	7.3	57.9 (26.3–117.2)	8.1	207.8 (99.0–367.4)	7.5	1.5

[a] In thousands.

[b] Per 1000 live births.

**Source: Shefali Oza et al, Neonatal cause-of-death estimates for the early and late neonatal periods for 194 countries: 2000–2013. WHO, 2013*

Annex II - Inflammatory response in bacterial sepsis*

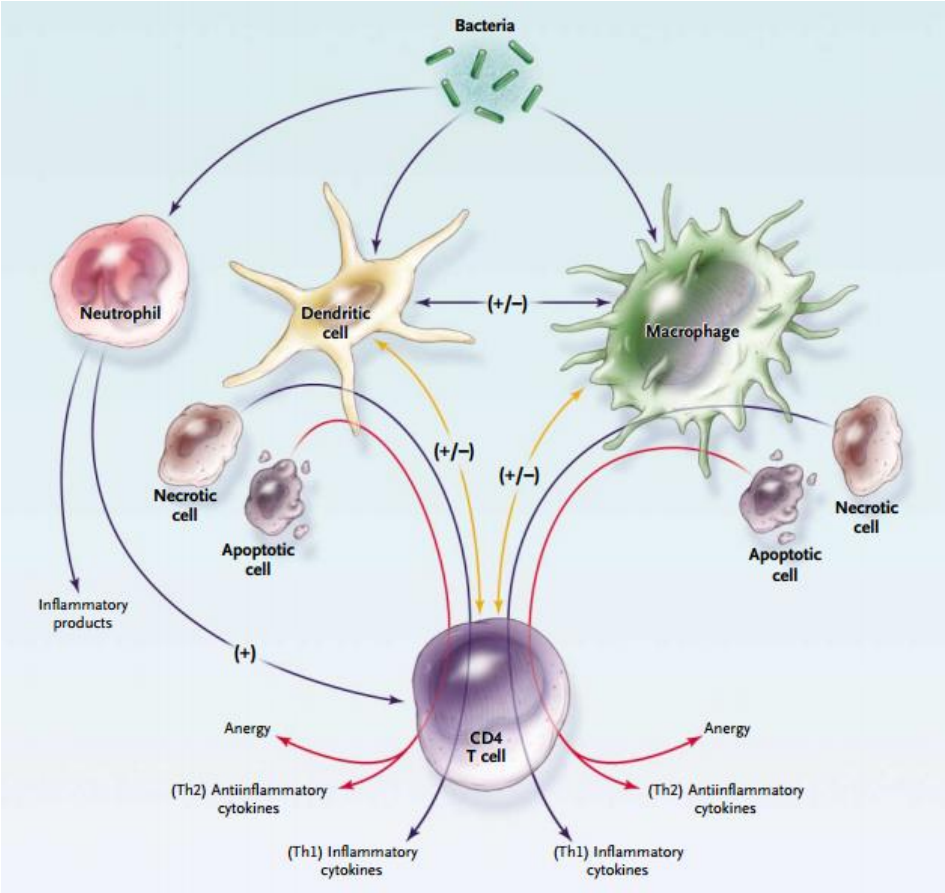
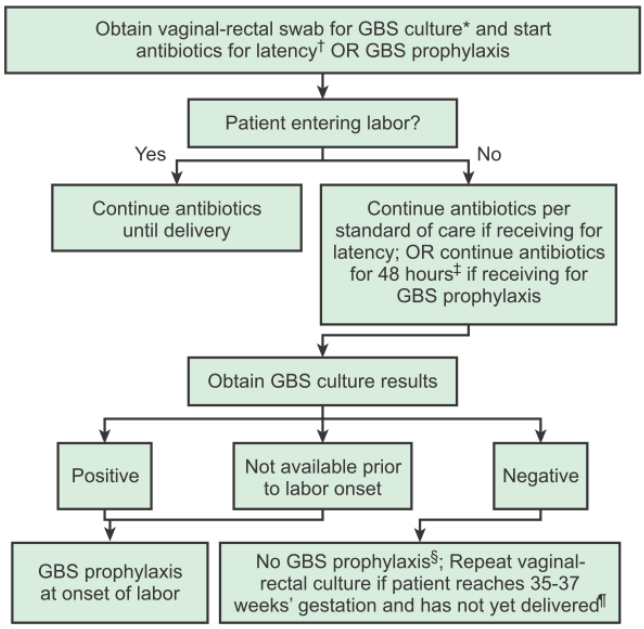


Figure 1. The Response to Pathogens, Involving "Cross-Talk" among Many Immune Cells, Including Macrophages, Dendritic Cells, and CD4 T Cells.

Macrophages and dendritic cells are activated by the ingestion of bacteria and by stimulation through cytokines (e.g., interferon-γ) secreted by CD4 T cells. Alternatively, CD4 T cells that have an anti-inflammatory profile (type 2 helper T cells [Th2]) secrete interleukin-10, which suppresses macrophage activation. CD4 T cells become activated by stimulation through macrophages or dendritic cells. For example, macrophages and dendritic cells secrete interleukin-12, which activates CD4 T cells to secrete inflammatory (type 1 helper T-cell [Th1]) cytokines. Depending on numerous factors (e.g., the type of organism and the site of infection), macrophages and dendritic cells will respond by inducing either inflammatory or anti-inflammatory cytokines or causing a global reduction in cytokine production (anergy). Macrophages or dendritic cells that have previously ingested necrotic cells will induce an inflammatory cytokine profile (Th1). Ingestion of apoptotic cells can induce either an anti-inflammatory cytokine profile or anergy. A plus sign indicates up-regulation, and a minus sign indicates down-regulation; in cases where both a plus sign and a minus sign appear, either up-regulation or down-regulation may occur, depending on a variety of factors.

**Source: Hotchkiss et al. The pathophysiology and treatment of sepsis. N Engl J Med 348(2): 138-150, 2003.*

Annex III. Algorithm for GBS intrapartum prophylaxis for women with preterm premature rupture of membranes*



**Source: Enrione M, Powel K. Sepsis, Septic Shock, and Systemic Inflammatory Response Syndrome. Kliegman R. Nelson's Textbook of Pediatrics (20th Ed.). Saunders, 2016)*

Annex IV. Culture-Based and Non–Culture-Based Diagnostics for Neonatal Sepsis*

CATEGORY	PARAMETER	OPTIMAL TIMING, VOLUME OF SPECIMEN, ROUTINE/INVESTIGATIONAL	APPLICABILITY FOR NEONATAL SEPSIS
Culture-based			
Blood	Culture	>1 mL of whole blood ROUTINE*	Gold standard for bacteremia
Cerebrospinal fluid (CSF)	Culture	When clinically feasible ROUTINE	Optimize antimicrobial therapy
Urine	Culture	>72 hr of life ROUTINE	Not useful for EOS; potential benefits for LOS
Tracheal aspirate	Culture	ROUTINE	Usually reflects colonization
Non–culture-based			
Immune function	MHC II TNF-α	INVESTIGATIONAL† INVESTIGATIONAL	Both decreased in chorioamnionitis and sepsis
Neutrophil indices	Neutropenia Absolute neutrophil count Absolute immature neutrophil count	After 12 hr of life Consider GA, delivery mode, altitude, arterial versus venous sampling, time since birth ROUTINE	Neutropenia better predictor for sepsis than leukocytosis
Neutrophil markers	CD64	Elevated for 24 hr after infection Requires 50 μL blood Results within hours INVESTIGATIONAL	Cut points between 2.38–3.62 optimal sensitivity, specificity and NPV for EOS
Platelet count	Thrombocytopenia and thrombocytosis	Late findings; slow to respond ROUTINE	Thrombocytopenia associated with fungal infection
CSF cell count	CSF WBC	Uninfected neonates mean 10 cells/mm^3. Range up to 20 cells/mm^3 ROUTINE	Does not predict culture-proven meningitis
CSF chemistries	CSF protein CSF glucose	Term <100 mg/dL preterm higher; 70–80% of serum glucose ROUTINE	Elevated in fungal meningitis Low glucose specific for bacterial meningitis
Acute phase reactants	CRP Procalcitonin	8–24 hr after infection 2–12 hr after infection ROUTINE/INVESTIGATIONAL	Good NPV Better sensitivity but less specificity than CRP
Sepsis panels/scores		After 24 hr of life INVESTIGATIONAL	Most useful for NPV and discontinuation of antimicrobial therapy

**Source: Enrione M, Powel K. Sepsis, Septic Shock, and Systemic Inflammatory Response Syndrome. Kliegman R. Nelson's Textbook of Pediatrics (20th Ed.). Saunders, 2016)*

Annex V - Antibiotics commonly used in pediatric practice for neonatal sepsis*

Penicillin G Injection Tablets	**Penicillin active against most Gram-positive cocci; *S. pneumoniae* (resistance is increasing), group A *Streptococcus*, and some Gram-negative bacteria (e.g., *N. gonorrhoeae, N. meningitidis*)** Neonates: Postnatal age ≤7 days weight 1,200-2,000 g: 50,000 units/kg/24 hr divided q 12 hr IV or IM (meningitis: 100,000 units/kg/24 hr divided q 12 hr IV or IM); weight >2,000 g: 75,000 units/kg/24 hr divided q 8 hr IV or IM (meningitis: 150,000 units/kg/24 hr divided q 8 hr IV or IM); postnatal age >7 days weight ≤1,200 g: 50,000 units/kg/24 hr divided q 12 hr IV (meningitis: 100,000 units/kg/24 hr divided q 12 hr IV); weight 1,200-2,000 g: 75,000 units/kg/24 hr q 8 hr IV (meningitis: 225,000 units/kg/24 hr divided q 8 hr IV); weight >2,000 g: 100,000 units/kg/24 hr divided q 6 hr IV (meningitis: 200,000 units/kg/24 hr divided q 6 hr IV) Children: 100,000-250,000 units/kg/24 hr divided q 4-6 hr IV or IM (max dose: 400,000 units/kg/24 hr) Adults: 2-24 million units/24 hr divided q 4-6 hr IV or IM	*Cautions:* β-Lactam safety profile (rash, eosinophilia), allergy, seizures with excessive doses particularly in patients with marked renal disease. Substantial pathogen resistance. Primarily renally eliminated *Drug interaction:* Probenecid
Ampicillin Polycillin, Omnipen Capsule: 250, 500 mg Suspension: 125 mg/5 mL, 250 mg/5 mL, 500 mg/5 mL Injection	**β-Lactam with same spectrum of antibacterial activity as amoxicillin** Neonates: Postnatal age ≤7 days weight ≤2,000 g: 50 mg/kg/24 hr IV or IM q 12 hr (meningitis: 100 mg/kg/24 hr divided q 12 hr IV or IM); weight >2,000 g: 75 mg/kg/24 hr divided q 8 hr IV or IM (meningitis: 150 mg/kg/24 hr divided q 8 hr IV or IM). Postnatal age >7 days weight <1,200 g: 50 mg/kg/24 hr IV or IM q 12 hr (meningitis: 100 mg/kg/24 hr divided q 12 hr IV or IM); weight 1,200-2,000 g: 75 mg/kg/24 hr divided q 8 hr IV or IM (meningitis: 150 mg/kg/24 hr divided q 8 hr IV or IM); weight >2,000 g: 100 mg/kg/24 hr divided q 6 hr IV or IM (meningitis: 200 mg/kg/24 hr divided q 6 hr IV or IM) Children: 100-200 mg/kg/24 hr divided q 6 hr IV or IM (meningitis: 200-400 mg/kg/24 hr divided q 4-6 hr IV or IM) Adults: 250-500 mg q 4-8 hr IV or IM	*Cautions:* Less bioavailable than amoxicillin, causing greater diarrhea *Drug interaction:* Probenecid
Vancomycin Vancocin, Lyphocin Injection Capsule: 125 mg, 250 mg Suspension	**Glycopeptide antibiotic active against most Gram-positive pathogens including staphylococci (including MRSA and coagulase-negative staphylococci), *S. pneumoniae* including penicillin-resistant strains, *Enterococcus* (resistance is increasing), and *C. difficile*–associated colitis** Neonates: Postnatal age ≤7 days, weight <1,200 g: 15 mg/kg/24 hr divided q 24 hr IV; weight 1,200-2,000 g: 15 mg/kg/24 hr divided q 12-18 hr IV; weight >2,000 g: 30 mg/kg/24 hr divided q 12 hr IV; postnatal age >7 days, weight <1,200 g: 15 mg/kg/24 hr divided q 24 hr IV; weight 1,200-2,000 g: 15 mg/kg/24 hr divided q 8-12 hr IV; weight >2,000 g: 45 mg/kg/24 hr divided q 8 hr IV Children: 45-60 mg/kg/24 hr divided q 8-12 hr IV; *C. difficile*–associated colitis; 40-50 mg/kg/24 hr divided q 6-8 hr PO.40-50 mg/kg/24 hr divided q 6-8 hr PO	*Cautions:* Ototoxicity and nephrotoxicity particularly when co-administered with other ototoxic and nephrotoxic drugs Infuse IV over 45-60 min. Flushing (red man syndrome) associated with rapid IV infusions, fever, chills, phlebitis (central line is preferred). Renally eliminated *Target serum concentrations:* Peak (1 hr after 1 hr infusion) 30-40 mg/L; trough 5-10 mg/L
Gentamicin Garamycin Injection Ophthalmic solution, ointment, topical cream	**Aminoglycoside antibiotic active against Gram-negative bacilli, especially *E. coli, Klebsiella, Proteus, Enterobacter, Serratia*, and *Pseudomonas*** Neonates: Postnatal age ≤7 days weight 1,200-2,000 g: 2.5 mg/kg q 12-18 hr IV or IM; weight <2,000 g: 2.5 mg/kg q 12 hr IV or IM; postnatal age >7 days weight 1,200-2,000 g: 2.5 mg/kg q 8-12 hr IV or IM; weight >2,000 g: 2.5 mg/kg q 8 hr IV or IM Children: 2.5 mg/kg/24 hr divided q 8-12 hr IV or IM. Alternatively may administer 5-7.5 mg/kg/24 hr IV once daily Intrathecal: Preservative-free preparation for intraventricular or intrathecal use: neonate: 1 mg/24 hr; children: 1-2 mg/24 hr intrathecal; adults: 4-8 mg/24 hr Adults: 3-6 mg/kg/24 hr divided q 8 hr IV or IM	Cautions: Anaerobes, *S. pneumoniae*, and other *Streptococcus* are resistant. May cause ototoxicity and nephrotoxicity. Monitor renal function. Drug eliminated renally. Administered IV over 30-60 min *Drug interactions:* May potentiate other ototoxic and nephrotoxic drugs *Target serum concentrations:* Peak 6-12 mg/L; trough >2 mg/L with intermittent daily dose regimens only
Cefotaxime sodium Claforan Injection	**Third-generation cephalosporin active against Gram-positive and Gram-negative pathogens. No antipseudomonal activity** Neonates: ≤7 days: 100 mg/kg/24 hr divided q 12 hr IV or IM; >7 days: weight <1,200 g 100 mg/kg/24 hr divided q 12 hr IV or IM; weight >1,200 g: 150 mg/kg/24 hr divided q 8 hr IV or IM Children: 150 mg/kg/24 hr divided q 6-8 hr IV or IM (meningitis: 200 mg/kg/24 hr divided q 6-8 hr IV) Adults: 1-2 g q 8-12 hr IV or IM (max dose: 12 g/24 hr)	*Cautions:* β-Lactam safety profile (rash, eosinophilia). Renally eliminated. Each gram of drug contains 2.2 mEq sodium. Active metabolite *Drug interaction:* Probenecid

**Source: Enrione M, Powel K. Sepsis, Septic Shock, and Systemic Inflammatory Response Syndrome. Kliegman R. Nelson's Textbook of Pediatrics (20th Ed.). Saunders, 2016)*