Diagnostic criteria for early onset neonatal sepsis

Klobučar, Branimir

UNIVERSITY OF ZAGREB
SCHOOL OF MEDICINE

Branimir Klobučar

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Abbreviations:

ANC- Absolute neutrophils counts

CAM- Chorioamnionitis

CFR- Case fatality rate

CRP- C-reactive protein

ELBW- Extremely low birth weight

EOS- Early onset neonatal sepsis

GA- Gestational age

GBS- Streptococcus group B

I:M- Immature to mature neutrophil ratio

I:T- Immature to total neutrophil ratio

LBW- Low birth weight

LOS- Late onset neonatal sepsis

MBP- Mean blood pressure

PCT- Procalcitonin

PMN- Total polymorphonuclear leukocytes

SD- Standard deviation

SIRS- Severe systemic inflammatory response

TNF-α- Tumor necrosis factor-alpha

VLBW- Very-low-birth weight

PPH- Persistent pulmonary hypertension

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1.0 Summary

Title: DIAGNOSTIC CRITERIA FOR EARLY ONSET NEONATAL SEPSIS

Author: Branimir Klobučar

Neonatal sepsis is one of the leading causes of neonatal mortality worldwide. Unlike in the adult population, there is a large variability in the clinical picture and therefore the difficulty to recognize and diagnose neonatal sepsis. In addition to this, there is a lack of clear diagnostic criteria that would aide clinicians in diagnosis and rapid treatment of sepsis. There has been much ongoing research in this area without any striking success.

Neonatal sepsis can be divided into different categories based on the age of disease onset. Early onset neonatal sepsis occurs in the first 72 hours of the child's life and is associated with various risk factors and physiological differences when compared to other age groups. The risk factors may be related to the mother, the child, or the process of delivery.

Early onset neonatal sepsis also has a specific set of pathogens that are most likely to cause disease. The two most common pathogens, E. coli and Group B streptococcus, have a tendency to exploit the neonates immature immune system resulting in possible complications. Several components of the neonates' physiological inflammatory response have been researched for their value in diagnosis. The current evidence for C-reactive protein, procalcitonin, interleukin-6 and several other inflammatory reactants have shown variable results. The lack of a clear biomarker that would consistently confirm or exclude the diagnosis of neonatal sepsis continues to elude us. The continued exploration into the various markers and their combinations still needs to be researched so that a list of criteria can be developed in order to reduce the devastating results of the disease.

Keywords: Sepsis, Early-onset sepsis, newborn, diagnosis

2.0 Sažetak

Naslov: DIJAGNOSTIČKI KRITERIJI ZA RANU NOVOROĐENAČKU SEPSU

Pisac: Branimir Klobučar

Sepsa je jedan od vodećih uzroka smrtnosti novorođenčadi širom svijeta. U usporedbi sa odraslom populacijom, u novorođenčadi je klinička slika sepse nespecifična i šarolika. Zbog toga je rano prepoznavanje novorođenačke sepse uvelike otežano. Pored toga, nedostaju jasni dijagnostički kriteriji koji bi omogucili ispravnu i brzu dijagnozu sepse. Ovaj problem predmetom je konstantnog istraživanja, zasad bez nekog znatnijeg uspjeha.

Novorođenačka sepsa može se podijeliti u različite kategorije ovisno o vremenu pojavljivanja bolesti. Rana novorođenačka sepsa javlja se u prvih sedamdeset i dva sata života i povezana je s različitim rizičnim čimbenicima i razlikuje se od sepse u drugim dobnim skupinama. Čimbenici rizika mogu biti povezani s majkom, novorođenčetom ili s procesom rađanja.

Rana novorođenačka sepsa također ima specifičan skup patogena kao najvjerojatnije uzročnike. Dva patogena, Escherichia coli i Streptococcus B skupine, najčešći su uzročnici rane novorođenačke sepse.

Neke osobitosti upalne reakcije novorođenčadi istraživane su s ciljem vrijednovanja u postavljanju dijagnoze. Istraživanja o dijagnostičkoj vrijednosti C-reaktivnog proteina, procalcitonina, interleukina-6 i nekoliko drugih upalnih reaktanata pokazala su različite rezultate. Još uvijek nije nađen biljeg koji bi dosljedno potvrdio ili isključio dijagnozu sepse u novorođenčadi. Neophodno je nastaviti istraživanja te pronaći dijagnostičke alate čime bi se omogućilo pouzdano i pravovremeno postavljanje dijagnoze ove opake bolesti.

Ključne riječi: sepsa, rana novorođenčka sepsa, novorođenče, dijagnoza

3.0 Introduction

This review will be geared towards the difficulties that come along with the diagnosis of early onset neonatal sepsis (EOS). Clinicians have long been able to have a clear definition of sepsis in adults. Having a clear definition enables the physicians to administer the proper treatment as soon as possible, improving the patients' chance of survival. This also avoids the overuse of antibiotics in patients where sepsis is not the correct diagnosis. Overuse of antibiotic therapy is a growing concern throughout the world; avoiding their unnecessary use can halt the inevitable resistance bacteria will develop to antibiotics. Unfortunately, clear diagnostic criteria are unavailable to the neonatal population. The clinical signs and symptoms of EOS are none specific and inconsistent making it difficult for a clinician to make a diagnosis (1). Due to this challenge, this review will focus on the most common and highly researched components in early onset neonatal sepsis that may assist the clinician in the process of diagnosis.

4.0 Classifications and Definition

Sepsis can occur at any age, but it is in the youngest and oldest age groups of the population where a fatal outcome is a frightening reality. It is important that the definition of sepsis is clear and consistent, enabling clinicians and researchers to be on the same page discussing its identification and treatment. Generally sepsis is defined as severe inflammatory response syndrome (SIRS) with confirmed infection. SIRS is the general body inflammatory response that typically leads to physiological alterations from baseline in the absence of a known cause. Body temperature >38 or < 36 degrees Celsius, tachycardia and mean respiratory rate >2 SD relative to age and leukocyte count elevated or suppressed for the relative age are all features of SIRS. SIRS can be a systemic reaction to several different causes that can be either infectious or non-infectious. Sepsis can progress further, being then classified as severe sepsis. Severe sepsis is the combination of sepsis with either organ dysfunction; hypoperfusion abnormality or sepsis

induced hypotension. Sepsis can progress as far as septic shock, which is defined as sepsis induced hypotension persisting despite adequate fluid resuscitation (2).

Due to the physiological differences between adults and the pediatric population the definitions of SIRS, severe sepsis and septic shock have been altered. For children less than a year of age the definition of SIRS would be considered with the presence of bradycardia or tachycardia (3). It must also be taken into consideration that there is the absence of any external stimulation, congenital malformations or an unexplained depressed heart rate for over a 30 min period. Severe sepsis is defined as sepsis in the presence of cardiovascular organ dysfunction, acute respiratory distress syndrome or two or more other organ system dysfunctions such as hepatic, renal, neurological and hematologic. Septic shock in children does not require observable hypotension to make a diagnosis as it does in adults. Shock may occur long before hypotension sets in, and as a result septic shock in children is defined as tachycardia with signs of decreased perfusion. Due to the high levels of overlapping criteria, septic shock in children is considered as sepsis with cardiovascular dysfunction (3).

The terminology defining the age of a child can become quite confusing and often terms are mixed and interchanged. Due to the focus of this paper, it is important to define the age group of interest. According to an international consensus done on June 8th 2010 at the European medical association, the term “neonate” is classified for children < 1 month of age. The term “infant” is reserved for children between 1 month and 2 years, whereas children greater then this age fall into categories defined as toddlers, preschool children, school age children and adolescents (4). The term “neonatal sepsis” is reserved for sepsis that occurs in newborns up to 28 days of life. Neonatal sepsis can be further divided into early and late. The classification between early and late neonatal sepsis has had several different definitions throughout time due to the unfamiliarity of where one would end and the other begin. The most widely used definition of early onset neonatal sepsis is sepsis that occurs within the first 72 hours after birth. Onset of sepsis between 72 hours and 7 days of life would be defined as late onset neonatal sepsis (5). Some

literature defines early neonatal sepsis occurring less then or equal to 4 days of life and late neonatal sepsis up to 120 days of life (6, 7). Some authors have also gone further to divided neonatal sepsis into 3 separate categories opposed to the more common 2 categories of early and late. EOS occurring within the first 4 days, LOS between 5 and 30 days and "late, late onset neonatal sepsis" occurring on or beyond the 30^{th} day of life (8). EOS also can have a wider time range defined as positive microbiological cultures within the first 7 days of life (9). Making the distinction between early and late onset neonatal sepsis (LOS) is an important one due to the difference in pathogens, modes of treatment and risk factors that are more frequently associated with each type of sepsis.

5.0 Epidemiology

Neonatal sepsis continues to be a heavy burden on the world's health care and a major cause of neonatal death worldwide. It has been estimated that 36% of the worlds 4 million neonatal deaths are a direct result of invasive neonatal infections, with most of these cases occurring in low and middle-income countries (10). The risk of neonatal mortality due to infections in low-income countries has been estimated to be 11 times higher then that of high-income countries (11). Only approximately 3.6% of the neonatal deaths due to infections occurred in high-income countries and still in 2007 it was established that neonatal sepsis was one of the top ten causes of infant mortality in the United States (12, 13). It is estimated that incidence rate of EOS ranges from 0.77 to 1 for every 1000 births in the United States. However, when we divide neonates into different categories based on birth weight (<1500g) and gestational age (<32 weeks) we see a rise in the incidence rate to 25 per 1000 and 8 per 1000 births respectively. Selected populations are also at an increased risk, as is seen in black preterm infants who hold the highest incidence of infection (5.14/1000 live births) and mortality (24.4% case fatality ratio) (5).

6.0 Pathophysiology

Sepsis is a systemic inflammatory reaction of the body to an infectious microorganism that can lead to septic shock and death. The body produces several different types of proinflammatory cytokines in attempt to protect itself. However when the pathogen is in the victims' blood, the body response is usually an overreaction that continually proves to be detrimental. The process begins by the bacterial agent entering the circulatory system and interacting with the complement cascade causing the release of several proinflammatory mediators such as C3 and C5a. These mediators cause vessel vasodilation and a release of proinflammatory cytokines such as interleukin-1, 6 and 8 (IL-1-6-8) (14). These cytokines facilitate the adhesion of leukocytes to the endothelial wall, releasing nitric oxide and reactive oxygen species that then cause injury to the endothelial cell wall. This cell injury coupled with vessel vasodilation leads to leakage of the fluid into interstitial space (15). In addition to vessel vasodilation the bacterial antigen can facilitate the formation of microthrombi in the microcirculation, further compromising tissue perfusion (14).

The majority of the complications associated with bacteremia are due to the lack of counterbalance in proinflammatory cytokines. The immaturity of the neonates immune system fails to produce adequate anti-inflammatory cytokines (14). Due to this immaturity of the immune system compared to adults, they have an increased rate of infections (16). Neonates are also not able to proficiently combat the effects of the polymicrobial flora that they are exposed to during and after birth. It is therefore during this period their major source of defense against any pathogen is their innate immunity and the passive protection that is acquired in utero from their mothers (17).

7.0 Etiopathogenesis

7.1 Risk Factors

Neonatal sepsis can be the result of a wide range of microorganisms with several different routes of transmission. EOS and LOS not only differ in the timeline in which infection occurs, but also on the organisms and routes of entry. Sepsis that occurs in the early days of the neonates' life can be due to factors related to the mother or the child. Maternal factors have shown to be of great influence to the development of EOS. The child can be affected by maternal factors either when it is in the uterus or during the process of birth. Throughout her pregnancy the mother can be exposed to several different procedures, all of which carry some risk of harming the child. A procedure such as amniocentesis is no exception; it is a procedure that is frequently used for diagnostic purposes concerning the development of the fetus. It carries a risk of exposing the fetus to microbial agents within the uterus (18). The mother's dietary habits prior to labor or delivery can also put the infant at risk for certain microbial agents that contaminate food (5). One of the maternal risk factors that have been heavily studied is prolonged rupture of membranes (PROM). This is a condition of pregnancy where there is a rupture of the amniotic sac prior to the onset of labor and can occur at any gestational age. It is defined as the rupture of the membranes longer than 24 hours before delivery and carries the increased risk of ascending infection which can put both the mother and child at risk (19). In developed countries such as the United States, PROM complicates approximately 3% of all pregnancies (20).

Several studies have also presented the case of fever in the mother and child induced by epidural analgesia. It can pose as a risk factor in the development of neonatal sepsis. It appears that the increased frequency of neonatal sepsis evaluation and treatment occurred more often in children of mothers receiving epidural analgesia compared to those that did not (21). A maternal fever that is greater then 38 degrees Celsius usually prompts the clinicians to evaluate the infant for sepsis and as a result may lead to the unnecessary administration of antibiotics (22). Vaginal colonization of group B streptococcus (GBS) is a common occurrence. The source of maternal colonization often occurs from the gastrointestinal tract. Some women may present with subtle urinary tract

related symptoms; however, the vast majority is asymptomatic. A strong link between EOS and maternal colonization has been seen over the years, raising considerable investigation into the subject (23). Chorioamnionitis (CAM) is a bacterial infection that results in the inflammation of the fetal membranes, which consists of the amnion and the chorion (24). CAM is defined as a group of signs consisting of maternal fever, maternal-fetal tachycardia, uterine tenderness, foul odor of amniotic fluid and leukocytosis of >15 000 WBC/mm^3 and is considered a strong risk factor for the development of neonatal sepsis (5). CAM complicates approximately 1 to 4% of all pregnancies in the United States, however its frequency varies with the diagnostic criteria used, associated risk factors and gestational age. Up to 70% of preterm births and 13% term births are complicated by CAM (25).

Several important and well-studied risk factors are related to the development of the infant. Preterm birth is defined as any births that occur before the 37^{th} completed week of gestation. We can further classify preterm birth into moderately preterm (33-36 completed weeks), very preterm (<32 weeks) and extremely preterm (<28 weeks). Several factors can contribute to increased values of preterm births, especially in high-income countries with rigorous caesarian policies implemented to reduce still birth rate, therefore artificially increasing preterm birth rate (26). In 2005 the Lancet published that preterm birth rates (percentage of births before 37 weeks) accounted for 28% of all neonatal deaths, slightly above the 27% of deaths directly due to neonatal sepsis and pneumonia (11). Not only is preterm birth one of the leading direct causes of neonatal death, it is also a leading risk factor for neonatal deaths due to infection. Complications that increase the risk of a preterm infant to infection are related to its immature immune system. Due to the fact that the child has less time to develop in the mothers' womb, it is also susceptible to missing the period in which passive immunity from the mother is acquired. Preterm birth is not always the cause of increased risk to developing infection, but also can be a result of another unidentified problem, such as systemic inflammation or congenital abnormalities (26). Another factor that is highly connected with preterm birth is a low birth weight. Reduced infant birth weight is usually a result of preterm birth or restricted intrauterine growth (11). It can be categorized according to the birth weight of

the child into low birth weight (<2500g), very low birth weight (<1500) and extremely low birth weight (<1000g) (27). Low birth weight is a common concern throughout the globe as approximately 18 million births fall under 2500g each year. Even if it contributes to 14% of all births, 60-80% of all neonatal deaths are in this low birth weight category (11). The highest proportion of low birth weights are in South Asia (28%) and the lowest occurring in industrialized countries (7%) (27). Both preterm and low birth weight are the risk factors most closely associated with EOS and therefore it is important we take a closer look into their usefulness in predicting neonatal sepsis (28).

7.2 Pathogens

Just like the risk factors between EOS and LOS differ, the pathogens that result in infection also differentiate. The route of infection for EOS typically tends to occur either through vertical transmission in utero, during birth (perinatal infections) and through breast feeding (postnatal infections) (14). The two most common pathogens making up 70% of all the early neonatal infections are Streptococcus group B (GBS) and E. coli (5, 14). Other less common pathogens making up the final 30% are other streptococci (Viridians and pneumonia), Staphylococcus aureus, Enterococcus, H. influenzae (excluding group B) and Listeria monocytogenes (14).

Sepsis caused by GBS in the early neonatal period consists of approximately 47% of all neonatal infections (14). According to a collection of data between the year 2005 and 2008 it was shown that EOS due to GBS was 0.77 cases for every 1000 live births. The vast majority of EOS cases due to GBS also tend to occur more often in term neonates (73%) (29). Pregnant woman who are colonized with GBS tend to be asymptomatic. African American, < 20 years of age, low parity and diabetic woman have a higher degree of susceptibility to colonization by GBS when compared to the rest of the population (30). The 12.1% mortality rate in EOS due to GBS was almost double that of LOS which was only 6.8% (31). Neonates who are infected with GBS are also at a high risk of developing persistent pulmonary hypertension (PPH). This condition is associated with a high neonatal mortality rate of 10 to 20% (32). Children suspected of having acute respiratory syndrome through imaging must be investigated for possible GBS infection.

Due to the increase in evidence of neonatal sepsis and maternal colonization of GBS, prophylactic antibiotic therapy has become a common practice. This has translated to a reduction in the incidence of EOS caused by GBS; however, at the same time has risen concern with the possible increase in resistance in ampicillin-resistant E. coli over time (5, 14).

The second most frequent pathogen, attributing to approximately 23% of the EOS cases and 81% of all preterm cases is E. coli. This relationship appears to be even stronger when looking at VLBW neonates (33). Like GBS, E. coli is also another microbe that populates the mucosa of the vaginal canal. Most of the neonatal infections are acquired during passage through the canal or just before delivery. Even if the incidence of infection due to E. coli is lower when compared to GBS, the percentage of deaths from E. coli is much greater. When taking into account both E. Coli and ampicillin-resistant E. coli, the case fatality rate (CFR) found in a surveillance program from 2005-2008 was 22.9%. This is significantly greater when compared to the CFR of GBS, which was 6.8% (29). Figure 1 below shows the reduction in the incidence rate in EOS due to GBS over a 10-year period.

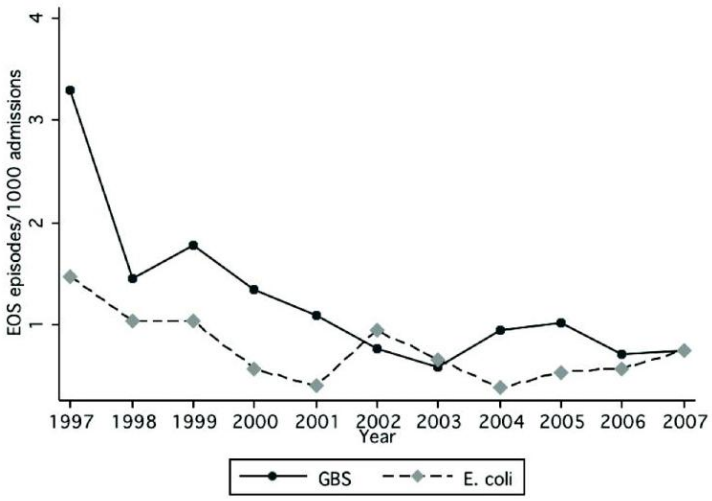

Figure 1. GBS and E. coli early onset sepsis annual cumulative incidence per 1000 late preterm NICU admissions between 1997 and 2007 (7).

8.0 Diagnostics

8.1 Clinical Presentation

Table 1 below displays the normal vital sign values of a child depending on their age. However, not only do the values vary according to age, they also change with the neonate's weight. In 2008 the hemodynamic consensus depicted different mean blood pressure (MBP) values for children between 1 to 3 days of age, their weight and their gestational age (GA). ELBW who were between 23-27 weeks of gestation neonates had a MBP equal or higher than their GA. Neonates with VLBW and GA of 28-33 weeks had a normal MBP value of $\geq$ 30 mmHg and LBW children with a GA 34-37 weeks had normal values $\geq$ 35. Term and normal weight neonates had normal values $\geq$ 40 mmHg (15). The clinical presentation of a newborn child can be very difficult to identify and as a result, diagnose. The clinical presentations can vary between preterm, term, low birth weight neonates and type of pathogen involved. Symptoms also vary by being broad and unspecific making the physicians task that much more difficult. Delayed weight gain, pale skin, lowered tone, reduced movements, reduced crying and reduced eating all are common features. Body temperature tends to fluctuate and can be high, low or even normal, and therefore making it difficult to use to detect a diagnosis. Sepsis being a systemic disease can result in symptoms of any organ system. Determining the system that is being affected can also prove to be of great value to localize possible source and the likely pathogen.

Table 1: Vital signs for Age specific groups. Modified from International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus: definition for sepsis and organ dysfunction in pediatrics (3)

Age Group	Heart Rate (bpm)	Respiratory Rate (breaths/min)	Systolic Blood Pressure (mmHg)
0 to 7 days	100 to 180	>50	<65
7 to 28 days	100 to 180	>40	<75
1 mo to 12mo	90 to 180	>34	<100

Changes in the newborns arousability, sunken or bulging fontanelle, reduced femoral pulse and seizures can point to neurological involvement. Nausea, vomiting abdominal distention can direct the physician to a gastrointestinal cause. It is important to also observe skin changes due to their common occurrence. Cutaneous and mucosal petechiae, impetigo, cellulitis, infections in and around the umbilicus and abscesses can all be observed. Other more general symptoms such as metabolic acidosis, jaundice hypoglycemia, hyperglycemia may be due to the metabolic alterations of the neonate (9). Being aware of any possible clinical signs and symptoms that result form neonatal sepsis is important to recognize so that investigation into the child's status can be initiated.

8.2 Laboratory

In the diagnostic process, laboratory analysis has become a critical part in our decision-making. In neonatal sepsis this has been an area of ongoing debate and research. For the most part, results have been unclear and a single perfect diagnostic tool has yet to be discovered. The absolute best marker for diagnosis would have to contain high sensitivity, specificity, positive predictive value and negative predictive value (34). Taking this into consideration, there have been many different diagnostic markers researched that may be used in combination in order to assist in diagnosis.

The gold standard for a definitive diagnosis is a blood culture. However, there are many problems with this test that reduces its value. Blood cultures can take as long as 48-72 hours making them an unreliable tool in determining if treatment is needed in the critical hours once the disease has begun. They also lack a high positive predictive value with less than 50% of the cases being positive. In suspected cases, blood cultures should still be taken before antibiotic therapy is initiated. The results of the cultures should then guide further treatment for usage of narrow spectrum antibiotics, further assessment or discontinuation of treatment (14).

Blood tests are routinely done for virtually all diseases including neonatal sepsis. There has been an increase in their use for the screening of neonates with suspected cases of sepsis. According to a study published in 1988 Rodwell et al, a hematological scoring system was developed in order to assist in the early diagnosis and treatment of neonatal

sepsis (35). Immature to total neutrophil ratio (I:T), immature to mature neutrophil ratio (I:M), total polymorphonuclear leukocytes (PMN) count, immature PMN count, degenerative changes in PMNs and platelet count were the parameters included in the scoring system. Each of the parameters contributes a point in the overall score. A score > 2 would indicate the increased likelihood of sepsis, whereas a score ≤ 2 would point to almost certainty of the absence of sepsis (35). This would be an ideal system if it predicted sepsis with a high accuracy. However, the scoring system had a weak positive predictive value and inconsistent sensitivity and specificity make it unreliable as a soul indicator for treatment and diagnosis. Levels of neutrophils vary considerably in children depending on their gestational age, birth weight, age in hours and various other maternal, intrapartum and perinatal factors (36). Blood results overall tend to have a high negative predictive values but poor positive predictive values (5).

Biological markers have emerged as the most researched area in the journey to find a perfect test. Two acute phase proteins, C-reactive protein (CRP) and procalcitonin (PCT), are among those heavily researched. CRP is a peptide that is synthesized in the liver in response to an infection or the inflammatory process. Its levels rise within the first 6 to 8 hours and peaking at the 24^{th} hour (5). With a half-life of 24-48 hours, CRP has the best predictive value with serial measurements when compared to individual measurements (34). PCT levels in the blood rise as quickly as 2-4 hours after exposure and can peak between 6-8 hours (34). It is a peptide produced in liver cells and monocytes with a half-life of about 24 hours. Its normal values in a neonate younger then 72 hours are usually < 0.1 ng/liter. However, there is a normal physiological elevation that occurs in the first 24 hours after birth, possibly resulting in confusion during result interpretation. Its levels are more likely to be elevated due to bacterial infections and tend to decrease as a response to treatment. Promising studies have show the PCT levels can be of use and is being increasingly used in the real-time guidance of therapy (5).

The inflammatory response of the body to a foreign antigen results in the production of cytokines. Cytokines are small peptides that are produced by the body's immune cells. They act on other cells in the body, further amplifying the inflammatory

response. Interleukin-6 and 8 are produced by leukocytes and act on other leukocytes (37). The release of IL-6 results in the production of CRP and has thus raised interest as a possible early detector in neonatal sepsis (38). IL-6 has a very short half-life and falls quickly to undetectable levels once treatment is initiated, and as a result to this quick drop in serum levels, the sensitivity of IL-6 as a marker drops from 67% to 58% from 24 to 48h hours after infection (39). IL-8 is produced in response to polysaccharides, TNF and IL-1. It was found to increase proportionally to the severity of the infection, giving it value in the early detection (40). Tumor necrosis factor-alpha (TNF-α) is another pro-inflammatory cytokine that has been known to have a role in activation of several other inflammatory pathways and as a result studied to find a possible role in neonatal sepsis (41). It has similar kinetic to IL-6 and has found to have been higher in groups with confirmed sepsis versus the controls (41, 42). Even with promising data, research on TNF-α and as diagnostic marker in sepsis has been limited. In addition to cytokines, certain soluble cell surface antigens have been researched. CD64 is found on macrophages and monocytes and binds IgG antibodies with a high affinity. Its expression is known to be increased in the presence of other inflammatory cytokines like INF-γ (43, 44). Due to the costly procedures required to obtain values for CD64, it is likely an unreliable method of diagnosis. It has been suggested that many laboratories may not be willing to perform such tests individually and rather in batches, defeating the purpose of quick testing to diagnose sepsis (45).

CD11β is a α-subunit of an adhesion molecule involved in neutrophil adhesion, diapedesis and phagocytosis. The molecule is located at very low levels on the surface of neutrophils, with the majority located in granules (46). Upon interaction with a foreign pathogen an increased expression occurs in a very short period of time. Its expression is also influenced by IL-8, a strong neutrophil activating agent (47). Compared to adults, a newborn show reduced ability to enhance CD11β expression due to lower levels located in granules (46). Even with reduced levels compared to adults, CD11β levels are detectable within the first 5 min of a bacterial infection and peak within 30 minutes (48, 49). It was observed in the first day of life there was a 2-4 times increase in its expression on neutrophils in blood culture positive children, results which were also comparable to

adults (50). Due to its quick increase at the onset of an infection, both IL-8 and CD11β have show promising results as reliable detectors of EOS.

9.0 Discussion

9.1 Risk Factors

Maternal factor have a large influence on the developmental wellbeing of the child prior to, during and after birth. There has been difficulty in separating the risk factors for sepsis because of their high influence upon one another. PROM > 24 hours, CAM, maternal GBS colonization, gestational age, birth weight and maternal fever all have an impact on the risk of the child developing sepsis. Whether one has a greater influence on sepsis development over the other, is still an ongoing debate. Previous studies observed that the incidence of culture proven EOS was between 2.6% - 8.1% when premature rupture of membranes was the main risk factor studied (51). In a study by Alam et al, premature rupture was defined as >18 hours before birth. They found that neonates had a culture proven EOS rate of 4%. However, this was difficult to attribute solely to PROM due to the presence of maternal fever in 88% of the cases and CAM in 77% of the cases. Other risk factors commonly associated with the children in this study were low birth weight (47%) and GA < 34 weeks (41%). Longer PROM of > 48 hours was identified in 41% of the septic neonates (51). Other studies showed an increased risk of sepsis when PROM present for > 48 hours. Seaward et al showed a 2.75-increased risk in neonatal infection when compared to PROM < 12 hours (52). A study conducted in Thailand had an incidence rate of sepsis in newborns follow PROM as high as 27.9%. However the diagnostic criteria for sepsis were wider in this study, which included culture negative neonates with signs and symptoms of sepsis (53). Linder et al had an incidence rate of sepsis in 8% of the neonates with PROM >24 hours. However ten out of the eleven children with sepsis were born prematurely, whereas the eleventh child was small for gestational age. Neonatal sepsis was found in 15% of premature infants with PROM (54). Monitoring of children for development of symptoms for 72 hours after birth should be practiced if born to mothers with PROM > 18 hours (53).

Children born to mother with PROM also have a high probability of other risk factor contributing to the development of neonatal sepsis. CAM appears to be a condition that parallels PROM. One of the risk factors for the development of CAM is in fact PROM (25). Both the Center for disease control (CDC) and the American academy of pediatrics (AAP) consider CAM as the second risk criterion in EOS (55). Some studies linked EOS to CAM using strict diagnostic criteria, with one or two clinical finding in addition to maternal fever (56). Whereas, in more recent studies the criteria of maternal fever is all that may be observed to conclude a diagnosis of CAM (57). This can lead to over diagnosis and overtreatment of both the mother and child, as women who receive epidurals have a higher likelihood of fever (21). Yancey et al found an overall relative risk of 4.4 in neonates who developed sepsis, born to mothers with CAM (58). However, these results often differ when studies differentiate the GA between infants born to mothers with CAM. Gestational ages $\geq$ 35 week showed a low correlation (0.47%-1.24%), where children < 35 weeks showed rates ranging from 4.8%-16.9% (55). Other studies showed a high specificity (98%) and negative predictive value (98%) for CAM, but low sensitivities (14%) and positive predictive values (7%) (59). Due to the evidence presented, it is difficult to predict the probability of developing sepsis solely based on these risk factors. Both CAM and PROM can be influenced by other factors making them unreliable.

Children born prior to the 37th week of gestation carry with them a host of complications. The frequency of neonatal sepsis in preterm neonates occurs at a greater rate when compared to term neonates. Vergnano et al found that 82% of neonatal infections occurred in children born prior to the 37th week, with 71% of those children being born with < 32 weeks of gestational (60). This was also found in another study considering children infected by E.coli. They concluded that children with a gestational age $\leq$ 33 weeks were at 26.5 times more likely of infection when compared to the controls (61). However it continues to be difficult to separate the maternal risk factors form one another. Preterm neonates also carry a higher likelihood of other maternal risk factors, which is apparent when the evidence is provided. As previously mentioned, clinical CAM occurs more frequently among women who deliver preterm babies versus

those that deliver term infants at a rate of 5.7% and 1.7% respectively (62).

Table 2. Predictive power of clinical risk factors Modified from Flidel-Rimon et al(59), PPV- Positive predictive value, NPV-Negative Predictive Value

	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Prematurity < 37 weeks	28	48	0.7	98.1
PROM >24 hr	14	62	0.5	98.2
Maternal Fever ≥38°C	19	68	0.75	98.5
Chorioamnionitis	14	98	7	98.9
2 Risk Factors	28	79	1.7	98

Schrag et al compared preterm infant with maternal risk factors such as PROM and fever and found an increased risk of infection of 5.2 and 7.4 respectively when compared with term infants with the same maternal risk factors (61). The table above shows the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the major maternal risk factors associated with EOS in the population studied (59). In the absence of the risk factor there is high reliability in predicting the absence of neonatal infection, however none of the risk factors are reliable enough to predict neonatal sepsis. As mentioned before it is difficult to separate all the maternal risk factors because they often happen in parallel. More studies need to be conducted to evaluate each risk factor on their own. Until then it is best to use maternal risk factors as a tool to anticipate possible high-risk cases and to guide the need for further diagnostics.

Vaginal colonization appears to be a more independent maternal risk factor when compared to the previous risk factors. Figure 1 shows an evident decline in the occurrence of GBS neonatal sepsis since the introduction of prophylactic antibiotic therapy in mothers colonized with GBS. As with any other maternal risk factor, preterm neonates have higher mortality rates from GBS infection (20%) when compared to term neonates (2%-3%) with an even higher mortality rate seen in preterm children ≤ 33 weeks

of gestation (30%) (63). Mother colonized with GBS were shown to be > 25 times more likely to give birth to early onset GBS disease. Without any intervention, colonized mothers would give birth to GBS infected children at a rate of 1%-2% (63, 64). In a collection of studies investigated by Verani et al, there was shown to be a high correlation with mothers that had positive GBS urine sample and neonatal infection (63). When taking into consideration other possible maternal risk factors, colonized mothers with no other maternal risk factors gave birth to GBS infected children at a rate of 5.1/1000 births. Whereas mother not colonized with GBS and one other maternal risk factor gave birth to GBS infected children at a rate of 0.9/1000 births (64). In this time where we are more aware of GBS infections and the risk factors related to them, screening has become much more widespread. Due to this, now we see > 60% of all early onset GBS infections in mothers testing negative for GBS colonization. False-negatives are also common due to testing of mother between 35-37 weeks of gestation will miss some women with intrapartum colonization (63).

Table 3 on the next page illustrates the rates of neonatal sepsis collected over a 15-year period for both early and late depending on the newborn birth weight. It is evident that the rates of neonatal sepsis are higher in lower birth weight neonates, with onset occurring more frequently after the 4th day of life. Even if the rates of neonatal sepsis are much lower in the first three days of life, there is a significant inverse correlation between birth weight and rate of EOS. Gram-negative organisms occur 51%-53% of the time in LBW neonates with E.coli being the most frequent (65, 66).

Table 3. Birth Weight-Specific Sepsis rates for Newborns. Table modified from Bizzaro et al.(8) EOS- Early onset neonatal sepsis, LOS- Late onset neonatal sepsis

Birth weight (g)	**Sepsis, Cases /1000 births**		
	EOS (0-4 days)	**LOS (>4days)**	**Total**
ELBW <1000	54.5	441.8	496.3
VLBW 1000-1499	14.8	79.2	94
LBW ≥1500	3.52	23.43	26.95
Total	72.82	544.43	617.25

9.2 Laboratory

9.2.1 Complete blood count

Drawing of blood from neonates suspected of having sepsis has become a routine procedure. However, the information gathered can be difficult to interpret. White blood cell counts vary in the neonatal population depending on several different factors mentioned earlier. In term neonates, normal WBC count tend to peak between 12-14 hours after birth, ranging between 7,800 and 14,500/mm^3 (36). Several studies have been conducted assessing the total WBC count and absolute neutrophils counts (ANC) in relation to neonatal sepsis. Low values in both WBC count and ANC has been consistently associated with bacterial infection (38). In a study conducted by Hornik et al, WBC values < 5000/ mm^3 and < 1000/ mm^3 were found to have a high specificity in predicting the presence of infection, but with a poor sensitivity. The study also observed that subjects with normal WBC counts (5,000/ mm^3 – 19,999 mm^3) had positive blood cultures 60% of the time. However, when 4 different normal WBC counts were taken throughout the day, only 0.6% of the patients had positive blood cultures (67). These results are similar with an earlier study by Rodwell et al, who found low sensitivity and PPV values but a high specificity of 92% and a NPV of 94% (35). In addition to this, other studies observing higher cut off values (WBC count < 5,000 to 7,500 mm^3) also found specificity values of 91%, but even lower sensitivity values of 29% (68). In addition to WBC counts and ANC, I:T ratios have also shown similar results.

Various cutoff ranges have been examined for I:T ratio for the diagnosis of sepsis in neonates. Typically the lowest cut off ratio considered is >0.2, as was done in a study that observed 150 neonates with clinical suspicion of sepsis. They found a high specificity and NPV as well as low sensitivity and PPV. Hornik et al also observed higher cut off values of >0.25 and >0.5, however only had success in further improving the specificity and NPV while seeing a drop in sensitivity and PPV (67). The same trend was presented in earlier studies conducted in 2000 with cut off values of ≥0.25 and ≥0.30 (69). These results stayed consistent even when preterm neonates of <32 weeks were considered. Here they found that between 25% to 50% of the neonates had an elevated I:T ratio (70). It is evident that alone I:T ratio cannot prove neonatal sepsis. However, the

finding suggests that neonatal sepsis cannot be ruled out with an elevated I:T ratio (71).

9.2.2 Biomarkers

CRP is the highest researched biomarker in relation to the diagnosis of neonatal sepsis. It is an acute phase protein that rises in response to the body's inflammatory reaction. It is commonly used to monitor the progress and treatment of a disease in both the adult and pediatric population. Research considering the effectiveness of CRP in the diagnosis of EOS has been extensive. Wide ranges of cut off values have been considered, ranging from 0.2 to 95mg/L (41). The most frequently studied cut off value of 10mg/L has shown variable results. According to Meem et al. who reviewed a collection of 70 papers with the cut off range from 0.2 to 10 mg/L found that the specificity ranged 72% to 100% and a sensitivity range from 41% to 96% (41). Other studies not observed by Meem et al found sensitivity values as low as 9% for a cut off value of 10 mg/L (72). CRP had the most promising values between 24 and 48 hours (5). It is evident that if used alone, CRP can reliably exclude the diagnosis of sepsis. However due to its low sensitivity it is an unreliable factor to be used to confirm diagnosis. If measured serially it has shown strong evidence against bacterial sepsis and its use in the guidance of antimicrobial therapy (5).

According to the research, PCT appears to have a generally better sensitivity when compared to CRP. In their comprehensive analysis of 100 studies, Meem et al found the mean sensitivity of PCT to be 77.9% and mean specificity 81.8% (41). When taking into consideration the cut-off values observed, higher values of >11pg/ml had a sensitivity as low as 21% compared to the lowest value of 58% found with cut-off values between 0.34 and 10 pg/ml (41). When specifically looking at EOS, Vouloumanou et al found a pooled sensitivity and specificity of 76%. They concluded that PCT has a very good diagnostic accuracy but seem to be somewhat better in LOS then in EOS. Although they used a lower range of cut-off vales when compared to Meem et al, there was no noticeable difference in the results (73). Even with improved sensitivity compared to CRP, PCT cannot identify 100% of septic neonates alone, making it more useful when used in combination with other markers and tests.

The research up until this point shows promising insight in to the diagnostic validity of interleukin-6 in the early phase of sepsis. However, its strength as the sole detector decreases rapidly due to its short half-life. Through various studies observed by Delanghe et al, the average sensitivity and specificity of IL-6 was 76% and 84% respectively. When only taking into consideration studies specifically geared towards EOS, the average specificity dropped to 71%, whereas the sensitivity remained the same (74). The previous studies considered took lower cutoff values for diagnosis even if higher levels of IL-6 have been found in children with proven sepsis (75). Two studies in which the cut off values of ≥100 pg/ml was used, had considerable variation in both their sensitivity and specificity ranging from 83.3% to 96% and 77% to 90.3% respectively. Both showed very good NPV of 97% or higher, but failed to show high PPV (76, 77). Due to the short half-life of IL-6, combining it with inflammatory markers with a longer half-life shows to improve its diagnostic validity. When combined with TNF-α in detection for EOS, there is an improvement in sensitivity compared to either of them alone (39). In 1994 de Bont et al observed an improvement of both sensitivity and specificity for both TNF-α and IL-6 from 60% to 100% when they are combined. Therefore claiming that when both are positive, the diagnosis of sepsis is almost certain (78). Later studies also found high sensitivity vales of 98.5%, PPV of 61% and NPV of 90% with no reported values for specificity for the combination of IL-6 and TNF-α (79). When considered separately TNF-α had a mean sensitivity of 79% and specificity of 81% (41). Døllner et al found that combining both CRP and IL-6 also improved both the specificity and sensitivity of both when compared to each individually (80).

Interleukin 8 is considered a highly accurate marker when predicting the presence of infection in early neonatal sepsis with sensitivity and specificity values ranging from 80% to 90% and 76% to 100% respectively (42). However there have been several studies conducted finding a wider range of results. When choosing a cutoff value of 70 pg/ml the sensitivity had a range from 44% to 92% and 70% to 90% for specificity. PPV and NPV values found in these studies ranged from 43% to 65% and 83% to 94% (81-83). With a higher cutoff value of 300 pg/ml there was an increase in the sensitivity (91%), specificity (93%), PPV (91%) and NPV (97%) (84). However due to the small

sample size of this, more research need to be done with higher IL-8 cutoff values. When the combination of IL-8 and CRP was considered, they observed an improvement in their overall results. Sensitivity, PPV and NPV increased from 44% to 80%, 58% to 68% and 83% to 93% respectively with the specificity remaining practically unchanged (81).

Research on CD11β levels and its usefulness in diagnosis have been limited but promising. Various studies have also combined CD11β with IL-8 and CRP with positive results. Weirich et al were one of the first ones to study CD11β as a marker in neonatal sepsis. They found that serum CD11β was low in children in whom were later excluded for sepsis and that all the children with high levels were infected. Sensitivity and specificity were 96% and 100% respectively, with PPV and NPV 100% and 99% respectively. These neonates were suspected to have infection or were confirmed via blood culture. However, when only taking bacterial infections into account the specificity would drop to 81% and the PPV to 22% (46). Nupponen et al tested both CD11β and IL-8 and finding that both are superior to CRP for detecting early infection in children with EOS. They found CD11β values of 100% for sensitivity and specificity and for IL-8 having a sensitivity of 91% and a specificity of 100%. It is also interesting to note that in the study the authors found that the levels of IL-8 were higher in non-survivors when compared to survivors paralleling the finding of the previously discussed studies (47). The same values were not achieved by all the studies conducted at that time as Weinschenk et al. failed to show any elevation in CD11β levels in infected neonates (85). More recent studies conducted consistently found a rise in CD11β levels in septic newborns when compared to non-septic newborns and controls. High specificity of 100% and PPV of 100% were also found in the study by Adib et al. The sensitivity (75%) and NPV (86%) where also found to be 100% when combined with quantitative measurements of CRP (86). Similar results were also found in a study conducted in 2011, with improved sensitivity and specificity when CD11β was combined with CD64 and CRP (87). The most recent study from Norway conducted in 2016, used GBS III stimulation in vitro to test the rise in CD11β. They also found results similar to the others, with a specificity of 100% and sensitivity of 95% (88). Although promising, only a handful of studies have been conducted on the relationship between the levels of CD11β

and neonatal sepsis. More research needs to be conducted in order to establish a definitive relationship.

10.0 Conclusion

Early onset neonatal sepsis continues to be a devastating disease for the both the patient, family and the physician. The importance of physicians being aware of all the possible variables that contributes to the development, and therefore the anticipation and preparation of the disease has proven to save lives. Continuous research in the factors involved in the pathophysiological process of neonatal sepsis can uncover methods into recognizing and treating the disease. The current evidence shows no one factor that can be used to diagnose sepsis, however promising results have been seen when two or more of these factors are combined. Experts in the field are continuously attempting to establish a set of diagnostic criteria that can be reliable and easily used. However due to the lack of consistent evidence in this area, no such list has yet been developed. Currently the best methods involved in the investigation of EOS is using the combination of maternal risk factors, clinical signs and symptoms, and various laboratory markers that are available. It is also highly valuable observing the patients response to treatment, further helping guide the next actions that need to be taken. Much has been discovered considering the research done thus far on the risk factors, clinical features and laboratory results. Yet, the continuous research into the various risk factors and biomarkers needs to be continued in order to find a factor or combination of factors in order to make a reliable diagnosis that can be used world wide.

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12.0 Biography

Branimir Klobucar was born in Toronto, Canada in 1986. Branimir's parents are of Croatian origin and in 1971 fled Yugoslavia to come to Canada. After graduating from high school in 2004, he enrolled into York University situated in Toronto. Here he completed his degree in Kinesiology and Health science and graduated with honours in 2008. He then further enrolled into Sutherland-Chan school of massage therapy where a year later he obtained his Massage therapy Diploma. For the next several years he worked in various rehabilitation clinics as a registered massage therapist before enrolling into The University of Zagreb, School of medicine in 2011. He chose to study at University of Zagreb because he always has wanted to experience living in Croatia. Throughout his childhood he would visit Croatia on countless occasions and as a result it had a special place in his heart.