Diagnostic criteria for late onset neonatal sepsis

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UNIVERSITY OF ZAGREB
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Diagnostic criteria for late onset neonatal sepsis

GRADUATE THESIS

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Abbreviations

ANC	Absolute neutrophil counts
AUC	Area under the curve
BPD	Bronchopulmonary dysplasia
BW	Birth weight
C3	Complement component 3
C5a	Complement component 5a
CBC	Complete blood count
CD64	Cluster of Differentiation 64
CoNS	Coagulase-negative Staphylococci
CRP	C reactive protein
CSF	Cerebrospinal fluid
CVC	Central venous catheter
DIC	Disseminated intravascular coagulation
DOL	Day of life
ELBW	Extremely low birth weight
EOS	Early-onset sepsis
GA	Gestational age
GNR	Gram-negative rod
GPC	Gram-positive cocci
I:T	Immature-to-total neutrophil
IgG	Immunoglobulin G
IL	Interleukin
LBW	Low birth weight
LOS	Late-onset sepsis
NEC	Necrotizing enterocolitis
NICHD	National Institute of Child Health
NICU	Neonatal intensive care units
NPV	Negative predictive value
PAMP	Pathogen-associated molecular patterns
PCT	Procalcitonin
PDA	Patent ductus arteriosus
PPV	Positive predictive value
SAA	Serum amyloid A
SD	Standard deviation
SIRS	Systemic inflammatory response syndrome
TLR	Toll like receptors
TNF-α	Tumor necrosis factor-α
VLBW	Very low birth weight
WBC	White blood cell

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1.0 Summary

Title: DIAGNOSTIC CRITERIA FOR LATE NEONATAL SEPSIS

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Late-onset neonatal sepsis is one of the leading causes of morbidity and mortality in neonates. Late-onset sepsis is usually defined as sepsis >72 hours after birth. Low birth weight and gestational age are inversely related to the development of late-onset sepsis. Gram-positive organisms appear to be the cause of the majority of the infections. Particularly coagulase-negative staphylococci have been implicated in late-onset neonatal sepsis. The pathophysiological mechanisms underlying the development of sepsis is a complex interaction between the invading pathogen and the neonate's immune system. Diagnosing late-onset sepsis is extremely challenging due to unspecific symptoms and clinical signs which frequently can mimic other non-infectious etiologies. Blood cultures are time consuming and often yield false negative results. Markers and laboratory test used in the evaluation, such as white blood cell count, absolute neutrophil counts, immature-to-total neutrophil ratio, C-reactive protein, procalcitonin, tumor necrosis factor-α, serum amyloid A, IL-6, IL-8 and CD64 have been used to assist in the identification of late-onset sepsis. However, at present time no marker is individually sufficient to confirm the diagnosis. Usage of two or more markers with different properties, also measured in different time intervals often leads to increased diagnostic accuracy. This could be helpful for the clinicians in the diagnosis and management of late-onset sepsis. There are several novel biomarkers under investigation that appear promising for the future.

Keywords: Neonatal Late-Onset Sepsis • Diagnosis • Newborn • Infection • Cytokines

2.0 Sažetak

Naslov: DIJAGNOSTICIČKI KRITERIJI ZA KASNU NOVOROĐENAČKU SEPSU

Autor: Gustaf Sebastian Edström

Pojava kasne novorođenačke sepse je jedan od vodećih uzroka smrtnosti novorođenčadi. Kasna novorođenačka sepsa obično se definira kao sepsa koja nastaje nakon više od sedamdesetdva sata poslije rođenja. Niske težine novorođenčadi i gestacijska dob su obrnuto povezane s nastupom ove sepse. Čini se da su gram-pozitivni organizmi, a posebice koagulaza negativni stafilokoki, najčesći uzročnici infekcija koje vode u novorođenačku sepsu. Patofiziološki mehanizmi iza nastanka sepse, su složena interakcija između patogena i imunološkog sustava novorođenčeta. Dijagnosticiranje kasne novorođenačke sepse je iznimno zahtjevno zbog nespecifičnih simptoma i kliničkih znakova koji često mogu oponašati druga neinfektivna stanja. Bakterijske hemokulture uzimaju vremena i često su lažno negativne. Postoji vise markera i laboratorijskih testova, kao što su broj bijelih krvnih stanica, apsolutni broj neutrofila, omjer ukupnih neutrofila, C-reaktivni protein, prokalcitonin, čimbenik nekroze tumora, serum amiloid A, IL-6, IL- 8 i CD64 koji se koriste u postavljanju dijagnoze kasne novorođenačke sepse. Međutim, trenutno nema specifičnih markera za pouzdano postavljanje dijagnoze. Kombiniranje dva ili više markera s različitim svojstvima koji su uzeti u različitim vremenskim razmacima, povećava dijagnostičku točnost. To bi moglo biti korisno za kliničare i u dijagnozi i liječenju kasne novorođenačke sepse. U toku su istraživanja na više različitih markera sa obećavajućim izgledima.

Ključne riječi: Kasna novorođenačka sepsa • Dijagnoza • Novorođenčad • Infekcija • Citokini

3.0 Introduction

Late-onset neonatal sepsis (LOS) is a life-threatening event in the neonatal period and in the rest of infancy. There are inherent difficulties with diagnosing neonatal sepsis early due to the non-specific presentation, which leads to increased morbidity and mortality. The warning signs could easily be confused with other non-infectious causes. Blood culture is the gold standard for diagnosis of neonatal sepsis but is time consuming. It takes approximately 48 to 72 hours to obtain results and false negatives are troublesome as bacteraemia is often low in concentration and sporadic. This is further expressed with low volume samples and antibiotic treatment prior to blood culture (1). In turn, this leads to limited sensitivity of the blood culture (2). The signs of sepsis such as fever or hypothermia, tachycardia, hypotonia and lethargy are unspecific and can easily be mistaken for other non-infectious conditions. Currently there are no adequate specific markers for diagnosis of neonatal sepsis. The most used biomarkers such as C-reactive protein (CRP), white blood cell count, total neutrophil count and Immature-to-Total neutrophil (I:T) ratio are valuable for guidance, but none has shown to be sufficient in diagnosing sepsis (3). Nevertheless, there are many promising biomarkers that are currently being explored. A sensitive and specific biomarker could guide clinicians in deciding whether to start antibiotic treatment or not, and if the continuation with antibiotics is necessary. To determine if a laboratory test is clinically useful, it should rise rapidly and have a good diagnostic window. The ideal biomarker should have a well-defined cut-off value, with sensitivity and negative predictive value (NPV) approaching 100% to be able to rule out neonatal sepsis. The specificity and positive predictive value (PPV) should also be >85% (4).

This review focuses on late-onset neonatal sepsis with approaches toward aetiology, risk factors, clinical features and laboratory markers. Hopefully this could help to the development of diagnostic criteria that could help diagnosing LOS, in order to reduce the morbidity and mortality connected with neonatal sepsis.

4.0 Definitions and Classification

The clinical condition of neonatal sepsis is classified according to postnatal age. There are slight variations in the exact time frame used for classification. The most commonly used classification defines early-onset neonatal sepsis (EOS) as infection ≤ 72 hours of life and late-onset neonatal sepsis (LOS) >72 hours to 7 days of life (1, 5, 6). One study used the age of the neonate when a positive blood culture was obtained and then classified further into early-onset (≤ 4 days), late-onset (5-30 days) and late, late-onset (>30 days) (7). There are other studies that extend the days of life (DOL) and define LOS from 4 days to 120 days of life (8, 9). There are also studies that define neonatal infection proven by blood culture as EOS (<7 days or <72 hours in case of VLBW) and LOS (>7 days after delivery) (10).

Classification of the pediatric age group can sometimes be confusing and terms can occasionally be used interchangeably, like neonate and infant. For the purpose of this paper we use the age group defined by European medical agency in 2010 at the consensus conference that states neonates is defined as birth to less than 1 month, and defining infants as 1 month to less than 2 years (11).

Neonatal sepsis starts with infection in the newborn. This primary event can in some individuals develop further into a systemic inflammatory response syndrome (SIRS). SIRS with suspected or proven infection constitutes the definition of sepsis (12). The definition of systemic inflammatory response syndrome is classically defined by: fever or hypothermia, tachycardia, tachypnea or hyperventilation, and abnormally high or low white blood cell count. Importantly, two or more of these variables need to be present to be able to diagnose SIRS (6, 13). Due to the inherent problems of diagnosing neonatal sepsis and in making the definition of SIRS more applicable to the pediatric age group, a consensus definition of SIRS was developed with some changes. Currently the criteria requires two out of four criteria, with one of them being abnormal temperature (>38,5°C or <36°C) or abnormal leukocyte count (increased or decreased for age or >10% immature neutrophils). The other two criteria's are tachycardia (>2 SD above normal age) or bradycardia (<10^{th} percentile for age) and increased mean respiratory rate (>2 SD

above for normal age) (14). The term “SIRS” was developed to describe the nonspecific inflammatory process occurring after infection, but also from non-infectious causes (15). Non-infectious causes in the newborn period that could develop into SIRS are traumatic delivery, asphyxia, inborn errors of metabolism and surgical procedures among others (16).

Severe sepsis is described if the newborn has sepsis and cardiovascular dysfunction or acute respiratory distress syndrome or ≥2 other organs dysfunctions (14). Sepsis could be seen as an order of phases starting with infection leading to SIRS and hence sepsis. If deterioration progresses, this could result in severe sepsis, and later septic shock with multiple organ failure and eventually death. It could be problematic to determine the current phase that is present in a patient as a change between phases could occur quickly (17). Generally if there is cardiovascular dysfunction that cannot be resolved with initial fluid therapy this signifies septic shock (18).

5.0 Epidemiology

Around 135 million children are borne yearly, with infection (36%) in the neonatal period being the single largest cause of death worldwide (19). Sepsis in developing countries is estimated to cause 30-50% of total neonatal deaths (20). The incidence of LOS in hospitalised newborns is estimated to be from 0.61% to 14.2% with variations geographically (21). In The United States and Australasia the incidence of LOS is up to 6 per 1000 births (22). Even with the usage of antimicrobial treatment 39% of cases of neonatal sepsis result in death or major disability (12). Neonatal mortality is presumably to be under-reported by 20% in developing countries (22).

6.0 Pathophysiology

The consensus conference in 1991 established the definitions of sepsis. It is defined as a condition that results from infection leading the host developing systemic inflammatory response syndrome (SIRS) (15). The interaction between the host complement system and the pathogen causes the release of pro-inflammatory mediators including C3 and C5a which leads to vasodilatation, chemotaxis and the release of cytokines Interleukin (IL) 1, IL 6, IL 8. In addition, the coagulation cascade is excessively activated, natural anticoagulants inhibited and fibrinolysis suppressed which in combination leads to an increased risk of microthrombi and consequential local cellular hypoxia (23, 24). Ultimately this culminates in end organ damage and dysfunction. Disseminated intravascular coagulation (DIC) is not rare in septic shock. Petechiae, ecchymoses and hemorrhages can also be observed due to consumption of coagulation factors and platelets (17).

The pathogens have different pathogen-associated molecular patterns (PAMPs) that are recognized by the host innate immune system, more specifically the Toll-like receptors (TLR). Toll-like receptors are membrane bound receptors that have a fundamental role in the pathophysiology of sepsis and septic shock. Upon activation of TLR, nucleus activation occurs and transcription of genes that induce pro-inflammatory and anti-inflammatory mediators, particularly cytokines ensues (16). The cytokine response to sepsis in neonates compared to adults is faster and more prominent. Meanwhile, the compensatory anti-inflammatory system in neonates appears to be immature, this is seen in both term and preterm neonates. Polymorphonuclear neutrophils, macrophages and eosinophils also have imperfect opsonization, phagocytosis and antigen presenting properties, which leads to reduced response by the neonatal immune system (17).

7.0 Ethiopathogenesis

7.1 Risk factors

There are several factors that contribute and interact to increase the probability of developing late neonatal sepsis. With decreasing birth weight (BW) and gestational age (GA) there is an increase in the incidence of late-onset neonatal sepsis, showing an inverse relationship between birth weight and gestational age (25). In one study from The National Institute of Child Health (NICHD) and Human Development Neonatal Research Network it was shown that neonates with BW of 401 g to 750 g had a 43% risk of late-onset sepsis. For BW of 751 g to 1000 g the risk was 28%, BW 1001 g to 1250 g had a 15% risk and neonates with BW 1251 g to 1500 g a 7% risk of LOS. In respect to gestational age, a comparable relationship can be made with neonates born < 25 weeks gestation, which had an incidence of 46% for LOS. There was a decline to 29% for GA between 25 to 28 weeks, and further to 10% at 29 to 32 weeks. Neonates born after 32 weeks had 2% incidence of LOS (26).

With prolonged hospitalizations due to decreased BW, GA and other medical conditions, the usage of central venous lines (CVC) for administrating antimicrobials, parental nutrition and other medicines is not uncommon, but CVC itself is a risk factor for developing LOS (27). CVCs can cause a blood stream infection either from intraluminal or extraluminal contamination. Intraluminal contamination results either from contaminated intravenous fluids or catheter hub. Organisms colonizing the skin can travel along the catheter and to cause extraluminal contamination. This can be identified with isolation of the same organism in blood as on the tip of the catheter (28).

Furthermore, patients that are on mechanical ventilators for respiratory support also have an increased risk for LOS (26). The endotracheal tube may provide a site of entry of organisms into the respiratory tract and later cause a systemic infection. Mechanical trauma to the endotracheal mucosa during suctioning may further contribute to infection due to breaking the anatomical barrier (29). Transient bacteraemia around five minutes after suctioning has been performed has been shown in neonates (30).

Total parental nutrition (TPN) has also been identified as a risk factor with particular precaution to the duration of TPN in the development of LOS (31). Lack of enteral feeding has been linked with the development of candidiasis as well as the usage of cephalosporins (32). Patent ductus arteriosus (PDA), bronchopulmonary dysplasia (BPD) and necrotizing enterocolitis (NEC) require supplementary interventions such as mechanical ventilation, CVCs and prolonged parenteral nutrition which leads to added risk factors for LOS (26, 33).

7.2 Pathogens

Classification of neonatal sepsis into early-onset (EOS) and late-onset neonatal sepsis (LOS) is based on different timing (7). As aforementioned, in classification different authors use slightly different timing to define the EOS and LOS. Regardless what specific timing is used, the classification was created to imply the different mode of transmission and different microorganisms associated with EOS and LOS, and to help in choosing the appropriate antibiotic treatment (34). EOS is associated with transmission from the mother during the intrapartum period and thus typically reflects vertical transmission (35). LOS on the other hand, is seemingly acquired postnatally from environmental sources (36).

The predominant pathogens implicated in LOS are gram-positive organisms, accounting for between 45 to 77% of the infections, with coagulase-negative Staphylococci (CoNS) being the most prevalent (28). The National Institute of Child Health and Human Development Neonatal Research Network reported in a study on 6215 admitted neonates that CoNS accounted for 48% of all first time infections. The other gram-positive organisms included Staphylococcus aureus (7.8%), Enterococcus spp. (3.3%) and group B Streptococcus (2.3%). Gram-negative organisms constituted 18% of infections where Escherichia coli, Klebsiella, Pseudomonas, Enterobacter and Serratia were most prevalent. Fungal organism accounted for a total of 12% and Candida albicans was the most encountered of the fungi (26).

Gram-negative organisms are less commonly a causative factor in the development of neonatal sepsis, albeit they have been associated with a higher mortality (28). Roughly one-third of cases of LOS are due to gram-negative infections, nevertheless, 40-69% of the deaths associated with neonatal sepsis can be attributed to gram-negative organisms (37). Of the infections caused by Pseudomonas aeruginosa several investigators reported mortality rates around 40% (8).

Coagulase-negative Staphylococci are unable to produce free coagulase which differentiates it from other types of Staphylococci. Out of the CoNS, Staphylococcus epidermidis is the principal microbiological organism that has been implicated in LOS in VLBW neonates. In industrialized countries it has been associated with up to 77.9% of all cases of LOS and 46.5% in developing regions (38). Staphylococcus epidermidis is capable of adhering and proliferating on plastic surfaces of indwelling catheters due to its capability to create multi-layered agglomerations termed biofilms. The biofilms cause problems both for the antibiotics and the immune system to attack the microorganism (10). Normally, Staphylococcus epidermidis is found on the skin and on mucous membranes and does not cause harm to healthy tissue. Colonization by Staphylococcus epidermidis in the neonatal period has also been indicated as beneficial to the host by educating the innate immune system and inhibiting virulent microorganisms (38, 39).

Out of the Candida family the Candida albicans and Candida parapsilosis species are the most common in neonates. Collectively the Candida species are the third leading cause of LOS in premature infants. Candida's ability to express certain virulence traits as adherence factors and cytotoxic substances has been associated with higher mortality rate and neurodevelopmental impairment (37).

Cohen-Wolkowiez et al showed the relationship between incidence of LOS with DOL and organism involved (Figure 1). In their study the distribution of organism implicated in LOS changed little depending on DOL but the incidence of LOS increased with DOL (8).

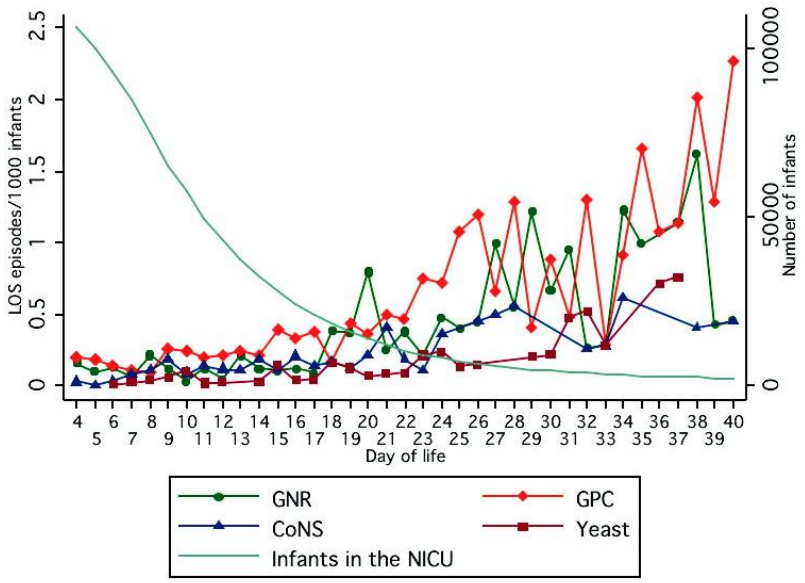


Figure 1. Infection episodes by organism group and postnatal age among late preterm infants with late onset sepsis. From Cohen-Wolkowiez et al (8).

8.0 Diagnostics

8.1 Clinical features

The clinical presentation of neonatal sepsis varies tremendously making it difficult to diagnose and the signs are often non-specific. The presenting sign are often variable and can also reflect non-infectious etiologies (6). Depending on the virulence of the pathogen and the host's immune system response there will also be an unpredictable clinical presentation. The immune system in premature neonates is also immature, leading to misleading signs and symptoms (10).

Normal range for heart rate, respiratory rate and systolic blood pressure in neonates was developed from the International Consensus Conference on Pediatric Sepsis in 2005. The values can be seen in table 1 below.

Table 1. Modified from International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definition for sepsis and organ dysfunction in pediatrics (14).

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

Clinical signs and symptoms are very variable, but most common include temperature instability, respiratory distress, apnea, cyanosis, bradycardia, tachycardia bulging fontanels, seizures, jaundice and feeding intolerance among others. Skin lesions could also be present and include cutaneous and mucosal petechial, impetigo, cellulitis and abscesses (10, 37). Motor functions are frequently diminished and initially the first symptoms might only be a neonate with lethargy or poor feeding (6). Fanaroff et al did a study on a total 2416 infants, 395 with culture-proven sepsis. They investigated the incidence, clinical presentation and risk factors for LOS in low birth weight infants. The main clinical features reported included increased apnea (55%), abdominal distension or guaiac-positive stool (43%), increased respiratory demand (29%), lethargy and hypotonia (23%) and feeding intolerance (25)

8.2 Laboratory findings

With fundamental difficulties to diagnose LOS based on solely clinical features, the quest for finding a marker that has sufficient sensitivity and specificity has been extensive. To date the definitive diagnosis still requires isolation of the pathogen from a normally sterile area, including blood and cerebrospinal fluid (CSF). Therefore in a suspected case of neonatal sepsis blood should be drawn (1 mL is recommended) and also lumbar

puncture should be considered (6). However, the results from culture can takes 24 to 48 hours before being obtained and the results can be false-negative especially if influenced by maternal antibiotic use or patients' antibiotic use (40). To decrease the excessive usage of antibiotics, several markers have been studied. Ng and Lam summarized the current view on the ideal biomarker for use in LOS and concluded that the biomarker should possess the following properties (4):

1. Well-defined cut off values, sensitivity and NPV approaching 100% for "ruling out" LOS. And specificity and PPV of >85%.
2. Detection of infection early
3. Be pathogen specific (e.g. viral, bacterial and fungal)
4. Help in antibiotic guidance and in monitoring disease progression
5. Predict severity
6. Predict prognosis
7. Require small volume of specimen, stable in laboratory, quick turnaround time, inexpensive.

There is a vast array of biomarkers studied that have been implicated in LOS, mostly acute phase reactants, cytokines and cell-surface antigens have been reviewed.

- Complete blood count (CBC)

Complete blood count (CBC) is widely accessible, rapid and relatively economical. A survey of neonatal intensive care units (NICU) showed that 99% of the NICU do obtain CBC count during the initial evaluation for LOS (41). Most frequently the white blood cell (WBC) count and differential with indices alike absolute neutrophil counts (ANC) and immature-to-total neutrophil (I:T) ratio are used to aid in the diagnosis of LOS. Although they are highly related to the timing of the sample and other non-infectious causes, this creates a very wide range of indices (42). Different pathogens could also induce different response, Gram-negative organisms seems to elevate the I:T ratio more than Gram-positive organisms (43).

- C reactive protein (CRP)

CRP is an acute-phase reactant that has a vital role in the humoral reaction to bacteria. It is widely available, cost-effective, fast and comprehensively studied as a biomarker in neonatal sepsis (44). Infection leads to release of IL-6 together with other pro-inflammatory cytokines that causes de novo CRP hepatic synthesis. CRP is released 4 to 6 hours after the onset of infection and peaks at around 48 hours. This in turn activates the complement system, monocytes, increase phagocytosis and increases the production of other proinflammatory cytokines (45). Generally, CRP is considered rather nonspecific, particularly in the adult population due to many non-infectious causes, such as rheumatoid arthritis and inflammatory bowel disease, which could be the cause of a rising CRP. Nevertheless, in neonates a significant rise in CRP has a narrower spectrum of diagnoses that could be responsible for its elevation (4).

- Procalcitonin (PCT)

Hepatocytes and monocytes are the main producers of PCT, that is a part of the acute phase reactants. PCT is a peptide prohormone of calcitonin and has been studied as a marker to differentiate non-infectious diseases from sepsis (46). In response to endotoxins it is seemingly released form hepatocytes and circulating macrophages. After 4 hours of exposure to bacterial endotoxins serum PCT begins to rise and reaches a peak in 6 to 8 hours. It then remains elevated for a minimum of 24 hours (47). The rise in serum concentration of PCT after infection is also faster when compared to CRP (3). In neonates however, there is a physiological postnatal increase of PCT, which is evident in both healthy term and preterm neonates with wide variations (40).

- Serum amyloid A (SAA)

Serum amyloid A is an acute phase reactant produced mainly from hepatic synthesis but also from smooth muscle cells, macrophages and endothelial cells. It is regulated by proinflammatory cytokines such as IL-6 and TNF-α. SAA has been reported to increase up to 1000 fold after the onset of inflammation (48, 49). SAA has been shown to be a useful marker in many conditions such as bacterial or viral infections, trauma and also

neonatal sepsis (48). Precisely how SAA works has not been established, but multiple functions have been attributed to SAA. For example SAA is involved in immunomodulatory actions, is a chemoattractant for monocytes and activates collagenases that aid in tissue regeneration (50).

- Cytokines

Several cytokines have been established to increase during response to neonatal infection. The increase of cytokines in neonatal sepsis occurs fast, even before the rise in acute phase reactants and the development of clinical signs (34). The most studied cytokines include Interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), IL-6 and IL-8. They are produced by a variety of cells like monocytes, endothelial cells and lymphocytes (51).

IL-6 is secreted rapidly in response to endotoxins, reaching its peak within 2-3 hours, followed by a rapid decline at around 6-8 hours and later a return to baseline by 24 hours (52). Il-6 induces hepatic production of CRP leading to the rise of IL-6 levels before CRP (53). Due to this feature, IL-6 is suitable for very early warning of sepsis, but due to the short half-life and uncertainty of knowing when the sample was drawn, Il-6 should not be relied on as the sole marker (53). In neonates with clinical sepsis or proven sepsis, IL-6 concentrations are significantly elevated compared to non-infected neonates (54). In a meta-analysis performed of 13 publications IL-6 revealed a pooled sensitivity and specificity of 79% and 84% respectively for neonatal sepsis (55).

IL-8 has comparable kinetics as IL-6 and has a part in chemotaxis and activation and migration of granulocytes (1). The levels of IL-8 increase 1-3 hours after infection and the half-life is less than 4 hours (56). A meta-analysis on IL-8 showed moderate accuracy in diagnosis of neonatal sepsis with a pooled sensitivity and specificity of 78% and 84%, respectively (56). IL-8 has also been shown to also be associated with the severity of infection and thus not merely beneficial as a marker of sepsis (57). TNF-α is also part of the cytokines that make up the acute phase reaction. The serum concentration of TNF-α has shown to be increased in infected neonates when compared to non-infected neonates (53).

- Cluster of Differentiation 64 (CD64)

Bacterial infection causes the up-regulation of leukocyte antigen that can be measured by flow cytometry. There are several advantages with using flow cytometry such as the requirement of a small volume of blood; the turnaround time is fast and there is a wide window for blood sampling. The disadvantages however, are that flow cytometry is usually not considered as a part of routine the evaluation in NICU and the process requires skilled technicians to be performed (4). The cell-surface antigen CD64 is present on neutrophil the surface, and is one of the three high-affinity antibody Fcy receptors. During sepsis inflammatory cytokines stimulate a quantitative up-regulation of CD64. This causes gradual increase in surface density and a stable expression of over 24 hours (58). Upon binding of immunoglobulin G (IgG) to CD64, phagocytosis and intracellular killing of opsonized microbes is enhanced. As opsonization requires encapsulated bacteria, no increase in expression of CD64 will be seen during viral infection. This up-regulation of CD64 is detected 1 to 6 hours after bacterial invasion (59). Up-regulation of CD64 has also shown to be an independent risk factor for developing LOS (60). A meta-analysis from 2013 consisting of 12 studies with a total of 1915 neonates showed a sensitivity of 78% and specificity of 81% in the diagnosis of neonatal sepsis with CD64 as a single test (61).

9.0 Discussion

9.1 What group of neonates have an increased risk of LOS?

VLBW neonates have an increased risk of developing LOS, generally the risk of developing LOS is inversely related to the gestational age and to the birth weight. The NICHD Neonatal Research Network showed data of birth weight and gestational age in correlation with infection and the highest rates revealed that neonates of 401 g to 705 g had a 43% risk of infection and 46% for less than 25 weeks gestation (62). This relates partly to the circumstance that less mature neonates and neonates of low birth weight require longer hospitalizations, mechanical ventilation, central venous access and parenteral nutrition, resulting in greater exposure to nosocomial infections and consequently increased risk of LOS (63). Bizzaro et al reported the incidence of EOS and LOS in relation to birth weight. As seen in table 2 there is an increase in sepsis per 1000 births with lower birth weight and it is more common for neonates of low birth weight to develop LOS compared to EOS (7).

Table 2. Birth weight-specific Sepsis rates for Newborns. Table modified from Bizzaro et al.(7). EOS- early-onset sepsis, LOS- Late-onset sepsis, ELBW- extremely low birth weight, VLBW- very low birth weight, LBW- Low birth weight.

Birth weight (g)	**Sepsis, Cases /1000 Births**		
	EOS	LOS	Total
ELBW <1000	54,5	441,8	496,3
VLBW 1499-1000	14,8	79,2	94
LBW ≥1500	3,52	23,43	26,95
Total	72,82	544,43	617,25

Some studies have presented that exposure to antenatal steroids has been shown to reduce the incidence of EOS, but on the other hand to increase the incidence of LOS (10). Although there seems to be conflicting evidence, Helen et al showed no increased risk of LOS in their study of combined use of maternal corticosteroid and antibiotic use (64). Nonetheless, the use of antenatal steroids should be balanced against the beneficial effects such as decrease risk of death, intraventricular haemorrhage and respiratory

distress syndrome (62). More studies are necessary on antenatal steroid use to draw any conclusion in the relationship to LOS.

Central venous catheters (CVCs) may provide an opportunity for interventions in LOS, though it is also contributing to LOS acting as a source of infection. Gaynes et al reported that blood stream infections were the most common nosocomial infection in all birth weights and that 88% were associated with umbilical or central venous catheters (65). Furthermore a cohort study showed that intraluminal contamination of CVCs accounted for 67% and extraluminal for 20% of the bloodstream infections (66). Hoffman et al presented in a cohort study that peripheral inserted central catheters removed due to adverse events were significant associated with LOS and that antibiotic usage before removal does not decrease the sepsis rate (67). The time length which a CVC could be kept before replacing it with a new one has also been of concern, but in a study of 135 cases of catheter related infection in the NICU, increasing dwell time was not linked with an increased infection rate and this could be due to decreased need of other invasive devices, improved nutrition and maturation of infants' immune system (68).

The low pH in the stomach is considered a protective mechanism against bacteria and antacids used in neonates could by raising the gastric pH decrease this defence. Terrin et al revealed that ranitidine was indeed associated with an increased risk of infections in VLBW infants (69). Singh et al evaluated 360 VLBW neonates, 64 whom received ranitidine and/or omeprazole. In their study they did not show any statically significant difference in the incidence of LOS (70). Noticeably there is conflicting evidence and more studies are needed to draw any definite conclusions. Several authors still recommend caution when prescribing antacids to neonates.

Mechanical ventilation is also a well-recognized risk factor for developing nosocomial infection, but also any type of respiratory tract device, tracheal intubation, nasopharyngeal and nasal cannula are associated with an increased risk (71). One study from Stoll et al showed that in neonates that were ventilated for more than 28 days, half of the neonates developed LOS compared to only 9% of those on ventilator for less than 7 days (26). When endotracheal suctioning is necessary, this must be performed gently to minimise the risk of breakage of the tracheal mucosa (29).

9.2 How could lab help with the diagnosis of LOS?

Serum biomarkers mostly investigated to aid in the diagnosis of LOS are either key proinflammatory or anti-inflammatory mediators involved in the inflammatory and infectious cascade. To date there is no single laboratory marker that satisfactorily diagnoses LOS. This relates to that many of the biomarkers are influenced by other factors than sepsis. Non-infectious conditions for example surgery or tissue injury also affects the biomarkers. Normal ranges are hard to establish due to the fluctuation in neonates. Often this leads to delayed diagnosis, excessive therapy, increased costs and emergence of resistant organisms. This leads to the possibility of combining two or more biomarkers and hence increases the sensitivity and specificity (4, 10).

CBC is commonly used in the initial evaluation of LOS, while it is difficult to interpret early in life because of the fluctuations with day of life and gestational age (37). Hornik et al reports an association between high or low WBC counts, high ANC, high I:T ratio and low platelet counts with LOS. The highest specificity was for WBC (99%) either $<1000/mm^3$ or $>50,000/mm^3$. With increasing postnatal age at the time of culture the associations became weaker. Even though these markers are associated with infection the sensitivity of these findings was low but the specificity was generally high, therefore no CBC count can sufficiently and reliably rule out LOS (9).

Of the acute phase proteins, CRP is studied extensively and used for diagnosis and monitoring of treatment. One problem of using CRP in the early days of life is the nonspecific physiological 3-day rise in CRP. This is due to stress during delivery and other maternal and perinatal factors. In the early stages of infection CRP has a low sensitivity due to delayed hepatic production (72). Several different cut-off values for CRP have been used, two studies with a cut-off value of 12 mg/L measured on the day of sepsis evaluation showed a sensitivity of 60% and 65% respectively and a specificity of 96% and 99% respectively. However after 24 hours both studies reported an increase in the sensitivity for CRP with a sensitivity of 82% and 72% respectively, the specificity was 96% and 100% after 24 hours respectively (53, 73). Hotoura et al used a cut-off value of 10 mg/L and also reported a good specificity of 90%, although the sensitivity was 68% (74). Higher cut-off values have been used; Terrin et al reported a sensitivity and specificity of 29% and 93% respectively in a study with 231 neonates using the cut-off value of 100 mg/L (75). To further increase the sensitivity the usage of serial measurements of CRP could be employed at 24-48 hours after onset of symptoms to increases the sensitivity (76, 77). Serial measurements of CRP are good for ruling out sepsis and normal levels for >48 hours could aid in the decision whether to continue or discontinue with antibiotic treatment (4). Another acute phase protein commonly implicated in LOS is serum amyloid A (SAA). SAA has shown to be a good prognostic marker in LOS. When comparing to CRP and WBC as a prognostic marker SAA is the earliest marker of LOS (78). Arnon et al reported that SAA is an accurate marker in the diagnosis of LOS at the first suspicion on sepsis. They reported that it was more sensitive marker for differentiating sepsis from non-sepsis preterm infants compared to CRP and IL-6, concluding that SAA could be used as an early marker for detection of LOS (79). Conversely, another studied focusing on SAA as a diagnostic marker during LOS, did not show any statistical significance (80). In a meta-analysis by Yuan et al involving 823 neonates (8-96 hours after infection), SAA showed moderate accuracy in diagnosing neonatal sepsis, the pooled sensitivity was 78% and the pooled specificity was 92% (81).

Procalcitonin has shown promising results. It has been suggested for usage in the international definition of sepsis (82). In one meta-analysis by Vouloumanou et al, a better diagnostic accuracy was reported comparing LOS to EOS when using PCT. They found the area under the curve (AUC) to be 0.95. The sensitivity of PCT for diagnosing LOS was 90% and the specificity was 88%. However they are highlighting that they did not have sufficient data for a well-founded conclusion (40). Yu et al showed in their meta-analysis on PCT, that PCT had moderate accuracy diagnosing neonatal sepsis but PCT had better accuracy than CRP in the diagnosis of LOS. Other studies have also reported better sensitivity and specificity, ranging from 87% to 100% with PCT compared to CRP and other acute phase proteins (83). A meta-analysis by Bokun Lv et al on TNF-α with 15 articles and 23 trials presented moderate accuracy with sensitivity of 68% and specificity of 89% for LOS. They also reported higher diagnostic accuracy for LOS compared to EOS when using TNF-α (84). Bokun Lv et al also compared PCT with TNF-α in their study and reported that the pooled specificity was 89% for TNF-α versus 77% for PCT, although the pooled sensitivity was marginally lower for TNF-α when compared to PCT (84). Ucar et al compared PCT, SAA, CRP and TNF-α and reported that CRP was the best reliable marker of inflammation aimed at diagnosing LOS, then PCT > TNF-α > SAA (80).

Interleukins are encouraging in the diagnosis of LOS. They are generally considered to be sensitive for neonatal sepsis. They can be detected in the blood early, but do have a short half-life which limit their clinical use (10). Ng et al studied IL-6 and used a cut-off value of 31 pg/mL and reported a sensitivity of 78% and a specificity of 92% at the time of evaluation of LOS. They found that 24 hours after the sensitivity and specificity was 44% and 93% respectively afterwards (73). Hotoura et al reported with a cut-off value of 30 pg/ml a sensitivity of 100% and a specificity of 74% using IL-6 (74). Another interleukin studied in diagnosis of LOS is IL-8, which has similar kinetics as IL-6 (1).

In a study by Boskabadi et al, IL-8 was measured in 93 neonates of ≥72 hours of age. The serum concentrations of IL-8 were 3.3 times higher in non-surviving neonates compared to surviving neonates. They also reported a sensitivity of 95% and a specificity of 100% using a cut-off value of 60 pg/ml, signifying that IL-8 could be a valid early predictive marker in diagnosing LOS (57).

Several different cell-surface antigens have been studied in neonates in the context of neonatal sepsis (85). CD64 seems to be the most promising in diagnosing LOS (86, 87). One study by Ng et al on CD64 investigating 80 infants showed a sensitivity of 95-97% and a negative predictive value of 97-99% at the time of sepsis evaluation and 24 hours after the onset. If combining CD64 with either IL-6 or CRP at the time of sepsis evaluation and CD64 at 24 hours the specificity was 88% and the positive predictive value was 80% (73). Other studies using different cut-off values reported different data. Mazzucchelli et al used 2.85 as cut-off reported a sensitivity of 87.5% and specificity of 100% for CD64 index used in culture proven LOS (86). Streimish et al used a cut-off value of 3.62 and demonstrated a sensitivity of 75%, specificity of 77% and negative predictive value of 96% (87).

The use of a combination of two or more markers has been studied; preferably to overcome one markers' disadvantage and in that way complement each other. Ng et al combined IL-6 and CRP on day 0 (day of sepsis evaluation) and TNF-α on day 1 or CRP on day 2 and presented a sensitivity and specificity of 98%, 91% respectively for diagnosing LOS (53). The biomarkers could also be categorized into early (IL-6, IL-8 CD64), mid (PCT) and late phase (CRP) based on the detection time after infection (88).

10.0 Conclusion

Late-onset neonatal sepsis has been and still is today a significant cause of morbidity and mortality, both in developing and developed countries. The risk factors are various and numerous, however preterm birth and low birth weight leads to prolonged hospital stay in a high-risk environment. Together they are the cornerstones in the development of late-onset neonatal sepsis. The combination of an indefinable clinical presentation that often resembles a wide array of conditions with biomarkers that is not sufficient to rule out or to rule in late-onset sepsis. There is a need for a biomarker that could reliable diagnose late-onset neonatal sepsis. Several biomarkers or combinations are promising, but larger studies are needed to be able to safely confirm late-onset sepsis. Of the studies available CRP and PCT seem promising. The use of multiple biomarkers such as IL-6 or CRP on the day of sepsis evaluation, and after 24 hours the use TNF-α has presented good sensitivity and specificity. Several new studies are in progress, in the research of potential novel markers that could revolutionize the diagnosis of late-onset neonatal sepsis in the future. In the meantime, suspicion to late-onset neonatal sepsis should relay on the presence of a wide spectrum of risk factors, unspecific signs of clinical presentation, different combination of laboratory (including microbiology) investigation, and on the response to antimicrobial and other, supportive treatment.

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13.0 Biography

Gustaf Edström was born in Uppsala, Sweden in 1988-11-19. He completed high school in natural science in June 2007. After high school he served his military service and later focused on paramedics in the military. In September 2011 he moved to Croatia to study medicine. During the summers he worked in Sweden in both surgery and emergency departments.