Incidence of and predictive factors for bacteremia in newborns upon admission to a neonatal intensive care unit level 1 in Germany

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INCIDENCE OF AND PREDICTIVE FACTORS FOR BACTEREMIA IN NEWBORNS UPON ADMISSION TO A NEONATAL INTENSIVE CARE UNIT LEVEL 1 IN GERMANY

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List of abbreviations

AGA - Appropriate for gestational age

APR - Acute-phase reaction

AWMF - Association of the Scientific Medical Societies in Germany

BC - Blood culture

bpm - beats per minute

BW - Birth weight

CFU - Colony-forming-units

CI - Confidence Interval

CoNS - Coagulase-negative Staphylococcus

CPAP - Continous positive airway pressure

CRP - C-reactive protein

CRT - Capillary refilling time

CTG - Cardiotocography

E.coli - Escherichia coli

e.g. - example given

ELBW - Extremely low birth weight

EONS - Early-onset neonatal sepsis

FIRS - Fetal inflammatory response syndrome

GA - Gestational age

GBD - Global Burden of Disease

GBS - Group B streptococcus

HCA - Histologically confirmed chorioamnionitis

I/T ratio - Immature-to-total neutrophil ratio

IAI - Intraamniotic infection

IAP - Intrapartum antiobiotic prophylaxis

IL-6 - Interleukin 6

LBW - Low birth weight

LGA - Large for gestational age

LONS - Late-onset neonatal sepsis

Max - Maximum

MIAC - Microbial invasion of the amniotic cavity

Min - Minimum

NPV - Negative predictive value

PPROM - Preterm premature rupture of membranes

PPV - Positive predictive value

PROM - Preterm rupture of membranes

SD - Standard deviation

SGA - Small for gestational age

SIRS - Severe inflammatory response syndrome

SOFI - Suggestive of fetal inflammation

TLC - Total leukocyte count

VLBW - Very low birth weight

WBC - White blood cells

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1. INTRODUCTION

1.1 Special characteristics of the newborn and its immune system

The neonate is defined as an infant within the first 28 days of life. This period is a critical stage in human development, which is characterized by significant changes in physiology compared to intrauterine life (1).

In order to ensure a more precise evaluation of the risk of disease, neonates are differentiated depending on their birth weight (BW), gestational age (GA) and both values in relation to each other, to discriminate between infants of the same birth weight (2), as we can see in Table 1.

Table 1. Classification of newborns based on GA and BW

Gestational age	GA < 37 weeks	Preterm infants
	GA 37 – 42 weeks	Term infants
	GA > 42 weeks	Post-term infants
Birth weight	BW <1000g	"Extremely low birth weight" (ELBW)
	BW <1500g	"Very low birth weight" (VLBW)
	BW <2500g	"Low birth weight" (LBW)
Birth weight in relation to gestational age	BW < 10^{th} percentile	Small for gestational age (SGA)
	BW 10^{th} – 90^{th} percentile	Appropriate for gestational age (AGA)
	BW > 90^{th} percentile	Large for gestational age (LGA)

Source: Speer CP. Neonatologie. In: Speer CP, Gahr M, Dötsch J, editors. Pädiatrie. Berlin, Heidelberg: Springer Berlin Heidelberg; 2019. p. 77-133. (2)

At the time of transition from intrauterine to extrauterine life, almost all organ systems undergo adaptive changes to adjust to the extrauterine environment.

Lung maturition takes place in the last weeks of gestation and is defined as sufficient production of surfactant in the alveoli. It starts around the 24^{th} week of gestation and sporadic thoracic movements can be detected. It is completed around the 35^{th}-36^{th} week of gestation (3). The fetal thoracic movements are interpreted as inhalation and exhalation of amniotic fluid and contribute significantly to the process of lung maturation. At the end of pregnancy, exhalatory phases predominate, allowing the lung to be filled with air after birth as fast as possible. Remaining amniotic fluid is reabsorbed from alveolar wall within a few hours after birth (2).

Since the immune system is still immature and limited to the exposure of maternal antigens, neonates represent a highly vulnerable population, with regard to infections and sepsis (4). The placental transfer of maternal IgG antibodies begins around the 20th week of gestation, with serum levels steadily rising until the end of pregnancy, approaching levels similar to those of adults as presented in Table 2 (5). GA and serum levels of IgG are inversely proportional to the risk of getting an infection, therefore most preterm infants are at an even higher risk for neonatal sepsis due to their inability to generate a quantitative and/or qualitative adequate humoral response against infectious agents (6-8). IgM antibodies are not passed from mother to child via placental route, due to their size. They are produced by fetal B-lymphoctes only in case of intrauterine infection (2).

Table 2. Lower limits of IgG depending on (gestational) age

Age	IgG serum level (in g/l)	Mg/dl
22^{nd} – 27^{th} week of gestation	1	100
28^{th} – 36^{th} week of gestation	2	200
37^{th} – 41^{st} week of gestation	6	600
3 months	2	200
> 12 years	7	700

Source: Jorch G, Schlüter D, Avenarius S, Böttger R, Brunner-Weinzierl M, Cornean S, et a. Unreifes Immunsystem. 2017 2017/08/07 [cited 2024/03/27]. In: Fetoneonatale Infektiologie [Internet]. Stuttgart: Georg Thieme Verlag KG. 1. Auflage. [cited 2024/03/27]. Available from: http://www.thieme-connect.de/products/ebooks/lookinside/10.1055/b-0037-145453. (7)

Not only antibodies, also other important parts of the immune system such as the complement system, granulocytes, macrophages and T-lymphocytes are not yet matured and can only exhibit decreased functionality in terms of pathogen defense mechanisms (2). Due to the aforementioned immaturity of the humoral immune response, neonates and particularly preterm infants rely more on an adequate innate immune response than adults do. However, neonatal neutrophils show qualitive as well as quantitative insufficienies (9).

1.2 Neonatal sepsis

Neonatal sepsis is a systemic inflammatory response with a clinical presentation similar to a severe inflammatory response syndrome (SIRS). It is commonly triggered by bacterial infection, rarely by viral or fungal pathogens (10). It is a serious and potentially fatal condition in pediatrics, which – if left untreated – rapidly leads to pneumonia, meningitis and/or septic shock and ultimately to neonatal death (11). Due to its complexity and the involvement of multiple organ systems, there is currently no agreed-upon internationally valid definition and its diagnosis rather relies on a range of clinical and laboratory parameters (12).

1.3 Epidemiology

Despite continuous advancements in prevention, diagnosis and treatment options, neonatal sepsis remains a leading cause of morbidity and mortality, especially in middle- and lower-income countries (13). Although there is a strong correlation between socioeconomic status and mortality (14), neonatal sepsis remains a significant health issue on a global scale. Even in industrial countries with adequate antibiotic therapy neonatal sepsis has the highest impact on neonatal mortality (15).

Understanding the epidemiology of neonatal sepsis is crucial for implementing effective preventive strategies and improving clinical outcomes. Approximately 1.3 million newborns are affected by systemic infections each year, which was estimated by the Global Burden of Disease (GBD) Study 2016/2017. This is equivalent to 9.37 cases per 1000 live births with a total number of 203 000 neonatal deaths that has been associated to neonatal sepsis (16,17). However, recent meta-analysis (2021) showed an increase in incidence of 28.42 cases per 1000 live births and a mortality of 17.9% (11), but incidence studies for neonates are lacking from many countries (17). Incidence of neonatal sepsis in Germany is estimated on 1.71 cases per 100 000 (18). While early onset sepsis is responsible for about 1% of neonatal unit admissions, late-onset accounts for 7% of admissions (19).

Thus, there is a strong necessity for a greater emphasis on conducting epidemiological studies, standardizing definitions of neonatal sepsis, and enhancing research within this area.

1.4 Classification

Depending on the time of onset neonatal sepsis is divided into early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS) (20). This classification allows a more specific approach particularly in terms of therapy and risk stratification. EONS is defined as sepsis within the first 72h after birth (21). Symptoms commonly start after 24-48h of life in 85% of cases. It is caused by vertical pathogen transmission (22). LONS occurs later than 72h of life with a peak incidence between 10th and 22nd day of life (23). In epidemiological studies cutoff points range from 72h up to 7 days, but most studies agree on 72h (24). The pathogen is often acquired from hospital settings with horizontal transmission of the microorganism (25). EONS is often accompanied by pneumonia, LONS rather goes with meningitis (2).

1.5 Route of transmission and pathogen spectrum

The etiology of EONS is multifactorial; however, pathogen transmission usually occurs vertically (26). Vertical transmission – also known as mother-to-child transmission – can occur at any stage of pregnancy: during the prenatal period in utero, during delivery (peripartum), or postnatal through breastfeeding. It can be subdivided in different mechanisms of infection.

Hematogenous transmission, as depicted in Figure 1, refers to the transplacental route of infection, when pathogens from maternal blood overcome barriers at maternal-fetal interface, enter the uterine compartment and lead to manifest FIRS. Hematogenous transmission happens exclusively in antenatal period (27).

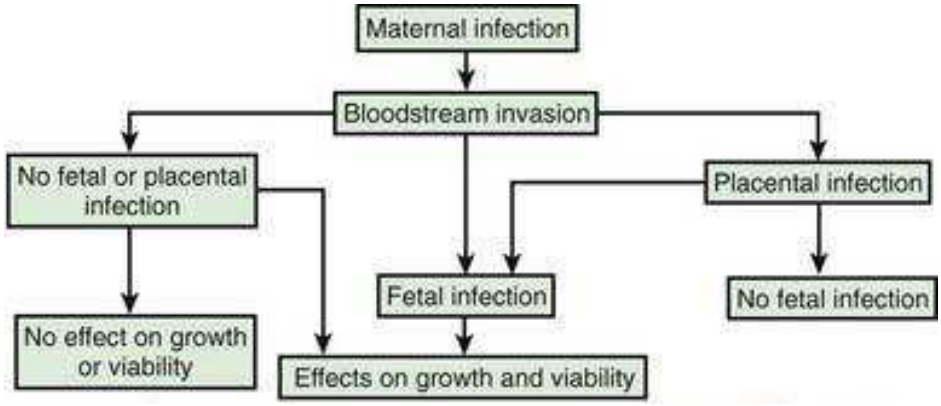


Figure 1: Pathogenesis of hematogenous transplacental infections

Source: Klein JO, Remington JS: Current concepts of infections of the fetus and newborn infant. In Remington JS, Klein JO, editors: Infectious disease of the fetus and newborn infant, ed 7, Philadelphia, 2011, WB Saunders (28)

Ascending infection however most commonly occurs prenatally or perinatally, during the process of vaginal birth. The causative microorganism is transmitted from the colonized genitourinary or gastrointestinal tract of the pregnant women to the amniotic membranes, the amniotic sac and to the fetus or newborn (27,29). Mechanisms of ascending and intrapartum infections can be seen in Figure 2. In both cases, aspiration of contaminated amniotic fluid and/or vaginal secretions can lead to adherence of the pathogen to the respiratory epithelium and subsequent invasive bacteremia. Ingestion of contaminated material can result in gastrointestinal colonization with potential invasion and subsequent bacteremia. Pregnancies with a history of preterm rupture of membranes and/ or cervical insufficiency are at higher risk due to a disruption of mechanical protective barrier (30,31).

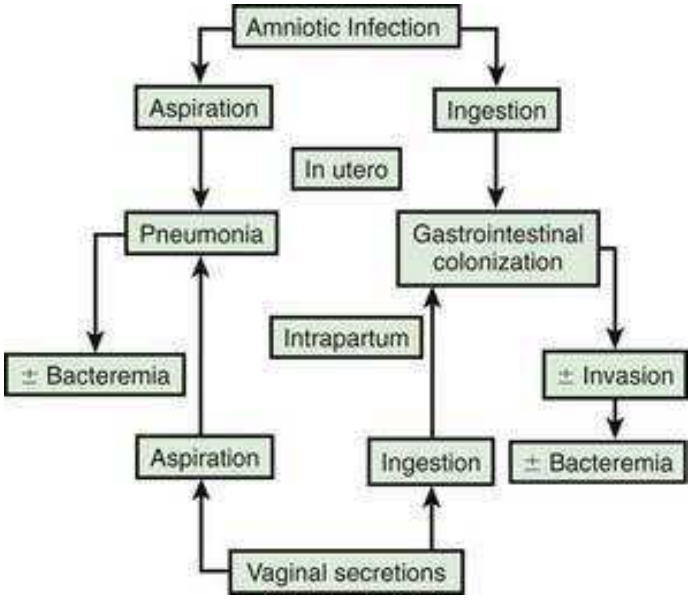


Figure 2: Pathway of ascending or intrapartum infections

Source: Klein JO, Remington JS: Current concepts of infections of the fetus and newborn infant. In Remington JS, Klein JO, editors: Infectious disease of the fetus and newborn infant, ed 7, Philadelphia, 2011, WB Saunders (28)

The most common causative agent of EONS among term infants is *Streptococcus agalactiae*, a gram-positive *Group B streptococcus* (GBS). Preterm infants and VLBW infants on the other hand are predominantly affected by pathogens that cause gram-negative sepsis like *Escherichia coli* (E. coli) (32, 33). 10-30% of pregnant woman show rectovaginal GBS colonization (34), in most cases with a subclinical or even asymptomatic course (35). However, the incidence of GBS associated EONS and LONS decreased over the last decades, due to improved maternal screening programs between 35-37 weeks of gestation and following

intrapartum antibiotic prophylaxis (IAP). This is leading to a relative increase in neonatal sepsis caused by *E. coli* (10,36).

Predominating pathogens causing LONS are coagulase-negative *Staphylococcus* strains (CoNS), mostly *Staphylococcus epidermidis*, followed by *E. coli.* (37,38). These infectious microorganisms are acquired postnatally from nosocomial or community environment (39). Invasive medical devices such as central venous catheters and parenteral nutrition, as well as prolonged hospitalization and mechanical ventilation are all potential risk factors for pathogen colonization and LONS (40). Table 3 summarizes the most common pathogens in EONS and LONS, respectively.

Table 3. Typical Pathogens of EONS and LONS in term infants

EONS	Frequency (%)	LONS	Frequency (%)
Group B Streptococcus	43-58	Staphylococcus epidermidis	39-54
Escherichia coli	18-29	Escherichia coli	5-13
Staphylococcus aureus	2-7	Staphylococcus aureus	6-18
Listeria monocytogenes	0.5-6	Enterobacter species	4-9
		Pseudomonas aeruginosa	3-5

Source: Zea-Vera A, Ochoa TJ. Challenges in the diagnosis and management of neonatal sepsis. J Trop Pediatr. 2015;61(1):1-13. (41)

1.6 Risk factors

The risk of infection in neonates is influenced by a multitude of factors encompassing maternal, neonatal, and environmental variables.

1.6.1 Maternal risk factors

The main risk factors for EONS with maternal origin are preterm rupture of membranes (PROM), bacterial vaginosis and intraamniotic infection (IAI) (42).

1.6.1.1 GBS colonization

Bacterial colonization of genitourinary and gastrointestinal tract with GBS is an important contributor in the pathogenesis of neonatal sepsis. As discussed in “route of transmission and pathogen spectrum”, 10-30% of pregnant women show rectovaginal

colonization with *Streptococcus agalactiae* (34, 43) and there is a significant association between maternal GBS colonization and the incidence of EONS (44).

Prevalence of microbial invasion of the amniotic cavity (MIAC) in preterm infants was 10,7%, in combination with PROM there was an increase up to 57,5% (45). In addition to that, GBS colonization is directly associated with an increased incidence of PROM, premature labor and preterm birth, which are strong risk factors for EONS themselves (46).

1.6.1.2 Preterm (premature) rupture of membranes

Preterm rupture of membranes is defined as such, if the rupture of amniotic membrane occurs before onset of labor. In 25% of cases rupture occurs before 37+0 weeks of gestation and is then called preterm premature rupture of membranes (PPROM), which affects approximately 3% of all pregnancies and 25-30% of all preterm births (47).

The amniotic membranes serve as a protective barrier to prevent invasion of external pathogens and to maintain a sterile environment. Therefore, P(P)ROM is often followed by IAI, caused by ascending vaginal pathogens. The incidence of chorioamnionitis after PPROM is 30% (48). When the rupture of membranes occurs >18h before the onset of labor, the risk of ascending infection is significantly increased. In that case, prophylactic (IAP) should be considered. Bacterial colonization however does not always result in the clinical picture of bacteremia. Factors that influence which patient will get the disease or not are not fully understood, but prematurity, immaturity of the innate immune system and underlying disease are included (49). Both the rupture of membranes and the resulting infection can lead to preterm birth. Preterm birth, in turn, is the most severe neonatal risk factor for neonatal sepsis. The increase in risk for an IAI is proportional to the duration of ruptured membranes (50).

1.6.1.3 Intrapartum fever and intraamniotic infection

Intraamniotic infection, or chorioamnionitis, is defined as an infectious event of fetal-maternal unit with resulting inflammation and changes in clinical picture and laboratory results. Inflammation affects amniotic fluid, placenta, fetus and fetal membranes or decidua (51). Diagnosis of chorioamnionitis at that time has been based on a histological examination of placental specimen (histologically confirmed chorioamnionitis (HCA)), nowadays its diagnosis is almost exclusively clinical (42). The definition “Triple I” (Inflammation, Infection or both) has been established to ensure a more precise diagnosis and evaluation of intrapartum maternal

fever because the clinical presentation of IAI is often subtle and difficult to recognize. Rapid diagnosis and intervention however is crucial to prevent fetal inflammatory response syndrome (FIRS) (48).

Clinical signs and symptoms of a manifest IAI include intrapartum maternal fever (>38,0°C), maternal tachycardia (>100bpm) and/ or leukocytosis (>15000/µl without administration of corticosteroids), uterine tenderness and pain upon palpation, fetal tachycardia (>160bpm) on CTG and amniotic fluid with foul odor (43,48). In clinical practice however, maternal fever during labor alone is often sufficient to establish a suspected diagnosis of chorioamnionitis, as all causes of neonatal sepsis are associated with elevated maternal temperatures of > 38,0°C and are therefore be considered a standalone risk factor of neonatal sepsis (42,52). Approximately 12% of cases of IAI result in subsequent neonatal infection. In 2 to 20% of cases of EOS, there is an associated clinical IAI (53).

1.6.2 Neonatal risk factors

The main risk factor for neonatal sepsis is preterm birth. All aforementioned maternal risk factors for neonatal sepsis are also enhancing the risk of premature birth and therefore the risk for neonatal sepsis to the same extent. Both, incidence as well as mortality are inversely proportional to GA (54). Immaturity of the immune system and the lung are the main contributors to the pathogenesis of neonatal sepsis in preterm infants. As previously discussed in “special characteristics of the neonate and its immune system”, placental transfer of antibodies starts around the 20th week of gestation. Full-term infants have approximately 600 mg/dl of IgG in their blood. Premature infants born before the 28th week of gestation only receive about one-sixth of the antibodies transferred from the mother, while infants born between the 28th and 37th week already have a twice as many antibodies in their serum (7). Premature infants, compared to full-term infants, also exhibit significant deficits in terms of lung maturity, which is completed around 35th-36th week of gestation. The lack of surfactant increases the risk of pneumonia by bacterial infection due to the insufficiency of the barrier between alveoli and capillaries (55). An important step in the process of lung maturation is the “inhalation and exhalation” of amniotic fluid. In case of PROM, a decrease in the amount of amniotic fluid impairs this process and can lead to lung hypoplasia (2).

1.7 Diagnosis

The identification of sepsis solely based on the above-mentioned clinical findings is very challenging and prone to delayed diagnosis. Early detection however is crucial, because neonates show an increased risk for neurocognitive complications including cognitive impairment and cerebral palsy (25). Even laboratory results are not individually sensitive or specific enough to prove or exclude an infection, especially since 24-36 h postnatally, a "physiological acute-phase-reaction (APR)" can take place, which leads to a general elevation of inflammatory parameters. The more criteria are present, the higher is the specificity and sensitivity of laboratory and clinical findings. Therefore, a combination of multiple symptoms and laboratory tests is necessary to enable a correct diagnosis of neonatal sepsis.

1.7.1 Clinical signs of infection

The clinical manifestations are unspecific and can vary from subclinical infection to severe systemic disease resembling the clinical picture of SIRS (21).

Early indicators and symptoms that support the suspicion of neonatal sepsis are lethargy, irritability and intestinal disturbances leading to a distended abdomen and feeding difficulties (56). Term infants frequently exhibit respiratory disturbances ranging from tachypnea to apnea resulting in oxygen desaturation and bradycardia, arterial hypotension with prolonged capillary refilling time (CRT) and reactive tachycardia as well as fluctuations in body temperature in both directions with a temperature gradient >2°C between central and peripheral sites (57). Furthermore, neurological abnormalities can occur. These include hypotony, apathy, lethargy as well as hyperexcitability.

Preterm infants show similar symptoms but in contrast to term infants they have a subtler presentation of those. However, in preterm infants, neurological abnormalities take precedence over the aforementioned symptoms (22).

1.7.2 Microbial analysis

Microbial culture remains the diagnostic gold standard for diagnosis of neonatal sepsis (58). A positive blood culture (BC) confirms the presence of pathogens in the blood stream (22). Sensitivity and specificity however are low, due to multiple variables affecting the growth

of bacterial cultures. These factors encompass the amount of blood volume that is obtained, timing of blood collection and the number of samples (59). Especially in CoNS sepsis it is recommended to collect two BCs to differentiate an actual infection from a contaminated sample. Furthermore, IAP is thought to be a possible cause of microbial growth suppression (60). The amount of inoculated blood volume is stated as the most important factor for successful recovery of the pathogen (61-63). Most studies recommend a volume of 1.0 ml of blood for neonates, but most samples are <1.0ml (64). However, the majority of septic newborns have low-level-bacteremia, with colony-forming-units (CFU) between 1-10 CFU/ml. Neonatal sepsis with a colony count <10 CFU/ml will show false-negative results in 60% of BC inoculated with sample volumes <1ml (62,65,66).

Therefore, decisions about treatment options should not only rely on BC results, because even if sepsis is clinically evident, results often remain negative (60). A positive BC result proves the diagnosis of sepsis, but inter-study comparison is difficult due to differing criteria for the diagnosis of suspected sepsis (67).

1.7.3 Leukocytes

Total leukocyte count (TLC) is a hematological test to assess the total number of white blood cells (WBC) or leukocytes in the blood. Leukocytes are nucleated cells that play an important role in the innate immune response (68). Counting specific subsets of leukocytes can be helpful for the identification of different diseases. Leukocytes, but neutrophils in particular, are responsible for the production of cytokines during infection, which then attract macrophages to the site of infection (69). Its relevance in diagnosis of neonatal sepsis however is very low. Norm values of TLC in neonates have a broad range from 10 to 35000µl. At the onset of an infectious process, leukocytes are mobilized from bone marrow pools. However, due to limited reserve volume, there is a rapid transition from leukocytosis to leukopenia (22).

Especially neutropenia is associated with multiple non-infectious complications during birth, but studies show that neutropenia and/or leukopenia, particularly in combination with postnatal respiratory distress, are significantly associated with EONS (70). In this situation, leukopenia and neutropenia are most likely caused by bone marrow depletion and therefore are bad prognostic factors for neonatal infection (60).

1.7.4 I/T ratio

Systemic inflammatory response in sepsis stimulates the new formation of neutrophils in bone marrow and the release of immature precursors (67). However, the total number of immature neutrophils in peripheral blood in the first 72 hours of life can be very high without a septic event, therefore its diagnostic value is very low (60). Because of that, the immature to total neutrophil ratio (I/T ratio) has been established. This quotient describes the total number of immature neutrophils in relation to the total number of neutrophils (including mature and immature cells) and is calculated as follows: immature neutrophils (promyelocytes + myelocytes + metamyelocytes + banded neutrophils) / total number of neutrophils (immature + mature). Immature neutrophils usually include banded neutrophils and metamyelocytes, earlier forms like myelocytes and promyelocytes are rarely seen (71).

The increase in immature neutrophils is called left shift. Information regarding the origin of the term "left shift" is not totally clear; however, it is most likely derived from the depiction of granulopoiesis, with left-sided depiction of precursor cells (Figure 3). With an increase in precursor cells, the ratio shifts metaphorically to the left (72).

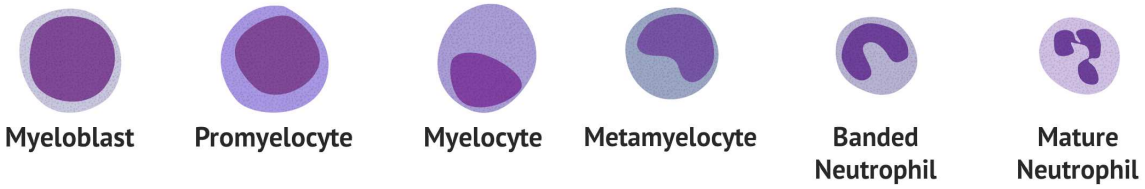


Figure 3: Stages of neutrophil maturation

Source: Tunney P; Douglas G; Hettige S. Neutrophil Morphology: The Medical Company 2021 (Available from: https://medschool.co/tests/blood-film/neutrophil-morphology) (73)

If the I/T ratio is ≥0.2, over 20% of granulocytes in peripheral blood are immature precursor cells, which is highly suggestive for EONS. It is however far from a perfect adjunctive test for early detection of neonatal sepsis, because this value shoes significant postnatal fluctuations. With a sensitivity of 70-75% and a positive predictive value (PPV) of 55% however, it is a useful tool to support the diagnosis when other tests show positive results as well. Values <0.2 in contrast show high specificity and high negative predictive value (NPV) for neonatal sepsis which may be useful in developing diagnostic options (67,74,75).

1.7.5 Interleukin 6

Interleukin 6 (IL-6) is a proinflammatory cytokine, that is produced by monocytes, endothelial cells and fibroblasts response to infection and tissue injury. Cytokine production is initiated by the recognition of antigens and/or pathogen-associated molecular patterns (PAMP) by toll-like-receptors (TLR) expressed on these cells (76-78).

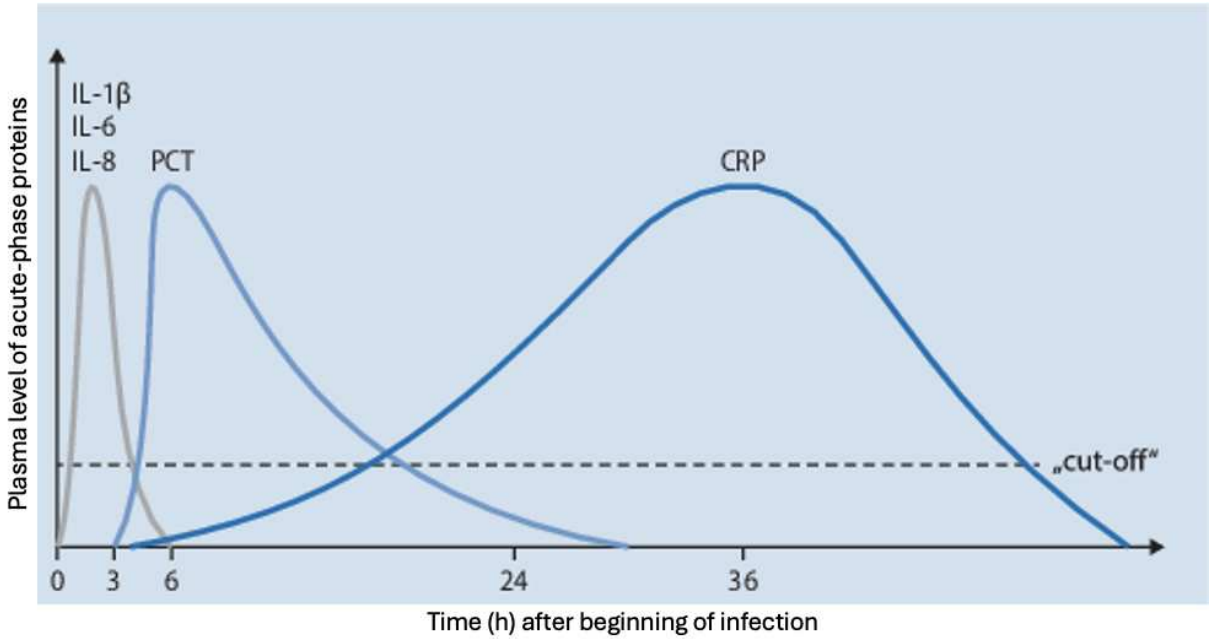


Figure 4. Schematic representation of the kinetics of CRP, PCT and IL-6 following an infectious event

Source: Niehues T. C-reaktives Protein und andere immunologische Biomarker. Monatsschrift Kinderheilkunde. 2017;165(7):560-71. (78)

As depicted in Figure 4, IL-6 has its peak early after onset of bacteremia and the half-life is short. Therefore, levels will rapidly return back to normal and sensitivity is highest in the first hours. Healthy neonates usually have IL-6 serum concentrations around 5 pg/ml (79), but during an inflammatory episode levels can rise up to a thousand-fold, reaching concentrations of several µg/ml (77). In contrast to term infants, preterm infants exhibit a 2-6fold greater increase of IL-6 in response to systemic inflammation in the first 48 hours of life. There is, however, no direct correlation between the magnitude of IL-6 serum levels and the severity of the ongoing infection (80). In clinical practice cut-off values of 80pg/ml are used in neonates on 1st day of life, 40pg/ml on 2nd day and 20pg/ml after 2 days of life (81).

Sensitivity and specificity of IL-6 regarding to the Association of the Scientific Medical Societies in Germany (AWMF) are 73% and 76%, respectively (22) and should not be used alone to exclude or prove the diagnosis of neonatal sepsis but rather in combination with CRP.

1.7.6 C-reactive protein

C-reactive protein (CRP) is an acute phase protein, that is produced in the liver as part of inflammatory response in sepsis. Due to the pathophysiology of an APR, CRP levels rise with a temporal delay compared to IL-6 and procalcitonin, because the release of CRP from hepatocytes is stimulated by IL-6. An increase in serum levels of CRP is therefore expected after the peak of IL-6 at 6-8 hours and typically has its peak between 24-48 hours after onset of infection. If the infection subsides, its levels rapidly decrease back to normal levels, due to its biological half-life of 19h (82).

The initial sensitivity of CRP immediately after suspected onset of infection is only 46%, increasing to 97% after 24 hours, with an initial specificity of (86%) (83). Furthermore, when two consecutive measurements yield values within the normal range, the negative predictive value exceeds 99%, making it a valuable indicator to discontinue ongoing antibiotic therapy. Combined with its high specificity, CRP is an important diagnostic marker for neonatal sepsis, in particular to decrease the exposure of unaffected neonates to broad-spectrum antibiotics and invasive procedures (84). From that it can be concluded, that during initial examination of the newborn elevated CRP values with clinical signs of infection strongly indicate the presence of infection with a high PPV. Conversely, negative CRP results at that stage do not effectively rule out infection, resulting in a low NPV (22). However, there are multiple noninfectious diseases that can cause CRP-elevation including maternal fever, perinatal asphyxia or meconium aspiration. Therefore CRP alone cannot confirm the diagnosis of EONS (85).

1.8 Treatment

1.8.1 Causative therapy

In the treatment of neonatal septicemia, the selection of an appropriate antibiotic therapy according to the results of microbial culture and antibiotic sensitivity screening would be the ideal approach.

In clinical practice however, antibiotic treatment usually has to be initiated immediately after blood cultures have been taken but before microbial tests grow cultures, to prevent the possible foudroyant progression of this disease. Upon identification of the causative pathogen, therapy should be narrowed, deescalated and adjusted (86).

Empirical antibiotic therapy is started, if clinical signs and early laboratory tests are positive (22). Therefore, 7% of term infants and post term infants in industrial countries receive "prophylactic" antibiotic therapy up to 3 days in case of suspected but not culture-proven sepsis. Only in 0.1% the pathogen can be ultimately confirmed (87). Calculated antibiotic therapy should rely on individual anamnestic data of the neonate and on local data about the most common pathogen spectrums and resistances. Time of onset can be important to narrow down possible causative pathogens (perinatal vs. nosocomial infection). There is currently no standard algorithm for antibiotic therapy of EONS and LONS (22).

When choosing antibiotic therapy, it is crucial that the most common causative pathogens, including *GBS*, *E. coli.*, *Listeria monocytogenes, Enterobacteriaceae, Enterococci* as well as hospital specific pathogens are targeted. Possible combinations that are used in clinical practice is 1. aminopenicillin or acylaminopenicillin + aminoglycoside or 2. cephalosporin group 2 or 3 + acylaminopenicillin (if necessary + aminoglycoside).

With *GBS* and *E. coli* as the most common causative organisms of EONS and LONS, respectively, the combination of broad-spectrum antibiotics (ampicillin or piperacillin + gentamycin) is effective in most cases (85). However, antibiotic therapy of neonatal sepsis should not be based on aminoglycosides (e.g. gentamycin) alone, due to its bad penetrative capability of liquor, cartilage, bone and bronchi. This is important to reconsider if meningitis and/ or pneumonia is suspected.

Aminopenicillins (e.g. ampicillin) and acylaminopenicillins (e.g. piperacillin) are not beta-lactamase resistant and therefore no effective treatment for infection caused by most staphylococci. In that case, treatment of choice are cephalosporins of group 2 (e.g. Cefuroxime), which shows high efficacy against beta-lactamase forming organisms.

40% of *E. coli* strains are resistant to antibiotic therapy with ampicillin, some even to aminoglycosides. In neonates with a history of IAI, risk for ampicillin-resistant *E. coli* due to IAP with ampicillin is even higher. Despite the risk of ampicillin-resistant *E. coli* the use of group 3 cephalosporins e.g. ceftriaxone should be carefully reconsidered, as every antibiotic therapy in neonates increases the risk for potential recolonization with resistant pathogens and subsequently nosocomial sepsis, especially with Klebsiella and other Enterobacteriaceae (22,88,89).

The duration of antibiotic therapy should be as short as possible and as long as necessary. An overview is shown in Table 4. Antibiotic therapy should be terminated, if microbial cultures, as well as clinical and laboratory signs of infection, remain negative. The British guidelines from the National Institute for Health and Care Excellence recommend termination after 36 hours, whereas American guidelines recommend termination after only 48 hours (86, 90). In case of positive blood culture, therapy should last 7-10 days (20,91).

Table 4. Duration of antibiotic therapy in neonatal sepsis

Duration	Comment
1 – 2 days	No clinical or laboratory sign for infection, stop therapy
5 – 7 days	No clinical signs of infection, CRP mediated (if CRP negative, stop therapy immediately)
7 – 10 days	Positive microbial culture
2 – 3 weeks	Meningitis
3 weeks	Osteomyelitis and invasive fungal infection
No therapy	Positive swabs (trachea, ear, eye) without clinical signs for infection

Source: Deutsche Gesellschaft für Pädiatrische Infektiologie e V, Abele-Horn M, Adam R, Adams O, Adamczick C, Aebi C, et al. DGPI Handbuch. Stuttgart: Georg Thieme Verlag KG; 2018. Available from: http://www.thieme-connect.de/products/ebooks/book/10.1055/b-006-160379. (20)

1.8.2 Supportive therapy

In addition to antibiotic therapy stabilization of the neonate and its vital functions is crucial. Depending on the needs of the patient, possible therapeutic measures should be considered.

In case of respiratory insufficiency or apnea, mechanical ventilation, either with nasal CPAP device or tracheal intubation, can be started. To keep up with circulatory disturbances, arterial blood pressure, body weight and urine output should be regularly controlled. 10% weight gain is common on the 1st day. Hypo- and hyperglycemias should be corrected (20).

1.9 Prognosis and complications

The most important prognostic factors for neonatal infection are the duration and severity of the infection. Both duration and severity are directly dependent on the timing of diagnosis. If early signs of the disease are recognized too late or not at all, the risk of subsequent diseases and long-term complications increases significantly.

Low birth weight and infections caused by Gram-negative pathogens are associated with a worse outcome (89,92). Premature infants, in particular, are affected by impaired neuronal development and have a higher risk of brain hemorrhages, periventricular leukomalacia, and cerebral palsy. Regarding delayed development, it is assumed that proinflammatory molecules negatively influence brain development (93).

Neurological long-term damage occurs in 15-30% of newborns with meningitis. Moreover, meningitis can be associated with periventricular leukomalacia and hydrocephalus. (94) Medication-induced complications include, among others, sensorineural hearing loss and nephrotoxicity associated with aminoglycoside therapy (95).

2. OBJECTIVES

2.1 Aim of the study

The aim of this study is to collect new and additional data on the incidence of neonatal sepsis among newborns that have been admitted to NICU as well as to obtain a more detailed picture about predictive factors of neonatal sepsis.

2.2 Hypothesis

Based on the findings of previously conducted studies, we hypothesize that the incidence of bacteremia upon neonates that have been admitted to a NICU in Bavaria, Germany, in the timeframe of this study, is lower than the number of positive blood cultures.

We also hypothesize, that the combination of multiple diagnostic tests improves sensitivity for the diagnosis of neonatal sepsis.

3. MATERIALS AND METHODS

3.1 Study design

This study is designed as a monocentric retrospective study conducted at the Department of Pediatrics of REGIOMED Hospital in Coburg, Bavaria, Germany.

3.2 Ethical approval

The Institutional Review Board of the REGIOMED Medical School ethically approved this study on 8th March 2024. All data and rights of patients were protected in accordance with the World Medical Association Helsinki declaration of 2013.

3.3 Data collection

All relevant data regarding the neonate as well as the mother could be obtained from the hospital server ORBIS of REGIOMED KLINIKEN Coburg. Data was collected from all newborns, that have been admitted to NICU in the time from 1st January 2023 – 31st December 2023 and from their mothers.

The resulting data set was anonymized, so that no personal and patient-related information cannot be traced back to the patient by third parties.

3.4 Variables

For all neonates, clinical profile data were collected and contained the following data:

- Clinical signs of infection
  - Breathing pattern
  - Hemodynamic status as capillary refill time
  - General condition as feeding difficulties
- Gestational age (GA)
- Birth weight (BW)
- Mode of delivery (vaginal or cesarean section (C-section))
- Duration of antibiotic therapy

Laboratory data included:

- Total leucocyte count (TLC)
- Immature to total leukocyte ratio (I/T ratio)
  (calculated from total count of mature neutrophils and total count of immature neutrophils)
- Interleukin 6 (IL-6)
- C-reactive protein (CRP)
- Microbial culture results

Maternal health related data included:

- Preterm rupture of membranes (PROM)
- Peripartum fever
- Pathological findings on cardiotocography (CTG)
- C-reactive protein (CRP)

3.5 Statistical analysis

The data collection was conducted using Microsoft Excel version 16.16 (Microsoft Corp., Redmond, WA, USA) for Macintosh. For the statistical analysis of the data, Statistical Package for the Social Sciences (SPSS) version 29.0.2 (IBM, Chicago, USA) for MacOS and PRISM 10 version 10.2.3 for MacOS was used.

For metric variables, means (M) standard deviations (SD), minimum (Min), maximum (Max) were determined. Categorial variables were shown as number (n) and percentage.

The normal distribution of the metric variables was tested by using the Shapiro-Wilk test. Metric variables were compared visually in form of boxplot graphs and for non-normally distributed metric variables, the Mann-Whitney U test was used for significance testing. Significance was considered present if $P < 0.05$

Categorial variables were visually presented in form of histograms, tested on statistically significant association with Fisher's exact test assuming no association (null hypothesis), with additional calculation of odds ratio.

For all significant variables, the statistical quality criteria in combination or alone sensitivity, specificity, PPV and NPV were calculated using cross-tabulation, given that:

- True Positives (TP): "Confirmed sepsis" with positive results
- True Negative (TN): "no sepsis" with negative results
- False Positive (FP): "no sepsis" with positive results
- False Negative (FN): "confirmed sepsis" with negative results

For correlation analysis of nominal-to-nominal variables contingency cross-table analysis with calculation of Cramér's V value was performed. Interpretation of Cramér's V test results is as follows: 0-0.1 (weak association), 0.1-0.3 (moderate association), 0.3-0.5 (strong association) and >0.5 (very strong association).

4. RESULTS

4.1 Characteristics of the neonate

In this study, 158 neonates and their mothers have been included, after considering the above listed data set.

Gender distribution shows 91 neonates of male sex (57.55%) with a predominance of 15.14% to female sex which accounts for 67 neonates (42.41%).

Out of the admitted patients, 88 newborns were born preterm before 37+0 weeks of gestation. This corresponds to 55.7%. On the other hand, 70 newborns had a gestational age of more than 37+0 weeks, thus full-term infants making up 44.3% of the study cohort. Mean gestation age upon admission was 36 + 1,7 weeks of gestation.

Mean BW of the total patient cohort is 2710.7g and a median of 2700g. BW has been additionally classified in ELBW, VLBW, LBW and normal BW. This cohort includes 9 neonates with a BW of <1000g (ELBW), 9 neonates with a BW of <1500 g (VLBW), 49 neonates with a BW <2500g (LBW) and 91 neonates with a BW >2500g (normal). Table 5 shows BW distribution depending on gestational age.

Table 5. Birth weight distribution among preterm and term infants

Birth weight (g)	Mean	SD	Min	Max	Median
Preterm	2128.5	722.2	370	3640	2210
Term	3442.6	615.8	1680	4735	3560
Total	2710.7	940.5	370	4735	2700

Another variable in this data set is birth mode, subdivided in vaginal birth and cesarean section. 39,91% were born vaginally, 60,09% have been delivered via C-section.

Table 6 shows the distribution of birth mode among preterm and term infants.

Table 6. Birth mode frequency distribution among preterm and term infants

Birth mode	Preterm		Term		**Total**	
	n	**%**	**n**	**%**	**n**	**%**
C-Section	69	43.7	37	23.4	106	67.1
Vaginal	19	12.0	33	20.9	52	32.9
Total	88	55.7	70	44.3	158	100

4.2 Laboratory variables of the neonate

Due to the limitations in the diagnosis of neonatal sepsis in terms of microbial culture, three groups have been determined during data collection with regard to the duration of antibiotic therapy. Antibiotic therapy lasting 3 or more days is considered indicative of confirmed neonatal sepsis (by microbial culture, laboratory results, or clinical signs) in this study.

Group 1: Neonates who received no antibiotic therapy at any time (no EONS)
Group 2: Neonates who received antibiotic therapy for less than 3 days (suspected EONS)
Group 3: Neonates who received antibiotic therapy for 3 or more than days (confirmed EONS)

Laboratory parameters that were collected from neonates include neonatal WBC, I/T-ratio, CRP, IL-6 and microbial culture. Distribution is shown in Table 7.

Table 7. Distribution of neonatal laboratory results

	n	Mean	SD	Min	Max	Median
WBC[a] ($x10^9$/l)	158	15.9	6.9	0.0	45.0	14.7
CRP[b] (mg/l)	158	3.6	7.7	1.0	56.6	1.0
IL-6[c] (pg/ml)	158	224.1	758.2	0.0	5000.0	14.5
I/T ratio[d]	114	0.1	0.1	0.0	0.6	0.1

a: White blood cell count; b: C-reactive protein; c: Interleukin 6; d: Immature to total neutrophil ratio

Out of 158 to NICU admitted neonates, from 145 blood cultures were taken, which is 91.7% of the whole group. Out of these 145 only 1 culture showed positive results with *Streptococcus agalactiae*. Overview is shown in Table 8.

Table 8. Frequency distribution of microbial results

	n	%
No culture taken	13	8.2
Negative culture	144	91.1
Positive culture	1	0.6

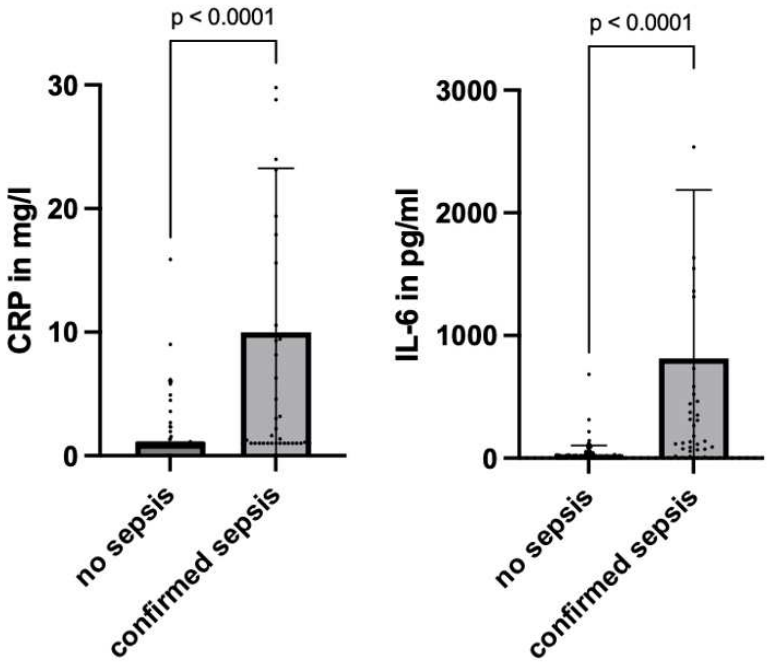
Data on duration of antibiotic therapy was collected from all neonates. It was subdivided in 3 groups: no antibiotic therapy, antibiotic therapy for less than 3 days and antibiotic therapy for more than 3 days (Table 9).

Table 9. Distribution of duration of antibiotic therapy

	n	%
No therapy	98	62.0
Therapy <3 days	21	13.3
Therapy >3 days	39	24.7

To simplify the determination of significance, the three previously defined groups are consolidated into two groups referred to as “no sepsis” (Group 1+2; n=119) and “confirmed sepsis” (Group 3; n=39). From that, total incidence can be calculated as 246.8 per 1000 admissions to on NICU in Germany, with 6.3 for BC positive and 240.5 for BC negative.

Neonatal CRP, IL-6, I/T ratio and TLC were tested on normal distribution with Shapiro-Wilk-Test, resulting in $P<0.001$ showing not normally distributed data. Figure 5 shows statistical significances of aforementioned diagnostic laboratory parameters. CRP and IL-6 are highly significant indicators of neonatal sepsis with $p < 0.001$, I/T ratio follows with $P=0.002$. TLC, however, is nonsignificant with $P> 0.05$. Therefore, TLC is not included in calculation, analysis and interpretation of sensitivity, specificity, PPV and NPV.



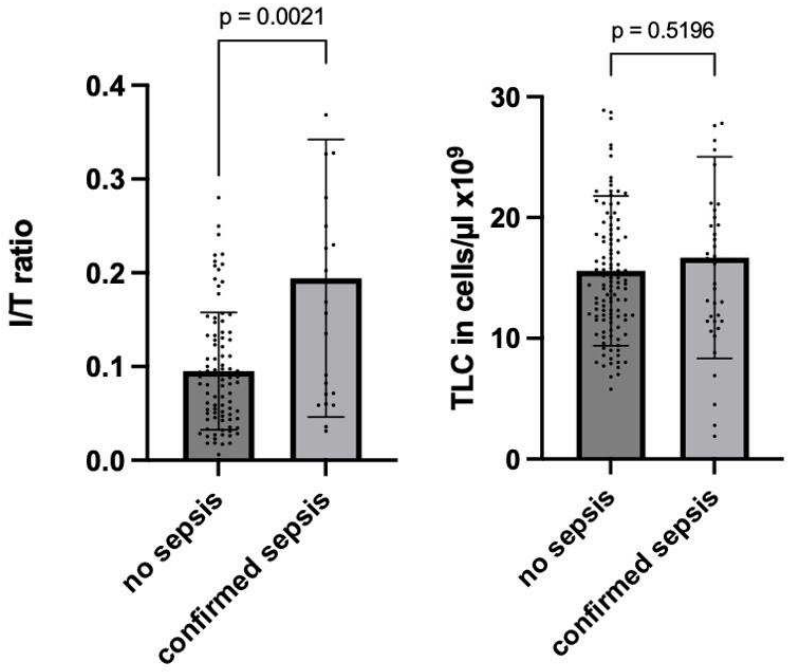


Figure 5: Diagnostic tests and their respective significances (Statistical Procedure: Mann-Whitney-U**)**

4.3 Clinical variables of the neonate

For clinical signs of sepsis, data on hemodynamic status in terms CRT, breathing pattern and general status in terms of feeding difficulties were collected.

Normal CRT is defined as <3 seconds, ≥3 seconds is the cut-off value for prolonged CRT. Out of 158 neonates, 40 neonates showed prolonged CRT, which accounts for 25,3% of the whole group. 118 patients (74,7%) had regular CRT.

Feeding difficulties could be observed in 33 neonates (20.9%), 125 neonates (79.1%) were without any signs of feeding difficulties upon admission.

Third clinical variable that has been analyzed is breathing pattern, subdivided in bradypnea, tachypnea and eupnea. 73 neonates (46.2%) showed eupnea, whereas 85 neonates showed abnormal breathing pattern. 21 patients (13.3) had bradypnea upon admission defined as <30 breaths per minute, whereas 64 neonates (40.5%) presented with tachypnea defined as >60 breaths per minute.

Table 10 shows the distribution of all clinical variables collected.

Table 10. Frequency distribution of clinical signs

Clinical variable		n	%
Breathing pattern	Eupnea	73	46.2
	Bradypnea	21	13.3
	Tachypnea	64	40.5
Hemodynamic status	Normal	118	74.7
	Prolonged	40	25.3
General status	No feeding difficulties	125	79.1
	Feeding difficulties	33	20.0

Capillary refill has been tested on statistical significance using Fisher's exact test with *P*=0.001. The difference in Groups "no sepsis" and "confirmed sepsis" can therefore be assumed as significant (Figure 6) with a high association between sepsis and prolonged CRT. There is a 3.8-fold higher probability to have a prolonged CRT in case of sepsis than without (Odds ratio: 3.779; CI (95%): 1.692 to 8.066).

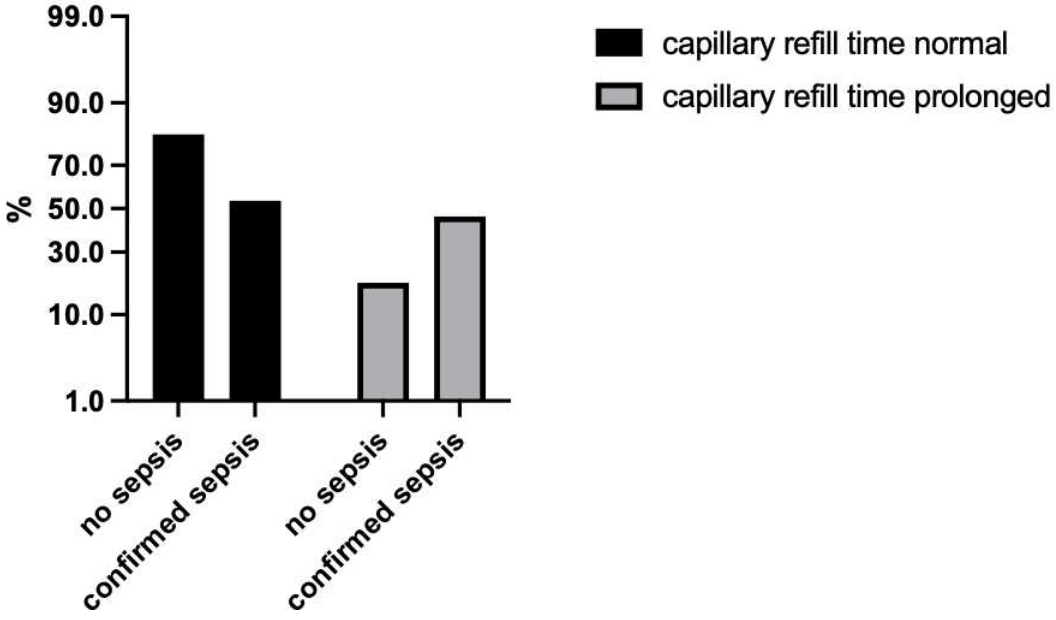


Figure 6: Statistical significance of capillary refill time in groups "no sepsis" and "sepsis" (Statistical procedure: Fisher's exact test)

For breathing pattern comparison, bradypnea and tachypnea have been analyzed as one under "pathological breathing pattern" in septic and non-septic neonates as presented in Figure 7. Fisher's exact test showed a significant difference (*P*<0.001) with an odds ratio of 5.288 (CI (95%): 1.980 to 13.24). Thus, observation of a pathological breathing pattern is 5 times more likely in septic patients.

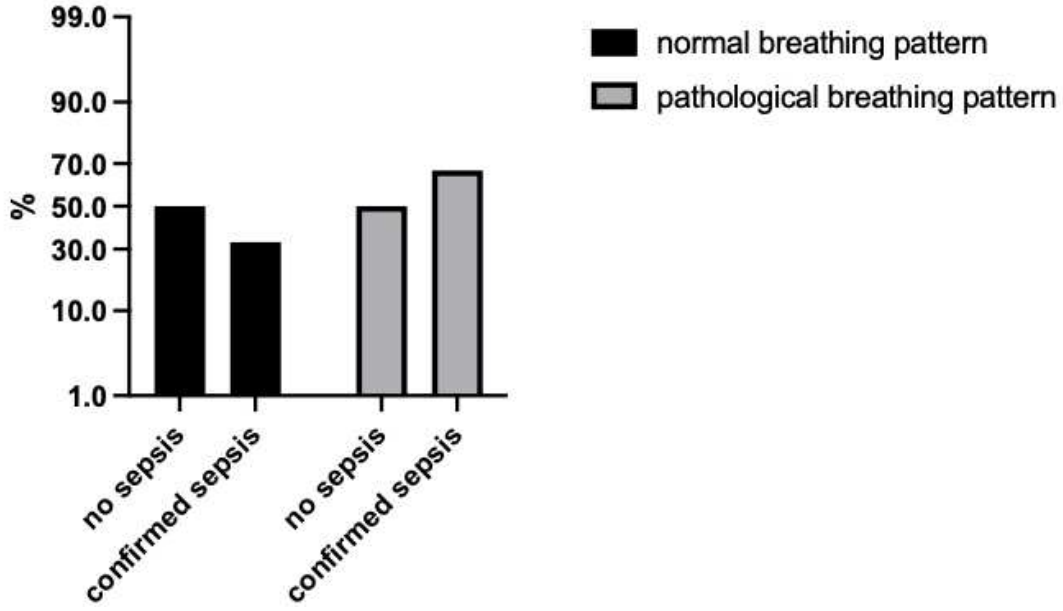


Figure 7: Statistical significance of breathing status in groups "no sepsis" and "sepsis" (Statistical procedure: Fisher's exact test)

For the clinical sign of feeding status, Fishers exact test gave *P*=0.826, which proves statistical non - significance. (Figure 8)

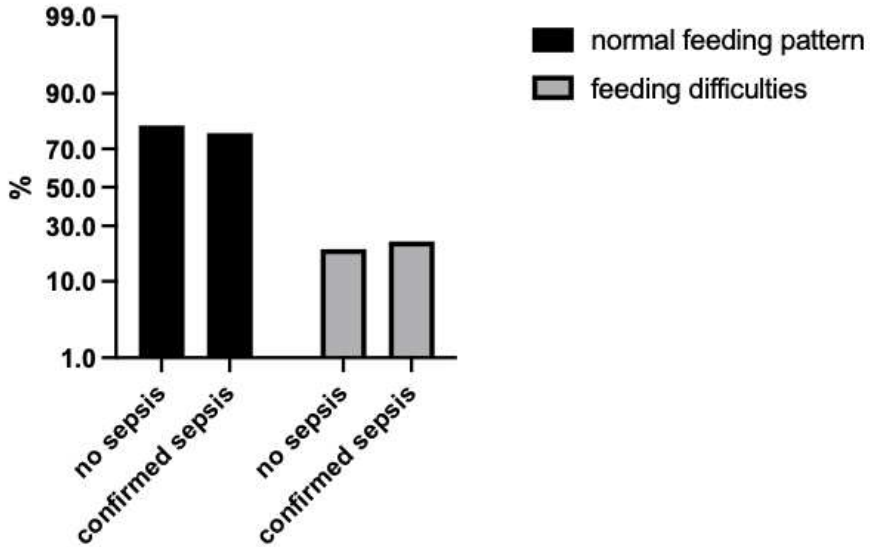


Figure 8: Statistical significance of feeding status in groups "no sepsis" and "sepsis" (Statistical procedure: Fisher's exact test)

4.4 Maternal variables

Table 11 summarizes the distribution of maternal CRP levels in the study population. The mean CRP level was 7.90 mg/L with a standard deviation of 17.54 mg/L. The CRP levels ranged from 0.00 to 143.73 mg/L, with a median value of 2.45 mg/L.

Table 11. Distribution of maternal CRP

	n	Mean	SD	Min	Max	Median
CRP[a]	158	7.9	17.5	0.00	143.7	2.5

a: C-reactive Protein

Table 12 presents the distribution PROM among the study participants. 39.9% of the neonates had PROM, while 60.1% did not.

Table 12. Frequency distribution of PROM

	n	%
No PROM[a]	95	60.1
PROM	63	39.9

a: Preterm rupture of membranes

Tables 13 and 14 summarize the frequency distributions of maternal fever during pregnancy and CTG results among the 158 neonates and their mothers.

Table 13. Frequency distribution of fever during pregnancy or birth

	n	%
No fever	154	97.5
fever	4	2.5

Table 14. Frequency distribution of CTG results

	n	%
Normal CTG[a]	131	82.9
Pathological CTG	27	17.1

a: Cardiotocography

Maternal predictive Factors maternal fever during pregnancy, maternal CRP, presence of CTG pathology and presence of PROM were analyzed on statistical significance. The presence of fever during pregnancy or birth resulted in a marginal significance (P=0.0468) and an odds ratio of 0.102 (CI (95%): 0.008 to 0) whereas the presence of a pathology on CTG shows the highest significance with P<0.001 and an odds ratio of 20.06 (CI (95%) = 8.325 to 46.76). Maternal CRP and presence of PROM, both showed non-significant p-values of P=0.701 and P=0.442, respectively.

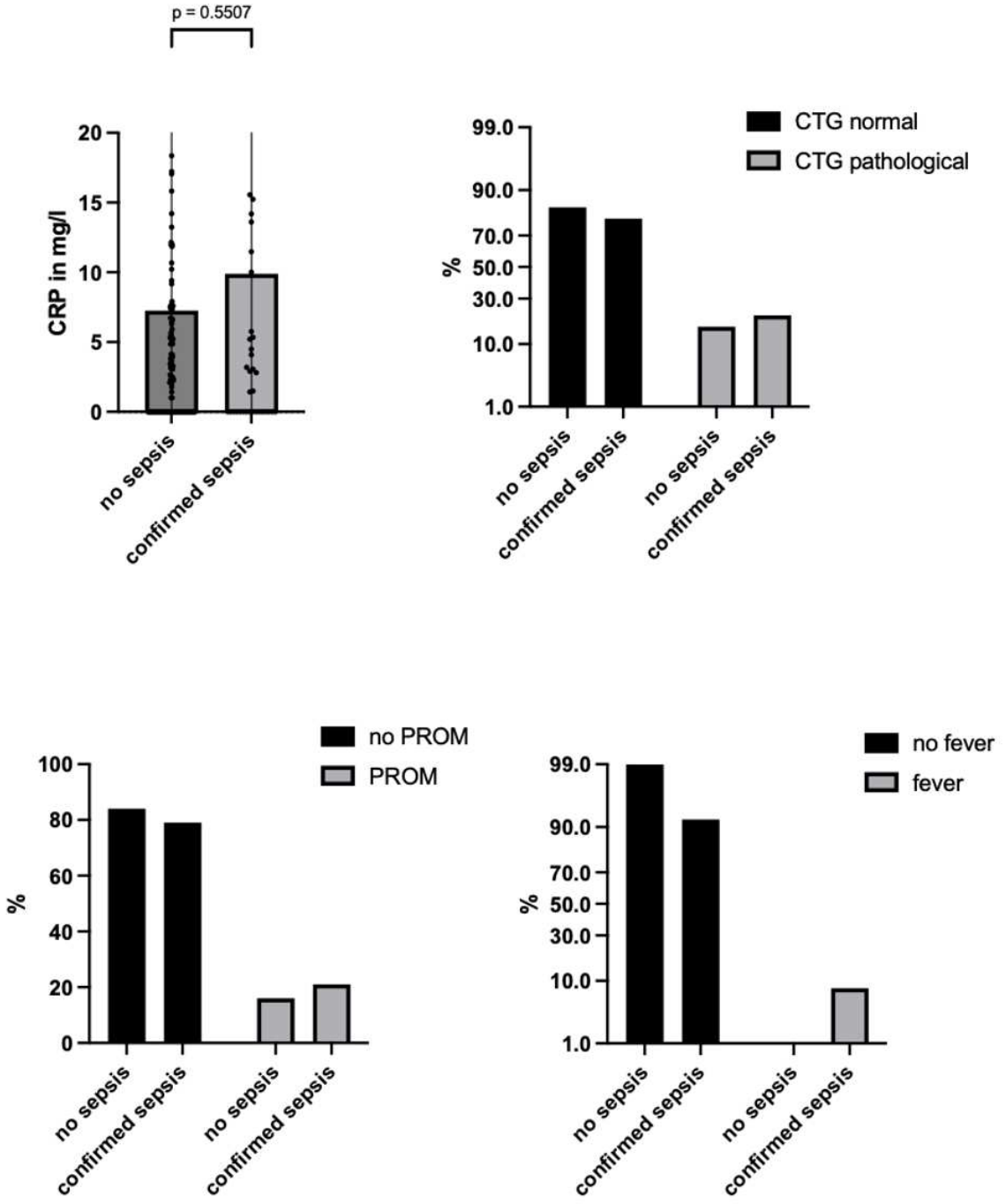


Figure 8: Maternal variables and their respective significances (Statistical Procedure: Mann-Whitney-U, Fisher's Exact Test)

However, investigation of the association between maternal antepartum or peripartum fever was difficult, because out of 158 mother-neonate pairs, fever was only documented in 4 mothers. Since maternal health data besides the birth report is documented in a different system than Orbis, which was used for this study, extended analysis on antepartum fever could not be performed. Data only shows tendencies.

4.5 Analysis of statistical quality criteria

Determination of statistical quality criteria has been performed for variables that showed a significant difference between the two groups “no sepsis” and group “confirmed sepsis”.

Table 15 shows the distribution of all previously tested significant variables among these two groups. For CRP positivity and negativity, a cutoff value of 10mg/L has been used. For differentiation of I/T ratio negativity and positivity, a cut-off value of 0.2 has been used.

Table 15. Frequency distribution of significant variables

	No sepsis		**Confirmed sepsis**	
	Negative	Positive	Negative	Positive
Hemodynamic status	97	22	21	18
Breathing status	60	59	13	26
Neonatal CRP	118	1	27	12
IL - 6	107	12	12	27
I/T ratio	83	9	13	10
Maternal fever	118	1	36	3
CTG	107	12	12	27

Table 16 presents the results from calculation of statistical quality criteria sensitivity, specificity, PPV and NPV. Combined values for most commonly used laboratory variables CRP and IL-6 shows an increase in sensitivity up to 78.7% and combined specificity decreased to 89.1%.

Table 16. Quality criteria of significant variables

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Hemodynamics	46.2	81.5	45.0	82.2
Breathing	66.7	50.4	30.6	82.2
CRP	30.8	99.1	92.3	81.4
IL-6	69.2	89.9	69.2	89.9
I/T ratio	43.4	90.2	52.6	86.4
Maternal fever	7.7	99.2	75.0	76.6
CTG	69.2	90.0	69.2	90.0
IL-6 + CRP	78.7	89.1	44.5	97.4

4.6 Analysis of statistical correlations

Correlation analysis for significant neonatal variables and all maternal variables with the cohort of confirmed sepsis has been performed. For simplified analysis, metric variables have been transformed into a nominal scale (yes/no; negative/positive).

Table 17 presents the results from Cramér's V Test. It shows that maternal fever is the only maternal variable that shows a marginally significant and weak association between the outcome of sepsis and presence of maternal fever during pregnancy or birth. Out of neonatal laboratory data IL-6 shows the highest correlation factor with very high statistical significance.

Table 17. Cramér's V Test results

	Cramér's V value	*P*-value
Hemodynamic status	0.274	<0.001
Breathing	0.248	0.063
Neonatal CRP	0.470	<0.001
IL-6	0.609	<0.001
I/T ratio	0.338	<0.001
PROM	0.013	0.866
Maternal CRP	0.036	0.651
Maternal fever	0.188	0.047
CTG	0.052	0.513

4.7 Analysis of preterm and term group

An analysis of the incidence of sepsis among newborns admitted to the NICU reveals a higher percentage among term infants compared to preterm infants. Specifically, 27 term newborns were diagnosed with sepsis compared to 12 preterm newborns. This indicates that, within the study population with confirmed sepsis, 30.77% were preterm, while more than two-thirds (69.23%) were term. On the other hand, out of the preterm group 13.64% infants were diagnosed with sepsis, whereas 38.57% of term infants were diseased.

Table 18 presents mean of preterm and term group with respective significance results calculated with Mann-Whitney-U Test. There is a significant difference for all analyzed laboratory variables between both groups, with higher mean values for all variables in the term group than in preterm group, representing the fact that in this study, more term neonates were diagnosed with sepsis than preterm neonates.

The same accounts for mean BW, showing a higher value in the population with confirmed sepsis (2932.6g) than without (2638g). For mean BW P=0.016. However, when analyzing BW distribution among "no sepsis" and "confirmed sepsis" differentiated in preterm and term group it shows that in preterm neonates mean BW differs significantly within individuals without sepsis and confirmed sepsis (Table 19).

Table 18. Mean laboratory variables among preterm and term infants

Mean	CRP (mg/L)	IL-6 (pg/ml)	I/T ratio	WBC (/µlx10^9)
Preterm	2.0	165.0	0.10	13.6
Term	5.6	298.5	0.13	18.6
P -value	<0.001	<0.001	0.047	<0.001

Table 19. Mean BW among preterm and term infants in groups of "no sepsis" and "confirmed sepsis"

Mean birth weight (g)	No sepsis	Confirmed sepsis
Preterm	2231.7	1474.6
Term	3356.1	3580.6

Analysis of percentage of statistically significant pathological clinical and laboratory variables group “confirmed sepsis” among preterm and term infants showed, that there is no definite pattern, whether preterm ore term infants are more likely to present with pathological clinical or laboratory variables in case of sepsis. However, due to the small sample size results only show tendencies. This must be reconsidered upon evaluation.

Table 20: Frequency distribution of significant variables in preterm and term infants of “confirmed sepsis” group

	Preterm		**Term**	
	n	**%**	**n**	**%**
Hemodynamic status	5	41.7	13	48.2
Breathing pattern	9	75.0	17	63.0
CRP	4	33.3	8	29.6
IL-6	6	50.0	19	70.4
I/T	3	7.7	7	17.9
Maternal fever	1	8.3	2	7.4
CTG	4	33.3	4	14.8

Additionally, correlation coefficient for neonatal sepsis and low BW has been determined. Cramer’s V test showed a statistically significant correlation with P=0.015 and Cramér’s V result was 0.194, thus correlation is interpreted as weak.

5. DISCUSSION

5.1 Interpretation and evaluation of results in the context of current research

Neonatal sepsis remains a critical challenge in neonatology due to its high morbidity and mortality rates. Early and accurate identification of sepsis is vital for prompt and effective treatment, yet it remains complex due to non-specific clinical presentations and the limitations of diagnostic tests. This study aimed to collect additional data on predictive factors for neonatal sepsis, and both, clinical and laboratory parameters to enhance diagnostic accuracy.

Regarding neonatal characteristics, our data showed that there was a slight predominance of male neonates with confirmed sepsis accounting for 57.55% versus females accounting for 42.41%. These tendencies in gender distribution are in agreement with Salama et al. (96) who showed that, males were at 1.7 times more risk to develop neonatal sepsis compared to females. Rafi et al. highlighted that male neonates have an increased risk as well, but in particular for LONS. However, since this case-control study was conducted in a tertiary care hospital in Bangladesh, this result is probably attributed to a higher incidence of LONS in general, due to poorer hygiene standards in both nosocomial and community environments (97). A notable finding was the high proportion of septic neonates who were born at term (69.23%). This contrasts with current research emphasizing prematurity as a major risk factor for neonatal infections due to immature immune and organ systems (98). However, since this study focused solely on NICU admissions during a specific period, it cannot accurately assess the relative risk of early-onset neonatal sepsis between preterm and term infants. No data was collected on the total number of preterm and term neonates born during the study period who were not admitted to the NICU, making it impossible to calculate the proportion of affected neonates in each group. Thus, the study's results reflect the prevalence of neonatal sepsis among NICU-admitted infants, irrespective of their primary disease (99). Consequently, the higher proportion of sepsis cases among term infants compared to preterm infants does not indicate a higher disease risk in term infants, but rather reflects the likelihood of NICU admissions related to gestational age at birth, which is often due to prematurity alone. This discrepancy also leads to a higher mean BW in patients with confirmed sepsis (2932.6g) than in patients without (2638g), which contradicts the understanding that low birth weight (<2500g) carries a 3.5-fold higher risk for EONS (96).

Analysis of CRP, IL-6, I/T ratio, and TLC among preterm and term infants reveals significant differences, with higher mean values observed in term infants. This supports the hypothesis that preterm infants do not carry a lower disease risk, but rather are more frequently admitted to the NICU due to non-infectious diseases, resulting in lower mean values of inflammatory parameters.

Despite unspecific clinical presentation, most septic neonates exhibited abnormal respiratory patterns (66.7%), including both bradypnea and tachypnea. These observations are consistent with existing literature (100), highlighting the importance of vigilant clinical assessment in the early hours of life. Analysis of the proportion of septic newborns with pathological breathing pattern showed, that 75% of septic preterm infants and 63.0% septic term infants presented with pathological breathing pattern. Moreover, septic infants are 5.288 times more likely to show up with a pathological breathing pattern, although correlation analysis revealed a moderate but non-significant correlation. Sensitivity of pathological breathing pattern, including tachypnea and bradypnea, with regard to the whole study cohort showed the second highest sensitivity with 66.7% and therefore is the most sensitive clinical markers for neonatal sepsis in this study. Breathing related clinical findings can vary from described tachypnea and bradypnea to nasal flaring, retractions, grunting and other signs of respiratory distress. The causes for respiratory distress however are nonspecific and can be associated with a variety of pathologies including meconium aspiration syndrome, pneumonia and pulmonary dysplasia (101). Therefore, despite the comparatively high sensitivity, this finding alone is an insufficient diagnostic tool for neonatal sepsis.

Additionally, the hemodynamic status, assessed via CRT, differed significantly between groups. The likelihood of prolonged CRT was 3.779 times higher in septic infants compared to healthy ones, with a moderate correlation observed between CRT and neonatal sepsis. However, calculated sensitivity was relatively low at 46.2%, with specificity at 81.5%. While CRT is discussed in AWMF guidelines as an important and sensible marker of hemodynamic status in neonates, its relevance for diagnosing neonatal sepsis is limited due to its non-specific nature, but in suspected sepsis it can support further evaluation (22).

Among laboratory markers, IL-6 emerged as the most sensitive for detecting acute infectious events, with a sensitivity of 69.2%, specificity of 89.9%, PPV of 69.2% and NPV of 81.4%. This aligns with existing guidelines for EONS, which report an IL-6 sensitivity of 73%, specificity of 76%, PPV of 56%, and NPV of 85% (22). The correlation coefficient between neonatal sepsis and IL-6 indicated a strong association, representing the role of IL-6 as an early laboratory parameter in acute infections. A systematic review with meta-analysis performed by Shahkar et al. on the role of IL-6 in prediction of neonatal sepsis, states a pooled sensitivity and specificity of 79% and 84%, respectively, and presents IL-6 as an accurate marker in prediction of neonatal sepsis that is not confounded by other variables (102). CRP follows with a

sensitivity of 30.8% and specificity of 99.1%, and although sensitivity is low, is consistent with tendencies of values presented in guidelines with 46% sensitivity and 86% specificity (22). Tessema et al. conducted a study including 899 neonates (104 culture proven sepsis, 160 clinical sepsis, and 625 controls) and could show 89.4% sensitivity, 97.3% specificity, 94.5% PPV, and 98.3% NPV of CRP alone, 24h after suspected infection (103). This aligns with values from current guidelines, where CRP sensitivity increased from 46% to 97%, at 0h and at 24h after first sign of infection, respectively (22). Furthermore, it was reported, that combined specificity decreased, in contrast to combined sensitivity, which can be observed as well in the results of the present study. Publications that focused on CRP, as did Kaur et al. in 2021, included 39 neonates of which 16 had culture-positive sepsis. They state CRP as the most sensitive marker in their study with sensitivity, specificity, PPV, and NPV of 87.5%, 43.2%, 35.9%, and 90.5%, respectively (104).

Prenatal and mother related variables included maternal CRP before birth, presence of PROM, fever during pregnancy or birth, and tachycardic CTG. Statistical analysis of these variables, however, only revealed non-significant associations with PROM and maternal CRP. Results from this study are in contrast to those presented by Salama et al. in a case-control study involving 174 cases and 348 controls. PROM was highlighted as the most influential predictor of neonatal sepsis, with an odds ratio of 5.2 (96). Maternal CRP and its association with neonatal sepsis has been investigated by Jeon et al. showing a sensitivity 71% and specificity 84%, as well as a higher prevalence of neonatal sepsis when maternal CRP was >12.2 mg/L with an odds ratio of 10.68 (105). Therefore, pediatricians should be alerted and aware in case of elevated maternal CRP.

CTG is a promising predictive factor of neonatal sepsis with a sensitivity of 69.2%, specificity 90.0%, PPV 69.2% and NPV 90.0%. Due to the lack of detailed descriptions of observed pathologies in birth reports; distinguishing between tachycardic, dipping, or bradycardic CTG patterns was not possible.

In results of current research, maternal risk factors are among the most important factors in the development of neonatal sepsis. Correlation between intrapartum CTG findings and interleukin-6 levels in the umbilical cord arterial blood was investigated by Di Pasquo et al. (2024). This prospective study could demonstrate an association between “suggestive of fetal inflammation” (SOFI) - CTG pattern and elevated IL-6 levels in umbilical arterial blood. SOFI

was previously defined as a “persistent fetal heart rate (FHR) increase > 10 % compared with the observed baseline FHR observed at the admission” (106). This proves that both, tachycardic CTG and IL-6, are reliable markers of fetal inflammation.

The results presented in this study support the hypothesis, that there is currently no variable that can be used as diagnostic tool for diagnosis of neonatal sepsis alone. However, an ideal diagnostic test would show a sensitivity of 100% to ensure that infected neonates are treated with an appropriate antibiotic therapy, as well as an NPV of 100% to exclude the disease with certainty to prevent healthy neonates from exposure to prophylactic interventions.

5.2 Study limitations

5.2.1 Data evaluation

This study was conducted as a retrospective study. Most of the methodological limitations encountered during data collection and analysis can be attributed to the study design, as no new data could be collected in cases of missing documentation leading to lack of clinical data, errors in laboratory assessment or errors in the process of blood drawing. This problem occurred upon calculation of I/T ratio from differential blood count. A valid differential blood count could not be obtained from 43 newborns, which accounts for 27.22% of the study population. Since blood samples were taken from the study population consisting of newborns, this error can be attributed to the small volume of blood collected. However, quality criteria calculated for I/T ratio therefore cannot be evaluated in the same way as results may be distorted. Evaluating the CTG results also proved difficult. In the delivery room records, CTG results are often described as pathological without further differentiation of the pathology. Therefore, the tachycardic CTG, which is typical for neonatal bacteremia, could only be evaluated with low accuracy as a predictive factor for neonatal sepsis. This must be taken into account when considering it as a prenatal risk factor.

5.2.2 Study population

For this study, data were collected from 158 mother-infant pairs; however, only one newborn showed a positive blood culture, which significantly limits the group we planned to investigate, as no comparisons can be made within this group. Therefore, the newborns were

divided into three groups based on the duration of antibiotic therapy. For the purpose of this study, neonates who received antibiotic therapy for more than three days are considered to have confirmed sepsis, as it can be assumed that clinical and laboratory diagnostic criteria for EONS are met with an antibiotic therapy of more three days. Newborns that received antibiotic therapy of less than 3 days are considered to have no confirmed sepsis or being proven as noninfected, as antibiotic therapy was stopped or never initiated. Statistical significance of encountered results therefore only shows tendencies.

6. CONCLUSION

Despite ongoing research, uncertainty remains about the potential risk factors for neonatal sepsis, with delays in diagnosis and therapy initiation being major contributors to high morbidity and mortality. Although blood culture results are crucial for proper diagnosis and management, they are often delayed and have a low yield. Due to the limitations of blood cultures, laboratory biomarkers are commonly used but are unreliable on their own. This often leads to empiric antibiotic therapy without confirmed diagnosis, due to severe neurodevelopmental deficits and the potentially fatal outcomes that are associated with EONS.

The main challenge is promptly and accurately identifying infected infants, which is often difficult due to the nonspecific nature of clinical signs. An ideal screening test should detect all infected cases (high sensitivity) and have a high negative predictive value to easily exclude the disease. This would ensure that infected infants get a prompt and adequate antibiotic therapy, while avoiding exposure of non-infected neonates to antibiotics, to prevent adverse events that are associated with antibiotic therapy. However, such a test does not yet exist, necessitating a combination of clinical vigilance and multiple diagnostic markers to increase statistical quality of these tests.

These findings highlight the complexity of diagnosing neonatal sepsis, as no single variable or test provides sufficient sensitivity and specificity for a reliable diagnosis of neonatal sepsis. While this study contributes valuable data to the field of research, it also highlights areas for future research. Larger, multi-center studies are necessary to validate these findings and explore additional biomarkers that could enhance the predictive accuracy for neonatal sepsis. Serial measurements as well as combination of biomarkers have already proven to increase diagnostic accuracy, however, this approach is expensive and remains difficult in clinical practice, in particular in neonates. Promising approach for a more rapid diagnosis is offered by molecular diagnostics including PCR, mass spectrometry and gene sequencing. Investigation of genetic and immunological factors that are underlying sepsis susceptibility could also provide deeper insights into individual risk profiles.

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8. SUMMARY

Introduction:

Neonates, or infants within their first 28 days of life, undergo significant physiological changes after birth, which makes them particularly susceptible to infections, in particular, due to their immature immune systems. The focus is on understanding the early onset of sepsis, which is a severe condition caused by bacterial infections that can lead to high morbidity and mortality in neonates.

Objectives:

The primary aim of the study is to identify the incidence of bacteremia in neonates at the time of NICU Level I admission and to determine the predictive factors that contribute to its occurrence. The study also seeks to test the hypothesis that certain maternal and neonatal factors are significantly associated with the risk of bacteremia.

Materials and Methods:

Relevant data of 158 neonates born during the study period and their mothers were obtained from their case records. A diagnosis of early onset sepsis was established based on antibiotic treatment duration or positive blood cultures. Statistical analyses, including Mann-Whitney U test, odds ratio, and Fisher's exact test, were utilized to analyze the data.

Results:

Only one positive blood culture out of 145 tested cultures was positive for streptococcus agalactiae. Antibiotic therapy duration showed 62.0% no therapy, 13.3% therapy <3 days and 24.7% therapy >3 days. CRP and IL-6 were highly significant indicators of neonatal sepsis (CRP: $P<0.001$; IL-6: $P<0.001$). Combined CRP and IL-6 showed increased sensitivity (78.7%) and high, but decreased specificity (89.1%). Out of maternal factors CTG pathologies showed the highest significance ($P<0.001$, OR=20.06), while maternal fever was only marginally significant ($P=0.0468$).

Conclusions:

The study concluded that early identification of bacteremia in neonates is crucial for timely treatment and improved outcomes. Specific maternal and neonatal risk factors can help predict the likelihood of bacteremia, aiding in the early diagnosis and management of neonatal sepsis. The findings underscore the importance of vigilant monitoring and appropriate therapeutic interventions in the NICU to mitigate the risks associated with neonatal bacteremia.

9. CROATIAN SUMMARY

Naslov:

Novorođenčad, ili dojenčad unutar prvih 28 dana života, prolazi kroz značajne fiziološke promjene nakon rođenja, što ih čini posebno podložnima infekcijama, osobito zbog nezrelog imunološkog sustava. Fokus je na razumijevanju ranog nastanka sepse, što je ozbiljno stanje uzrokovano bakterijskim infekcijama koje može dovesti do visoke morbidnosti i mortaliteta kod novorođenčadi.

Ciljevi:

Primarni cilj studije je identificirati učestalost bakterijemije kod novorođenčadi u trenutku prijema na NICU razine I i utvrditi prediktivne čimbenike koji doprinose njenom nastanku. Studija također nastoji testirati hipotezu da su određeni majčinski i neonatalni čimbenici značajno povezani s rizikom od bakterijemije.

Materijali i metode:

Relevantni podaci o 158 novorođenčadi rođenih tijekom razdoblja studije i njihovim majkama dobiveni su iz njihovih medicinskih kartona. Dijagnoza ranog nastanka sepse postavljena je na temelju trajanja antibiotske terapije ili pozitivnih krvnih kultura. Statističke analize, uključujući Mann-Whitney U test, omjer izgleda i Fisherov egzaktni test, korištene su za analizu podataka.

Rezultati:

Samo jedna pozitivna krvna kultura od 145 testiranih kultura bila je pozitivna na *Streptococcus agalactiae*. Trajanje antibiotske terapije pokazalo je da 62.0% nije imalo terapiju, 13.3% je imalo terapiju <3 dana, a 24.7% terapiju >3 dana. CRP i IL-6 bili su vrlo značajni pokazatelji neonatalne sepse (CRP: $P<0.001$; IL-6: $p<0.001$). Kombinirani CRP i IL-6 pokazali su povećanu osjetljivost (78.7%) i visoku, ali smanjenu specifičnost (89.1%). Od majčinskih čimbenika, CTG patologije su pokazale najveću značajnost ($P<0.001$, OR=20.06), dok je majčina groznica bila samo marginalno značajna ($P=0.047$).

Zaključci:

Studija je zaključila da je rano prepoznavanje bakterijemije kod novorođenčadi ključno za pravodobno liječenje i poboljšanje ishoda. Specifični majčinski i neonatalni čimbenici mogu pomoći u predviđanju vjerojatnosti bakterijemije, što pomaže u ranoj dijagnozi i upravljanju neonatalnom sepsom. Nalazi naglašavaju važnost budnog praćenja i odgovarajućih terapijskih intervencija u NICU-u kako bi se smanjili rizici povezani s neonatalnom bakterijemijom.