DIAGNOSTIC UTILITY OF PROCALCITONIN IN EARLY DIAGNOSIS OF NEONATAL SEPSIS IN A TERTIARY CARE CENTER: A CROSS SECTIONAL STUDY

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ABBREVATIONS

APGAR	-	Appearance, Pulse, Grimace, Activity and Respiration
CBC	-	Complete Blood Count
CNS	-	Central nervous system
CONS	-	Coagulase negative staphlococci
ELBW	-	Extreme low birth weight
EOS	-	Early onset sepsis
GBS	-	Group B streptococcus
hsCRP	-	High sensitivity C-Reactive Protein
IL-6	-	Interleukin – 6
IQR	-	Inter Quartile Range
LOS	-	Late onset sepsis
LSCS	-	Lower segment Caesarean section
LBW	-	Low birth weight
NICU	-	Neonatal intensive care unit
NVD	-	Normal birth weight
NS	-	Neonatal sepsis
Pro-CT	-	Procalcitonin
PCT	-	Procalcitonin
PROM	-	Premature rapture of membrane
SGA	-	Small gestational age
WBC	-	White blood cells
VLBW	-	Very low birth weight

ABSTRACT

DIAGNOSTIC UTILITY OF PROCALCITONIN IN EARLY DIAGNOSIS OF NEONATAL SEPSIS IN A TERTIARY CARE CENTRE: A CROSS SECTIONAL STUDY

Background.

Neonatal Septicaemia is the leading cause of neonatal mortality and morbidity in developing countries like India. Neonatal sepsis diagnosis is a challenge because of its nonspecific presentation together with low sensitivity of the time-consuming bacterial cultures. So, many sepsis markers, like Procalcitonin (PCT) and high sensitive C-reactive protein (hs-CRP), are emerging to improve its diagnosis.

Objective.

- Estimation of Procalcitonin, hs-CRP and blood culture in neonatal sepsis patients.
- Correlation of Procalcitonin, hs-CRP with automated blood culture, gestational age and mode of delivery

Methods.

This hospital based cross-sectional study was conducted on 101 neonates with sign and symptoms of sepsis enrolled from the neonatal intensive care unit (NICU) of Paediatric Department, KLE's Dr. Prabhakar Kore Hospital and MRC in Belagavi, Karnataka. Blood cultures for these neonates were done before starting antibiotics. Also, biomarkers PCT and hs-CRP were analysed using fluorescence immunoassay method and immunoturbidimetric methods, respectively. Data were analysed using SPSS 27 statistical software.

Results.

Among 101 neonates, 58 cases (57.40%) were male and 43 (42.60%) were female. In our study, serum PCT levels showed the higher sensitivity (75%), specificity (72.4%), PPV (73.1%), NPV (74.3%) and hs-CRP having higher sensitivity (77.8%) in neonates suffering from sepsis. Elevated PCT levels were significantly associated with male preterm birth. The most common isolated organisms were Klebsiella (64.4%), followed by S.haemolyticus (25.7%) and S.pneumoniae (5%).Highest median levels of both PCT and hs-CRP in case of Klebsiella pneumoniae indicating PCT well correlated with it.

Conclusion.

Procalcitonin was found to be domineering indicator including high sensitivity C - reactive protein as a predictor of neonatal sepsis with sensitivity, specificity, and other indicator of good diagnostic marker were established. In addition Procalcitonin and high sensitivity C - reactive protein levels correlated well with Klebsiella Pneumoniae, indicating use of Procalcitonin as an alternative marker for culture. This has also paved way for further studies with large sample, multicentre studies to refine the biomarker, use and validate its efficacy across diverse neonatal populations for diagnosis of neonatal sepsis instead of culture.

The merits and demerits of these biomarkers in early recognition and management of neonatal sepsis must be readily available information to the neonatologist for immediate management of neonatal sepsis to prevent mortality and morbidity.

KEYWORDS: Neonatal sepsis, procalcitonin, hs-CRP, Blood culture, EOS

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

INTRODUCTION

Sepsis is a potentially fatal illness and sepsis in new-born babies is called Neonatal Sepsis (NS). It is a common paediatri problem.[1]Neonatal sepsis is a widespread bacterial infection in new-born infants < 28 days with a positive blood culture. Neonatal Sepsis kills around 1 million neonates annually. Severe sepsis seems to be the main source of mortality, ranging about 8-10% in infants.[2]Its incidence varies with each country and is much higher in developing countries. These infections lead to hospitalization with increase in the financial burden.[3].Early-onset sepsis (EOS) and late-onset sepsis (LOS) are two possible presentations of sepsis in neonates. Birth to seven days is when EOS occurs, and eight to twenty-eight days is when LOS happens. Sepsis in Neonatal Intensive Care Unit (NICU) is documented as 20% in Low Birth Weight infants (LBW), 30% in Extremely Low Birth Weight infants (ELBW). Risk of poor neurological development and its side effects are significantly greater among the infants who develop sepsis[4] .It is an invasive infection and can vary widely in its clinical presentation, from asymptomatic bacteremia to fulminant, life-threatening disease[5.]

Early-onset sepsis (EOS) typically occurs when organisms from the mother's genitourinary tract are transmitted into new-born. These organisms can travel upward and potentially contaminating the amniotic fluid. New-borns can also contact with infection via trans placental spread in the womb or via the birth canal. Group B streptococcus, H. influenza, E. coli, coagulase-negative staphylococcal along with Listeria monocytogenes are common bacterial pathogens that cause early onset sepsis. Onset of sepsis is most rapid in premature neonates.[6]

Late-onset sepsis (LOS) is predominantly an acquired infection (hospital or community). It leads to increased complications and prolonged hospitalization. Lower the gestational age, higher the rate of nosocomial infections in LOS.[7] The prolonged use of parenteral feeding has been found as a substantial risk factor for LOS. This connection is most likely because of necessity for central venous catheters, which can become a source of infection.[7]

Neonatal Septicemia being the major cause of infant mortality and morbidity in underdeveloped nations like India, where the availability of advanced healthcare facilities may be limited. According to (NNPD), National Neonatal Perinatal database (2020) , Incidence of neonatal sepsis is 18/1000 live birth.[8] Hence, early diagnosis and appropriate use of antibiotic treatment in neonatal sepsis is needed. Misdiagnosing sepsis postpones treatment and heightens the risk of mortality. Overusing broad-spectrum antibiotics in patients, whether they have sepsis or not, leads to antibiotic resistance. Therefore, quickly and accurately identifying sepsis is crucial for improved clinical outcomes and reduced medical expenses.

Blood culture is the gold standard procedure for identifying sepsis in neonates. However, because blood cultures require a lot of time and are influenced by various factors, such as when the blood sample is taken, how much blood is collected, whether the baby has previously received antibiotics, and whether live bacteria are present, that they are not very precise in relation to their specificity and sensitivity. Additionally, the delay in receiving blood culture results often results in unnecessarily prolonged broad-spectrum empiric antibiotics courses , exposing patients to potential adverse effects as well as leading to the emergence of antibiotic resistance.[9]

Recent few studies included biomarkers for the early diagnosis of sepsis in neonatal like Procalcitonin (PCT), hs-C-reactive protein (hsCRP), and interleukin-6 (IL-6) etc. Sepsis can be detected using a biomarker called Procalcitonin (PCT). It is the hormone calcitonin's precursor protein. It gets produced and secreted by the thyroid glands of C-cells and participating in the regulation of calcium levels in response to hormonal stimuli[10] and hs-CRP is involved in the body's response during acute inflammation.it is synthesized in the liver. It is the most commonly used biomarker for identification of neonatal sepsis in the NICU, because of its low cost, availability to perform at all the center and easy availability of test results.

Need of the study:

However, specific biomarkers for the hyper inflammation state causing organ dysfunction has not been established.Amongst PCT, hs-CRP, and IL-6, IL-6 has a very short half-life. So, few studies determined PCT and hs-CRP as biomarkers to find out a correction between these levels and blood culture but these studies have some limitations, like did not use automated blood culture, few of the studies used retrospective designs and these studies lagged clinical correlation with the parameters[10]. keeping these points in view ,the following study has been taken up to find out the diagnostic utility of PCT and hsCRP in diagnosis of neonatal sepsis prior to adminstration of antibiotics.

OBJECTIVE OF THE STUDY

- Estimation of Procalcitonin, hs-CRP and blood culture in neonatal sepsis patients.
- Correlation of Procalcitonin, hs-CRP with automated blood culture, gestational age and mode of delivery

REVIEW OF LITERATURE

Neonatal sepsis is a serious condition in new-borns. It takes place when the immune system's response to an infection results in extensive inflammation, which can harm organs and occasionally cause death. Its a major reason for sickness and death worldwide, especially in poorer countries.[3] Neonatal sepsis can cause different infection like blood infections, pneumonia, brain infections (meningitis), and urinary tract infections in babies. In places where healthcare is not easily available, new-borns infections are more common compared to areas with good healthcare. Neonatal sepsis affects about 1 to 5 out of every 1000 babies born alive.[4]

EPIDEMIOLOGY

The study of the distribution and determinants of neonatal sepsis has evolved progressively over time[11.] Since from 1990s, early-onset sepsis (EOS) has declined, because of implementation of universal screening for sepsis in women, who is pregnant and the administration of antibiotic prophylaxis (IAP) during labour. Currently, Escherichia coli is increasingly causing EOS[5,6.]

Early-onset sepsis affects 1 to 5 out of every 1000 live newborns. Although 7% to 13% of all neonates are tested for sepsis, only 3% to 8% of these cases result in positive blood cultures[7] .The decrease rate of positive blood cultures can be attributed to maternal antibiotic administration and the small volume of blood obtained for testing. Premature babies and those weighing less than 1000 grams have a higher risk of sepsis. Additionally, male infants are more prone to developing sepsis[3.] Nearly 70% Gram positive organisms especially CoNS cause LOS, which is frequently associated with invasive procedures like catheterization. Neonates admitted to

hospital after community exposures are at risk for infections caused by Streptococcus species and Staphylococcus aureus.[11.]

CLASSIFICATION OF NEONATAL SEPSIS

It is characterized as early-onset and late-onset based on the time of beginning of the symptoms. Early-onset neonatal sepsis is defined as clinical symptoms occurring within the first 3 days of life (<72 hours), while other studies extend this to the first seven days. Late-onset neonatal sepsis refers to instances diagnosed between the 4th to 30th days of life, or beyond the first seven days .This classification reflects differences in the underlying pathogenesis.[6]

Early-onset sepsis results from pathogens transmitted from the infected mother, which can occur via Trans placental spread, hematogenously route, or ascending infection from the cervix. The onset of sepsis is most rapid in premature neonates.[6]The incidence of EOS is 100 times more favorable in very low birth weight infants, than in full-term infants. Pediatrics caregivers have focused efforts on reducing the risk of EOS[7.]

LOS is primarily an acquired infection, whether from the hospital or the community. It leads to increased complications and prolonged hospital stays. When the gestational age is low, the higher the rate of nosocomial infections.[7]

ETIOLOGY

Neonatal sepsis has different causes, depending on a number of variables. Such as the timing of infection, ongoing hospitalization, prematurity, and also the use of central line catheters.Additionally, cultural values, the quality of life, and antibiotic therapy can influence the causative agents of sepsis[7.]

Typical infections that cause sepsis in new-born's include

- **Gram-positive organisms**: Staphylococcus aureus, Streptococcus species, Coagulase-negative Staphylococcus (CoNS), Enterococcus species, and Listeria monocytogenes.
- **Gram-negative organisms**: Proteus species, Klebsiella pneumoniae, Enterobacter species, Escherichia coli, Citrobacter species, Haemophilus influenza, Acinetobacter species, and Pseudomonas aeruginosa.[7]
- **Anaerobic organisms:** Bacteroides species, Fusobacterium, and Peptostreptococcus.
- **Miscellaneous organisms:** Chlamydia trachomatis, Mycoplasma, and Urea plasma.
- **Congenital neonatal infections:** Rubella, Human Immunodeficiency Virus (HIV), Cytomegalovirus, Varicella-zoster virus, Parvovirus B19, Hepatitis B & C virus, Plasmodium species, Mycobacterium tuberculosis complex, Toxoplasma gondii, Treponema pallidum, and Strongyloides stercoralis.[7]

Early Onset Sepsis (EOS):

When sepsis first appears, Group B Streptococcus (GBS) and E. coli (Escherichia coli) are the most frequently isolated bacteria. The severity of maternal colonization is directly linked to the neonate's risk of infection. Amniotic fluid contaminated with vernix caseosa or meconium supports the growth of GBS and E. coli.[6] Consequently, a few species in the vaginal vault can multiply rapidly following premature rupture of membranes (PROM), likely contributing to this issue. Bacteria

enter into the bloodstream when there is aspiration and ingestion of infected amniotic fluid by the fetus leading to bacteraemia.[6]

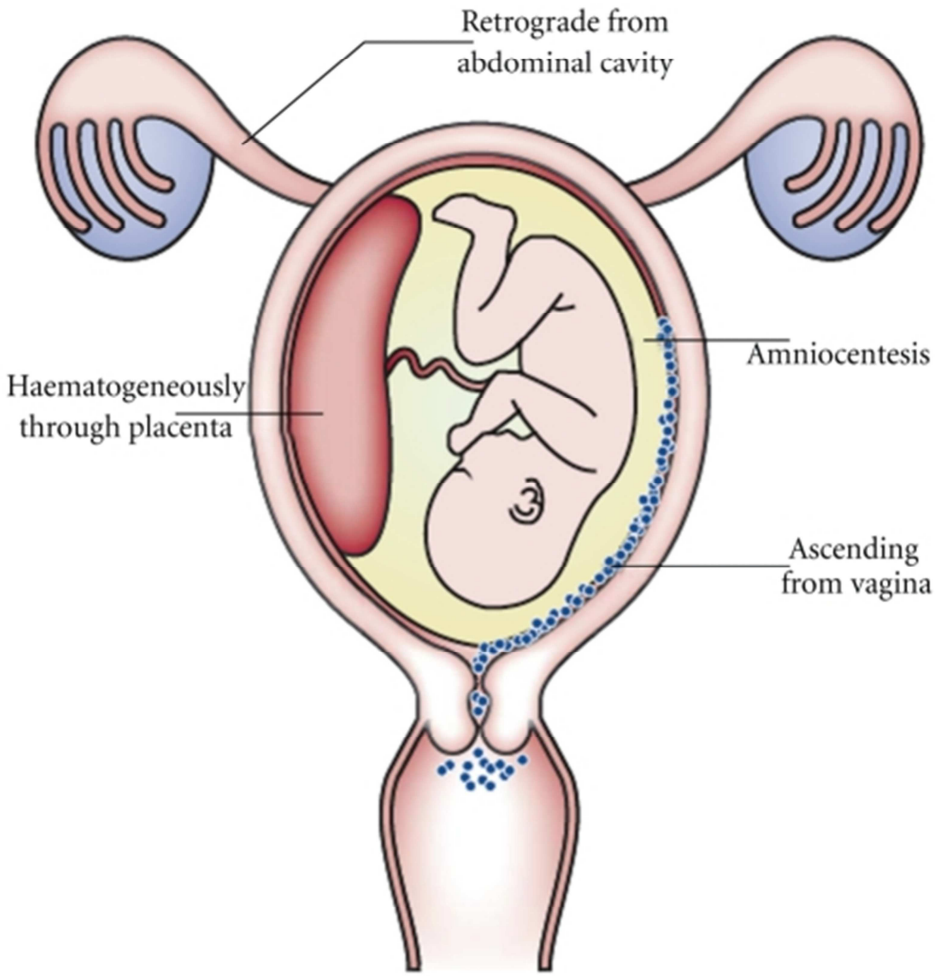


Figure 1 : Potential routes of intrauterine infection in EOS

Some pathogens, such as Rubella, Cytomegalovirus, Toxoplasma gondii, Treponema pallidum, Listeria monocytogenes, and Mycobacterium tuberculosis, can cause maternal infections that spread to the fetus either transplacentally or hematogenously. However, most infections are acquired via ascending infections or as the fetus passes through the infected birth canal during labor.[13]

Other agents of EOS include Viridans Streptococci, Streptococcus pneumoniae, Enterococcus species, and Listeria monocytogenes. Enteric gram-negative bacilli like Klebsiella, Citrobacter, and Enterobacter can also act as pathogens. In premature neonates Haemophilus influenza sepsis has been reported. Neisseria meningitidis has been rarely observed. Asymptomatic gonorrhea can occasionally develop during pregnancy, making Neisseria gonorrhoeae a potential pathogen. The incidence of GBS infections has declined due to the introduction of maternal intrapartum antibiotic prophylaxis in EOS cases.[13,14]

Late Onset Sepsis (LOS)

However it is sometimes connected to vertical transmission, late-onset sepsis is typically an infection picked up in a hospital. Pathogens that are frequently isolated include multi-drug-resistant gram-negative bacilli, Enterococcus species, Coagulase-negative staphylococci (CoNS), and Staphylococcus aureus[12]. Gram-positive bacteria are thought to be the cause of about 70% of LOS incidences particularly CoNS, often associated with invasive procedures such as catheterization. Neonates admitted to the hospital after community exposures are at risk for infections from Streptococcus species and Staphylococcus aureus.[15] decreased vitamin A levels in newborns and their mothers have been linked to a higher risk of late-onset sepsis, which highlights the need of taking vitamin A level assessment and adequate newborn and maternal supplementation into account[44].

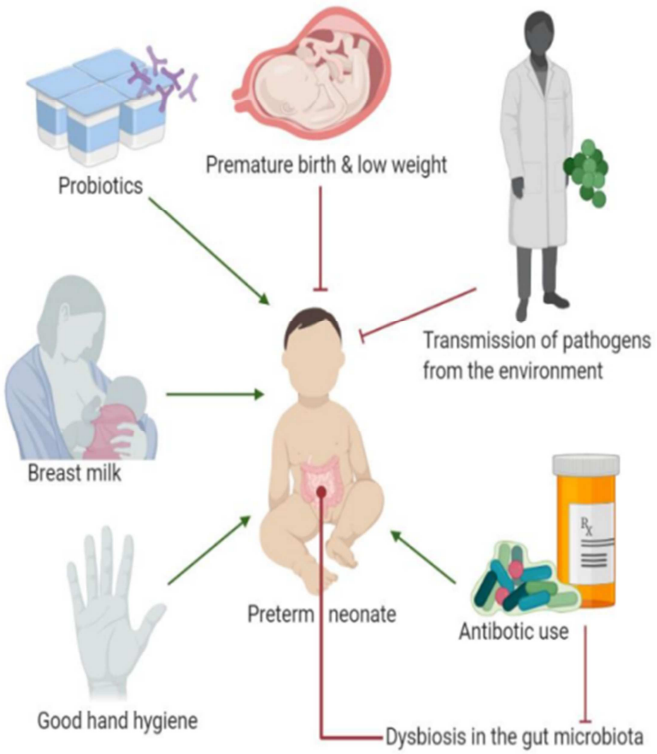


Figure 2: Multifactorial origin of LOS.

In extremely low birth weight (ELBW) infants, E. coli is a significant pathogen of LOS. Other frequently reported gram-negative bacilli include multi-drug-resistant Klebsiella species and Acinetobacter species. Outbreaks of Pseudomonas aeruginosa or hospital-acquired sepsis are often linked to contaminated respiratory equipment.[15]

Other pathogens include anaerobes, with Bacteroides fragilis being a notable causative agent of LOS. Mortality due to Bacteroides bacteraemia has been documented. Candida species are also cause of LOS, having 12% to 18% of cases in ELBW infants. Risk factors for candidemia include abdominal pathology, the use of third-generation cephalosporin's, hyper alimentation, and prolonged use of central intravenous catheters.[16, 17]

Risk Factors

Risk Factors for Sepsis with an Early Onset [14]

Major factors- Chorioamnionitis, low birth weight (less than 1500 grams), Prematurity (birth before 37 weeks), Intrapartum maternal fever (temperature >100.4º F), Premature rupture of membranes (PROM) lasting > 18-24 hours , Sustained fetal tachycardia.[18]

Minor factors are Maternal tachycardia ,Twin or multiple gestations, Tachypnea lasting less than 1 hour, PROM lasting more than 12 hours, Maternal GBS colonization, Low APGAR score (< 5 at 1 minute) Maternal leucocytosis, Maternal urinary tract infection (UTI),Foul-smelling lochia or amniotic fluid, Uterine tenderness, Previous child with GBS infection or sepsis ,Small for gestational age (SGA),Male sex.[18]

Risk Factors Associated with Late Onset Sepsis

Skin injury, invasive procedures, contaminated equipment, intra venous or enteral solutions, prolonged use of intravascular catheters, necrotizing enterocolitis, prolonged antibiotics, artificial feeding, poor sanitary habits, long-term hospitalization, lack of "Skin-to-skin" contact and antacid agents are the risk factors.[19,20,21]

Pathophysiology

Pathophysiology of sepsis is quite complex caused by circulating bacterial or inflammatory microbial products mediated by the release of cytokines. Previously published studies claimed that neonatal sepsis is having multifactorial

pathophysiology and there is no single pathogen, mediator or pathway to drive its pathophysiology of sepsis[31].

Preterm neonates are significantly more vulnerable to sepsis and infections compared to full-term neonates. This heightened susceptibility is primarily attributed to several factors:

Underdeveloped Immune System: Preterm infants have a deficient immune system, largely due to lower levels of IgG antibodies. This deficiency hampers their ability to effectively opsonize pathogens and activate the complement system, both of which are crucial for mounting a robust immune response[32.]

The adaptive immune response in newborns differs significantly from that in children and adults. Neonatal T cells have been classified as anti-inflammatory and toleragenic, with a functioning profile that appears to be programmed into neonatal hematopoietic stem cell (HSC) development[33.]

Immature Innate Immunity: The innate immune system which is the ―first line of cellular defense in preterm neonates is compromised, mainly because their epithelial barriers are not fully developed. This immaturity makes it easier for pathogens to invade and establish infections.These elements work together to increase the risk of infections in preterm new-born's, emphasizing the necessity for proactive infection prevention methods and close observation of this susceptible group.[32]

Adaptive Immunity

T-Cell

- Higher percentage of CD4 T-cells
- Abundant regulatory T-cells
- Predominance of Th17 cells
- Presence of anti-inflammatory and toleragenic T-cells
- Increased susceptibility of T-cells to apoptosis
- Diminished T-cell cytokine response
- Diminished mitogen-induce lymphocyte proliferation
- Diminished B cell receptor signaling
- Low serum immunoglobulin concentrations

Innate Immunity

Neutrophil

Dendritic cell

Monocyte

- Diminished pro-inflammatory response
- Deficiency in cytokine production
- Downregulation of nuclear factor-kB pathway
- Diminished upregulation of TNFα related genes
- Impaired neutrophil adherence, chemotaxis, phagocytosis
- Diminished neutrophil respiratory burst activity
- Delayed release of neutrophil extracellular traps
- Diminished dendritic cell maturation
- Diminished antigen presentation and pattern receptor signaling

Figure 3: Distinct features of the neonatal immunity

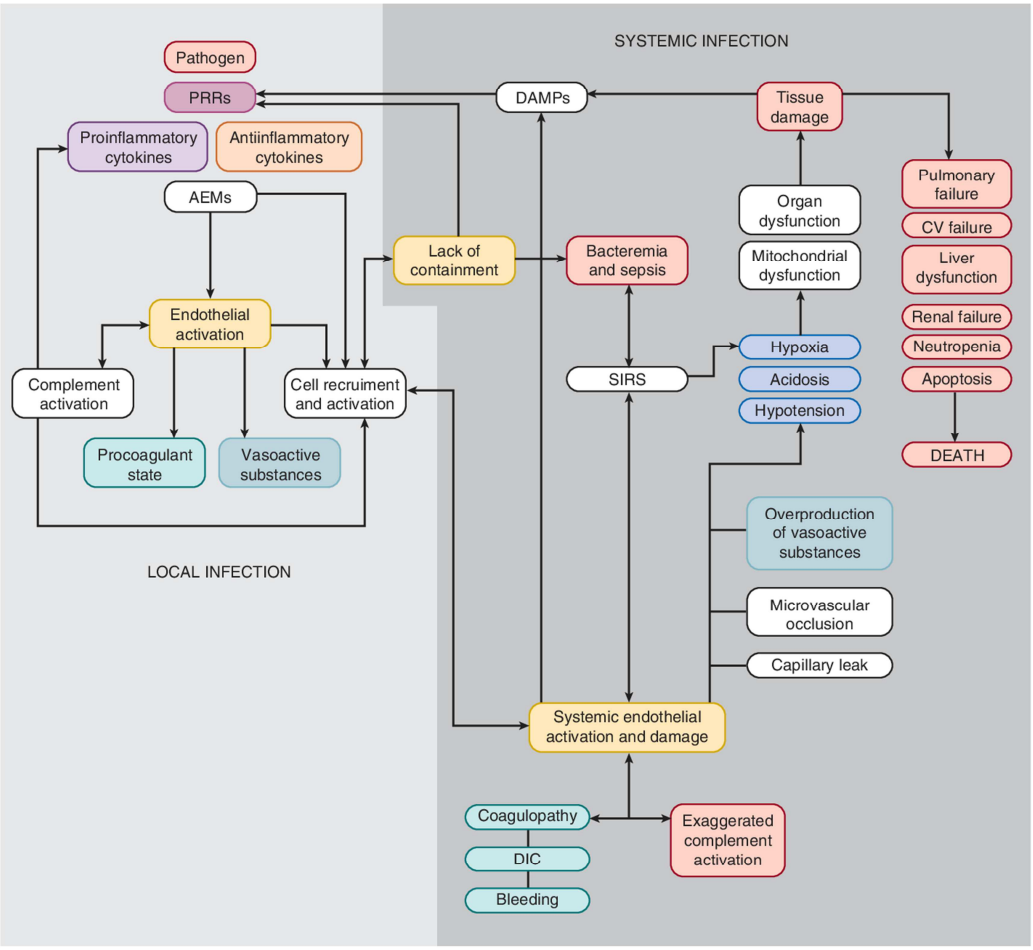


Figure 4: Pathophysiology of neonatal sepsis

New-borns having sepsis because of their underdeveloped immune systems. Key immune cells such as neutrophils, macrophages, and T lymphocytes, which are essential for combating infections, are not fully functional in infants. Additionally, new-borns lack a sufficient quantity of antibodies needed to effectively fight off pathogens. Premature infants, in particular, miss out on the critical time in the womb required to acquire antibodies from their mothers, placing them at an even greater risk for sepsis compared to full-term babies[11.]Immature Neutrophils:-These infection-fighting cells are not fully developed in new-borns. Immature Macrophages:-Another type of cell crucial for battling infections, macrophages are also not fully functional in

new-borns. Immature T Lymphocytes:-These cells, which help coordinate the immune response, are not fully developed in new-borns.Limited Immunoglobulins:-New-born's do not have an adequate supply of antibodies to effectively combat infections. Premature Birth: Babies born prematurely do not have enough time in the womb to receive the necessary antibodies from their mothers, increasing their susceptibility to sepsis.[11]

Clinical Manifestation

Early signs of neonatal sepsis (NS) are generally nonspecific, transient, and indirect, making it difficult to distinguish from infections caused by different organisms or other conditions like Respiratory Distress Syndrome. These clinical presentations are the result of various inflammatory and metabolic processes.

Bacterial sepsis acquired perinatally can quickly lead to death. The initial infection site may be the middle ear, genitourinary tract, paranasal sinuses, gastrointestinal tract, or lungs, and can later spread to the kidneys, joints, meninges, peritoneum, and bones.

Common early signs include anorexia, decreased spontaneous activity, apnea, temperature instability (either hypothermia or hyperthermia), bradycardia, poor sucking, poor appearance, and poor respiration.

A change in body temperature can be a potential indicator of infection. Temperature instability (greater than 99.6ºF or less than 97ºF) can be observed. Infection is associated with fever, and a temperature rise greater than 38˚C is seen in 10–15% of neonates who develop sepsis'[22, 23, 24,25.]

Signs of Neonatal Sepsis

1. Respiratory distress (90%): Tachypnea (>60 in min), apnoea, irregular respirations, flaring or grunting, hypoxia and inflating the nostrils are associated with NS.[24]

2. Gastrointestinal symptoms: Abdominal distention, diarrhoea, splenomegaly, dehydration signs with poor feeding and ileus (intestinal obstruction) are seen.[24]

3. Neurologic symptoms: High pitched cry, tremor or seizure, swelling of fontanel, irritability, lethargy and hyporeflexia or hypotonia are the indications.[24]

4. Cardiovascular signs: Tachycardia and hypotension are common.

5. Skin: Cyanosis, petechiae or purpura, pallor or skin mottling, jaundice and clammy skin are reported.

Many predefined clinical symptoms of infection include requirement of increased oxygen support or ventilator support, reduced perfusion in the peripherals (capillary refill period > 3 sec, increased serum bilirubin, decreased urine production and metabolic acidosis / base deficiency < −10mmol/l).[26]This also involves chronic infections such as septicaemia, pneumonia, osteomyelitis, meningitis, urinary tract infection, arthritis and profound diseases.[27]

Specific Site Infections

- Omphalitis Periumbilical erythema, bleeding, or discharge without a haemorrhagic diathesis
- Meningitis, Brain Abscess, or Encephalitis: Seizures, bulging fontanel
- Osteomyelitis or Pyogenic Arthritis: Decreased movement of an extremity, joint swelling, and tenderness
- Necrotizing Enterocolitis or Peritonitis: Unexplained abdominal distension, especially with bloody diarrhoea[29]

Multi organ failure – heart, lung, kidneys and liver, shock, and cerebral oedema are the short-term complications. The long- term complications include sensory or neurological dysfunction and delayed developmental milestones. [28] Most often sepsis is correlated with congenital heart disease. It can be seen in hemodynamically unstable infants with normal temperature. It affects up to 0.3% of live births. Early echocardiography is recommended.[29]

Diagnosis of Sepsis:

The diagnosis of neonatal sepsis (NS) relies on clinical or microbiological evidence. No single laboratory test can reliably diagnose NS in its early stages; therefore, a combination of laboratory tests is utilized to detect it.[14] Microbiology plays a crucial role in identifying the specific causative organisms. Additionally, other diagnostic methods are based on pathophysiological findings.[14]

- Conventional Blood culture
- CSF culture
- Urine culture
- Biomarkers – PCT, hs-CRP etc.
- Molecular methods

The possible samples for diagnosing NS includes blood, urine, cerebrospinal fluid, umbilical sample like cord blood and tracheal aspiration (rarely).[14]

Conventional Blood Culture:

1. **Blood Collection** - Blood can be collected from various sites such as central veins or umbilical catheters. It's essential to obtain the blood culture specimen through sterile vein puncture. Typically, blood is drawn from a site below the intravenous (IV) line to prevent dilution with infused fluids. Alternatively, blood can be collected from a newly placed sterile umbilical catheter. However, blood cultures may not be accurate from umbilical venous lines, especially after several hours, as they can become contaminated by bacteria on the umbilical stump. The specimen from the catheter should be taken out if sepsis related with the catheter is detected.[14]
2. **Skin Preparation**-The skin should be prepared using 70% isopropyl alcohol followed by an antiseptic solution such as chlorhexidine. Alternatively, clinicians may use iodine tincture and allow the area to dry thoroughly.[15]
3. **Timing of Collection**- Blood should be collected before starting antibiotic therapy to avoid false-negative results.

4. **Blood Volume-**The yield of isolating pathogens is higher with a larger blood volume. The minimum volume required for a blood culture is 1.0mL. However, obtaining a sufficient amount of sample from premature infants can be challenging.[15]

Staining: As a result of bacteraemia, organisms may be observed either independently or in association with polymorph nuclear leukocytes. Detection methods include Gram staining, methylene blue staining or fluorescent staining using acridine orange on the buffy coat.[15]

Blood Culture:

Identifying a specific pathogen offers numerous advantages, notably in selecting the optimal antibiotics and determining treatment duration. Despite being the diagnostic gold standard, obtaining cultures from neonates can be challenging due to low sample volumes and a high proportion of samples resulting in contamination or yielding negative results.[15]

Diagnosis in blood culture is confirmed by isolating a pathogen. Both aerobic and anaerobic organisms should be cultured simultaneously. Pathogens are cultured in blood culture bottles using automated BACTEC™ blood culture systems. Conventional methods, such as plating on media like Blood agar and broth culture like Brain Heart Infusion broth, may also be utilized. Growth typically occurs within 48 hours of incubation in over 90% of positive bacterial blood cultures. For Candidemia, Candida spp. grows on blood agar plates, while Sabouraud's dextrose agar (SDA) should be used if other fungi are suspected.[15]

Cerebrospinal Fluid Culture:

A lumbar puncture is typically reserved for neonates displaying neurological symptoms. In cases where Group B Streptococcus pneumonia could be mistaken for respiratory distress syndrome in the first day of life, lumbar puncture may be considered.[16]

Urine Culture:

Urine culture is essential for assessing sepsis caused by uropathogenic organisms. Urine should be collected using aseptic techniques. The presence of organisms or >5 white blood cells observed in Gram staining of a urine sample may indicate infection. However, the absence of pyuria does not rule out infection. Due to the poor yield, urine culture is not routinely performed.[17]

Other Potential Samples:

1. Umbilical Cord Blood : Early-onset sepsis (EOS) is prevalent in preterm infants, making umbilical cord blood a viable alternative for enhanced detection in high-risk neonates. It offers increased sensitivity and specificity.[18]

2. Tracheal Aspiration Cultures : While beneficial within the first 12 hours of life, tracheal aspiration cultures are not commonly utilized.

Biomarkers:

1. **Definition and Importance**: A biomarker is an objective measure used to indicate normal biological processes, pathological processes, or responses to treatment. The culture of blood is considered to be the gold standard for microbiological diagnosis, however because of its limited sensitivity and lengthy process, therapy may be postponed and complications may arise.[19]
2. **Pathophysiology of Sepsis**: Sepsis is triggered by microbial pathogens and cytokine-mediated responses. Activation of the coagulation and complement cascades occurs after interaction with bacterial proteins, leading to the release of acute-phase reactants, which serve as biomarkers.
3. **Inflammatory Biomarkers**: Improve laboratory diagnosis by the analysis of various inflammatory biomarkers. Acute-phase proteins, cytokines, and chemokines have garnered significant interest in detecting the progression of NS.[20]

Procalcitonin (PCT): In response to bacterial toxins, parenchymal cells such as liver, kidney, adipocytes, and muscle cells release procalcitonin, a type of prohormone (peptide precursor) of calcitonin. This process raises serum levels of procalcitonin (up to 5000fold) in 2–4 hours. On the contrary, procalcitonin levels are reduced in individuals with infections caused by viruses. PCT is widely used for NS diagnosis It is particularly useful for diagnosing early-onset sepsis (EOS) regardless of postnatal and gestational age[19,20.]

Human hsC-reactive protein - hsCRP pentameric acute-phase protein is produced and synthesized in the liver as a reaction to infection. hsCRP has long been a standard biomarker for detecting new-borns sepsis and is still a frequently used test for this

purpose, along with white blood cell counts and differential counts .hsCRP is produced by interleukin (IL-6, IL-1), tumor necrosis factor α (TNFα), and other factors. Bacterial membranes include Phosphocholine, which functions as the main hsCRP receptor. The complement cascade is started by hsCRP when it binds to this receptor, encouraging phagocytosis and the release of inflammatory chemicals.

During an infectious episode in neonates, hsCRP levels begin to rise within 6 to 8 hours and typically peak around 24 hours afterward. Consistently normal hsCRP levels strongly suggest the absence of bacterial sepsis. This correlation supports clinical decisions to discontinue antibiotics in neonates showing overall clinical improvement. Additional inflammatory markers, such as procalcitonin, haptoglobin, and cytokines, can supplement the diagnosis or assess treatment effectiveness. Chest radiography may also be conducted to examine pulmonary manifestations in neonates displaying respiratory symptoms or signs[19,20.]

Interleukin-6 : Interleukin-6 is an immune-regulatory cytokine detected in the bloodstream during the initial stages of infection. Elevated IL-6 levels seen in early bacterial infections may leads to early detection of new-born sepsis[19,20.]

Diagnostic Challenges and Criteria for Biomarkers:

1. Variability in Diagnostic Significance: Immunological markers identifying neonatal sepsis. However, investigations have revealed that their diagnostic significance varies, with fluctuating cut-off values and effectiveness across studies.

2. Other Diagnostic Tests: Additional diagnostic tests, such as presepsin, neutrophil CD64, and surface antigens chemokines, have been explored. However, further research is needed to establish their routine use.[19,20]

3. Criteria for Diagnostic Biomarkers: A reliable diagnostic biomarker should meet certain clinical criteria:

    - It should have a defined cut-off value, high sensitivity, and negative predictive value for ruling out disease.
    - It should enable early detection and identification of pathogen categories.
    - It should assess disease progression and guide antibiotic therapy.
    - It should predict disease severity and prognosis.

4. Current Limitations: Currently, no single marker is sensitive and accurate enough to guide antimicrobial treatment decisions across medical settings. While some cell surface markers show promising results in experimental settings, their practical use in clinical practice is limited by the requirement for specialized tools and skilled personnel.

Other Diagnostic Approaches:

a) Biochemistry Analysis:- Blood glucose measurement for hypoglycaemia treatment, creatinine and blood urea nitrogen for acute renal failure assessment, lactic acid test as a marker for septic shock severity, arterial or venous blood gas analysis for hypoxia and metabolic acidosis detection, and liver function tests for suspected sepsis-associated cholestasis.[21]

b) Pathological Findings:- WBC count (raised > 25000/mm3 or decreased < 5000/mm3) and immature to total neutrophil ratio more than 0.2 are included in the CBC.[22]Neutropenia is a better sepsis indicator than neutrophilia, particularly when obtained after 6 hours of life. Platelet count may indicate thrombocytopenia but is not useful for assessing NS.[21]

c) Role of Electrocardiogram and Imaging: ECG is indicated for tachycardia and cardiac auscultation findings. Chest X-ray and CT head may be useful for pneumonia and subdural hematoma assessment.[21,22]

Differential Diagnosis: Viral, fungal, or parasitic infection; Prematurity and its consequences, such as respiratory distress syndrome and intraventricular hemorrhage

Neonatal encephalopathy, metabolic illness, meconium aspiration, transient Tachypnea, congenital heart disease, and inflammatory biomarkers

Treatment / Management

Immediate initiation of antibiotic therapy is crucial upon clinical suspicion of sepsis, even in the absence of confirmatory laboratory results[30.]Antibiotic selection should be depends upon antimicrobial resistance patterns.

Early-onset sepsis (EOS) - intravenous ampicillin and aminoglycosides is typically prescribed to cover common pathogens.

Late-onset sepsis (LOS) - A combination therapy of vancomycin and an aminoglycoside is recommended for these cases.

Due to their limited penetration into the central nervous system (CNS), aminoglycosides may not effectively treat CNS infections. In such cases, consideration should be given to using a third-generation cephalosporin. Caution is advised with the use of ceftriaxone due to the risk of hyperbilirubinemia and the formation of calcium-ceftriaxone crystals. The escalating issue of antibiotic resistance underscores the importance of antibiotic stewardship teams in preventing unwarranted and prolonged antibiotic use in neonatal sepsis.

Prognosis

Babies born too early have a higher chance of dying compared to full-term babies. E. coli infection can be more deadly than GBS infection. Giving antibiotics during birth for GBS has helped reduce deaths from GBS. Treating babies suspected of having sepsis, even if their blood tests are negative, has also lowered death rates. Premature babies with sepsis might have problems with their brain development or vision. Giving certain antibiotics can also cause hearing and kidney problems in these babies.

Complications

Neonatal sepsis continues to cause severe morbidity and death in newborns. Prematurity and delayed medical care is frequently related with poor results. VLBW newborns have a higher risk of chronic lung illness, as well as extremely low birth weight infants are more likely to have neurodevelopmental issues such as hearing and visual deficiencies, cerebral palsy, and poor psychomotor and mental development . However, excessive antibiotic usage can raise the risk of severe candidiasis and multidrug resistance organisms.[34]

Procalcitonin

In 1975, Moya F et al. proposed the existence of a calcitonin precursor in chicken. The enormous biosynthetic molecule breaks intracellularly to produce the hormone, which they termed procalcitonin.[35] Allison's (1981) work on RNA extracted from human medullary cancer indicated that calcitonin is synthesized as a precursor protein molecule in humans[36]. Later investigations reveal that calcitonin is released following a series of Co and post-translational modifications such as glycosylation, proteolytic cleavage, and so on[37]. In 1993, French authors demonstrated that Procalcitonin (PCT) may discriminate between bacterial infections and non-bacterial inflammatory disorders.. PCT, a recently re-evaluated biomarker, meets many criteria for diagnostic accuracy, especially when compared to traditional biomarkers. It has demonstrated superior diagnostic accuracy across various infections, including sepsis.[30]

PCT structurally having 116 amino acid protein and cleaved into 3 different molecules;active calcitonin (32 amino acid), katacalcitonin (21 amino acid) and N-terminal procalcitonin (57 amino acid). [46,47,48,95]

- Molecular Formula[98] - $C_{153}H_{228}N_{40}O_{47}S_3$

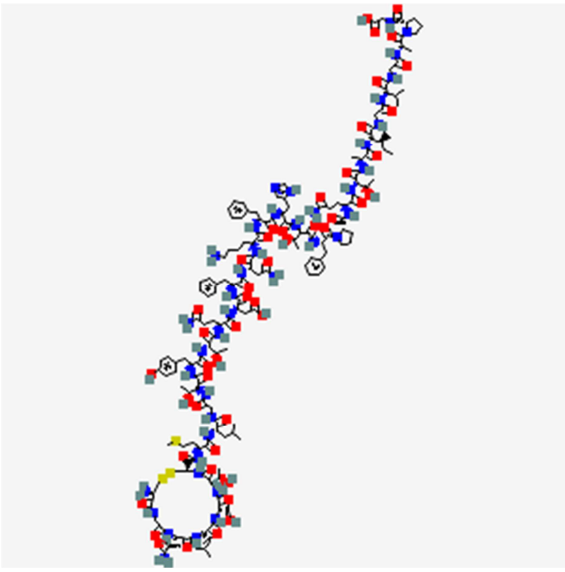


Figure 5: Chemical Structure of Procalcitonin

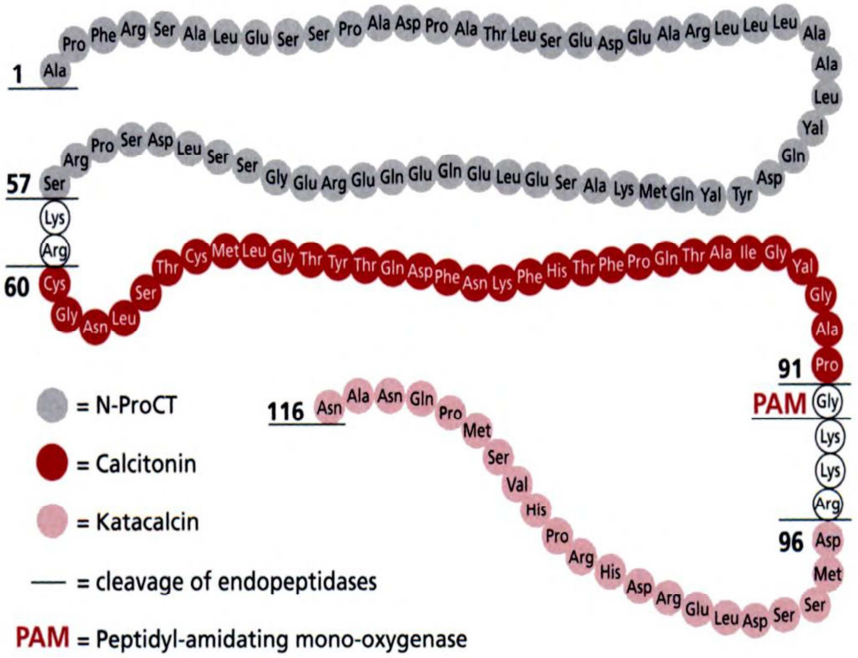


Figure 6: Structure of Procalcitonin

PCT, calcitonin precursor, is created by many parenchymal cells such as the liver, kidney, adipose tissue and muscle cells.. Its release is triggered by bacterial toxins within 2 to 4 hours, serum levels increase dramatically (up to 5000-fold). Conversely, PCT levels are suppressed in patients with viral infections[50]

Serum PCT levels in healthy persons are generally relatively low, at 0.01 µg/L. When exposed by pro-inflammatory triggers, particularly of endotoxins i.e. of bacterial origin, then PCT levels increase rapidly within 2 - 4 hours. The concentration high within 6 - 8 hours after exposure and remains increased for up to 48 hours after the stimulus is removed, making PCT an early rising biomarker.PCT synthesis is prompted by IL-6, IL-1β, and TNF-α, which also stimulate hsCRP production. Additionally, bacterial lipopolysaccharides can directly stimulate PCT production.

Conversely, interferon-γ, commonly produced during viral infections, downregulates PCT synthesis.[50]

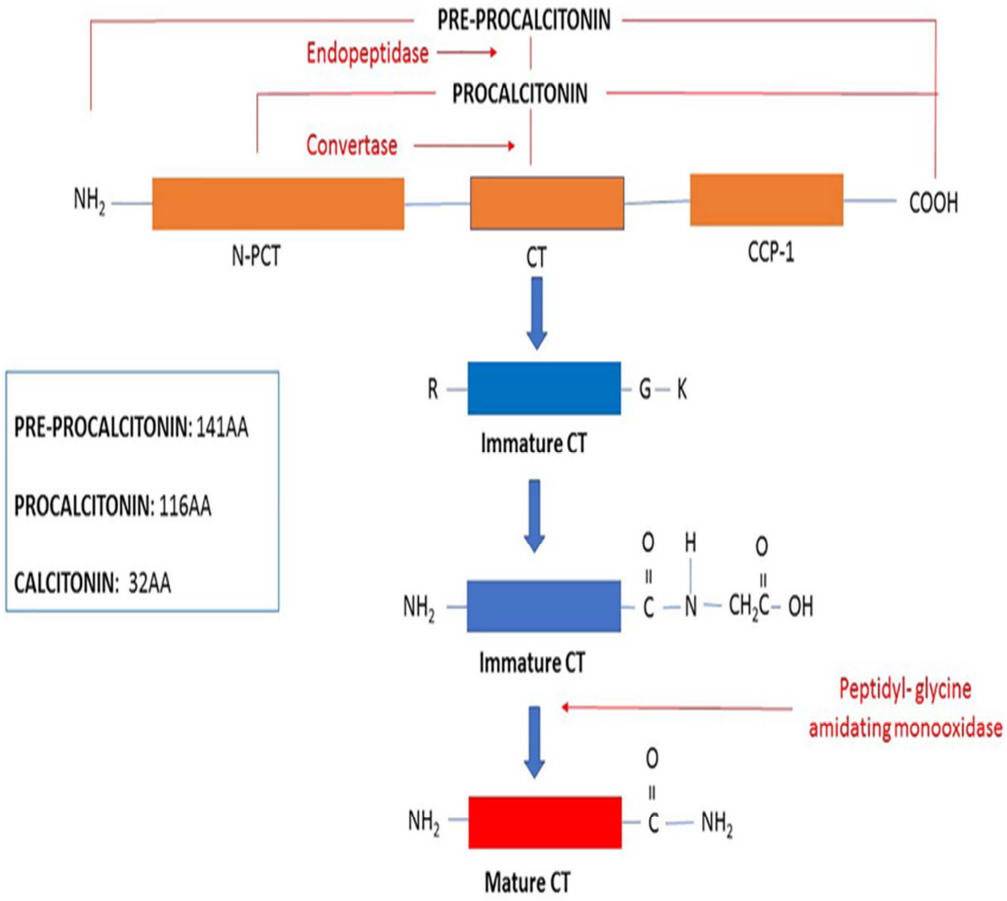


Figure 7: The process of biosynthesis of preprocalcitonin leads to mature calcitonin.

The CT-I transcription product, pre-ProCT, consists of 141 amino acids and is cleaved at the N-terminus. ProCT is made up of 116 amino acids and is converted to CT8 by an amination process. (Figure -5)Glycine terminates the nonaminated, immature 33-amino acid form of calcitonin that is seen in Procalcitonin. Posttranslational processing of Procalcitonin results in the production of immature Calcitonin and several extra free peptides. Additional modifications to the immature forms of Calcitonin provide a protein monomer with a changed core area and a bridge of disulfide joining its various chains. A large amount of the bioactivity of the mature hormones may be due to the amination of its carboxyl terminus.[68]

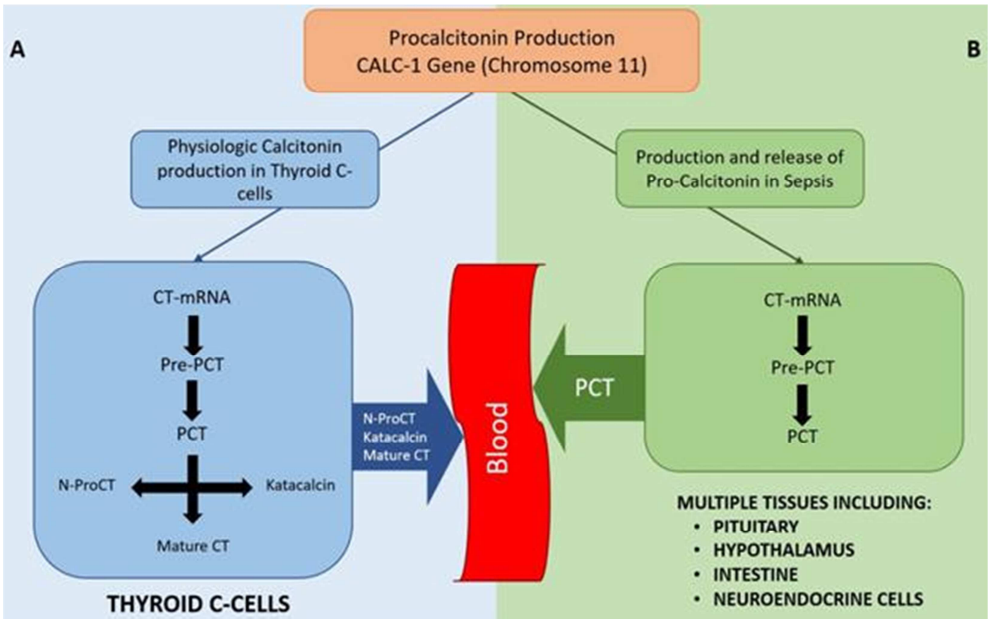


Figure 8: Fate of procalcitonin during inflammation and normal condition[82]

PCT is recognized for a purpose of reliable indicator for bacterial origin infection. However, similar to hsCRP, PCT levels in healthy new-born's experience a spontaneous increase after birth, peaking around 24 hours of age and gradually decreasing by 48 to 72 hours, although this timeline may vary based on gestational age (GA).

Preterm neonates exhibit a earlier, more pronounced, as well longer durationof PCT response compared to term neonates.

PCT's usefulness is hindered by its elevation in various non-infectious perinatal conditions, making it It is difficult to distinguish between viral and causes that are not infectious purely based on PCT levels. PCT has a shorter biological half-life of 22 to 26 hours compared to hsCRP and other acute-phase reactants, making it advantageous for early diagnosis.PCT overcomes hsCRP in EOS, showing 92% sensitivity, 97% specificity, 94% positive predictive value, and 96% negative

predictive value.PCT shows a more rapid response to early infection compared to CRP, aiding clinicians in the diagnostic process.PCT remains elevated longer than other biomarkers like TNF-alpha and IL-6, which enhances its ability to predict infection severity and treatment response.

PCT readings can also be mistakenly raised in non-infectious situations such cerebral hemorrhage, birth asphyxia, and newborn hypoxemia.

Positive PCT levels (>2.5 ng/mL) when combined with CRP considerably boost the diagnosis of bacterial infection, whereas negative CRP levels (<40 mg/L) improve the test's negative predictive value, which helps predict clinical outcomes and guide decisions about antibiotic therapy.

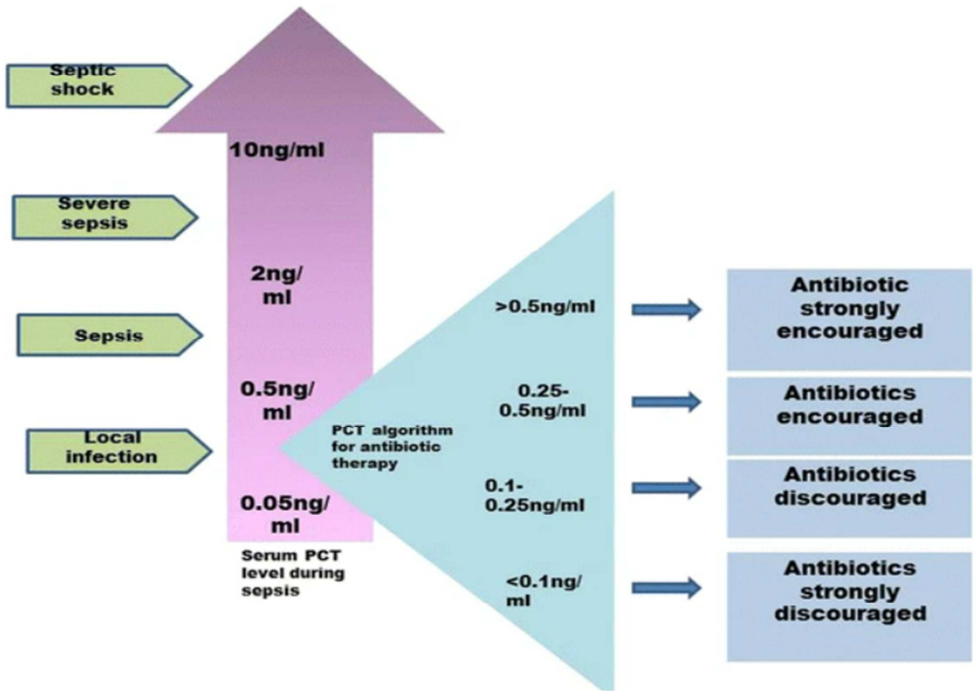


Figure 9: PCT algorithm for antibiotic therapy

The enforcement of antibiotic management helps to prevent needless prescriptions for antibiotics while also ensuring therapeutic efficiency [54]. Inappropriate medication use in patients can contribute to the emergence of antibiotic resistance [55]. A large number of neonates are treated with antibiotics for presumed infection despite having no organism detected in their blood cultures[41].It is necessary to decrease 'blind' drug prescription in order to prevent the development of secondary infections to antibiotics and an increase of drug resistance. Early detection of pathogens allows initiation of appropriate antimicrobial therapy that strongly correlates with positive outcomes[42] . The PCT is a superior choice than other markers that meet these requirements.

High sensitive C-Reactive Protein (hsCRP)-

The plasma contains a pentameric protein known as C-reactive protein (CRP), which has an annular (ring-shaped) structure. It is defined by a discoid arrangement of five identical subunits that are not covalently bonded; these subunits have a molecular mass of around 23 kDa and are each 206 amino acids long[39].

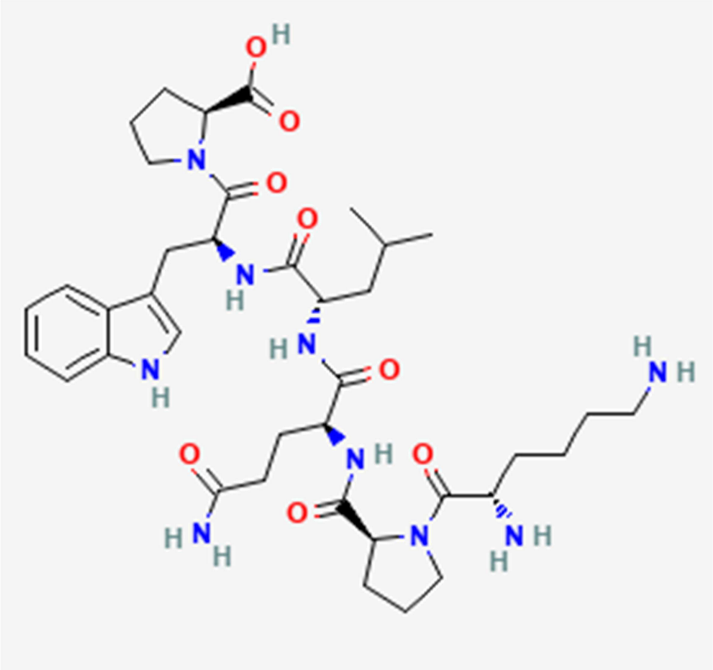


Figure 10: Chemical Structure of hsCRP

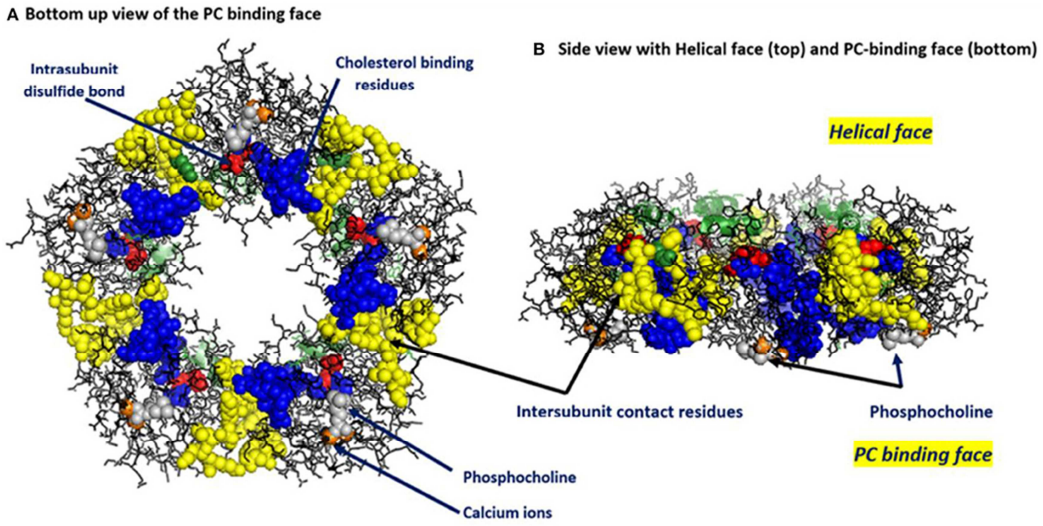


Figure 11: Structure of hsCRP

The liver produces CRP, a chemical that is involved in acute phase response. Increased CRP production can result from both infectious and non-infectious disorders, including cancer and inflammatory conditions[43].

When inflammation occurs, its concentration in the blood rises. After macrophages and T cells secrete interleukin-6, this liver acute-phase protein is released in greater amounts. The liver produces hsCRP[56] in reaction to substances secreted by adipocytes, T cells, and macrophages.[57] It belongs to the family of proteins called pentraxins.[56] It has nothing to do with blood coagulation or protein C (C-peptide, or insulin). The first known pattern recognition receptor (PRR) was hsC-reactive protein.[16]

Phosphocholine, which is expressed on the surface of bacteria like pneumococcus bacteria, is bound by hsCRP. This triggers the complement system, encouraging macrophage phagocytosis, which destroys pathogens and cells that are necrotic and apoptotic.[60,61] By doing this, hsCRP also binds to ischemia and hypoxic cells, that possess the capacity to regrow as time passes.

But they are eliminated too soon due to the binding of hsCRP[**23,24**] Antibodies also attach to the Fc-gamma receptor IIa, which is bound by the prehistoric antibody hsCRP. Furthermore, hsCRP binds to C1q, activating the classical complement pathway[58,59]. hs-CRP, thus, similarly to IgG antibodies, produces immunological complexes.

Hs-CRP levels between 2 and 10 mg/L are regarded as indicative of metabolic inflammation. Hs-CRP readings can be measured and graphed to help track the progression of a disease or evaluate how well a treatment is working. ELISA, nephelometry, immunoturbidimetric, and radial immunodiffusion 40 low: hs-CRP concentration less than 1.0 mg/L; average: 1.0 to 3.0mg/L superior to 3.0 mg/L

"hsCRP is the most widely studied marker of inflammation in cardiovascular disease, and its levels have been shown to predict future cardiovascular events in a variety of populations[40]."

The most researched acute-phase protein in EOS is high sensitive C-reactive protein (hs-CRP), even with the emergence of additional biomarkers[45].

MATERIALS AND METHOD

Source of Data

The present study comprises of Neonates [0-28 days] admitted in NICU, Pediatrics Department of KLES Dr. Prabhakar Kore hospital and MRC, Belagavi, Karnataka.

Study Design

A cross-sectional study conducted in a hospital.

Study Period

Period of one year from June 2022 to June 2023

Sample Size

Sample size at 95% Confidence Interval, 20% tolerable error and 5% attribution.

$$n = \frac{Z^2(1-\alpha/2).SD^2}{(20\% \text{ of SD})} \times 1.05$$

n = 100.84

n =101

Study Population

101 newborns admitted to the NICU at KLE's Dr. Prabhakar Kore Hospital and MRC in Belagavi, Karnataka, are part of the Pediatric Department.

Criteria for Patient Selection: -

The study included newborns who were suspected of having sepsis or who were brought to the NICU within 28 days after birth after exhibiting sepsis-related signs and symptoms.

Inclusion Criteria:

Neonates with suspected sepsis admitted to NICU.

Parents who give consent for the neonates.

Exclusion Criteria:

Neonates who were on antibiotics,

Neonates with history of congenital anomalies in the baby.

Authority's approval

All relevant authorities were consulted, including the Jawaharlal Medical College Belagavi Institutional Ethics Committee on Human Subjects Research, which granted permission to perform the study.

(Ref No. MDC/JNMCIEC/344) (Annexure-I)

Head of department, Pediatrics , KLES Dr. Prabhakar Kore hospital and MRC, Belagavi, Karnataka.

OBTAINING INFORMED CONSENT –

Informed parental consent was obtained for research participation. (Annexure-II)

SCHEDULING -

The duration of this study was one year. It was undertaken during June 2022 to June 2023

PATIENT INFORMATION -

The clinical data and data about the study participants was obtained using a standardized performa.

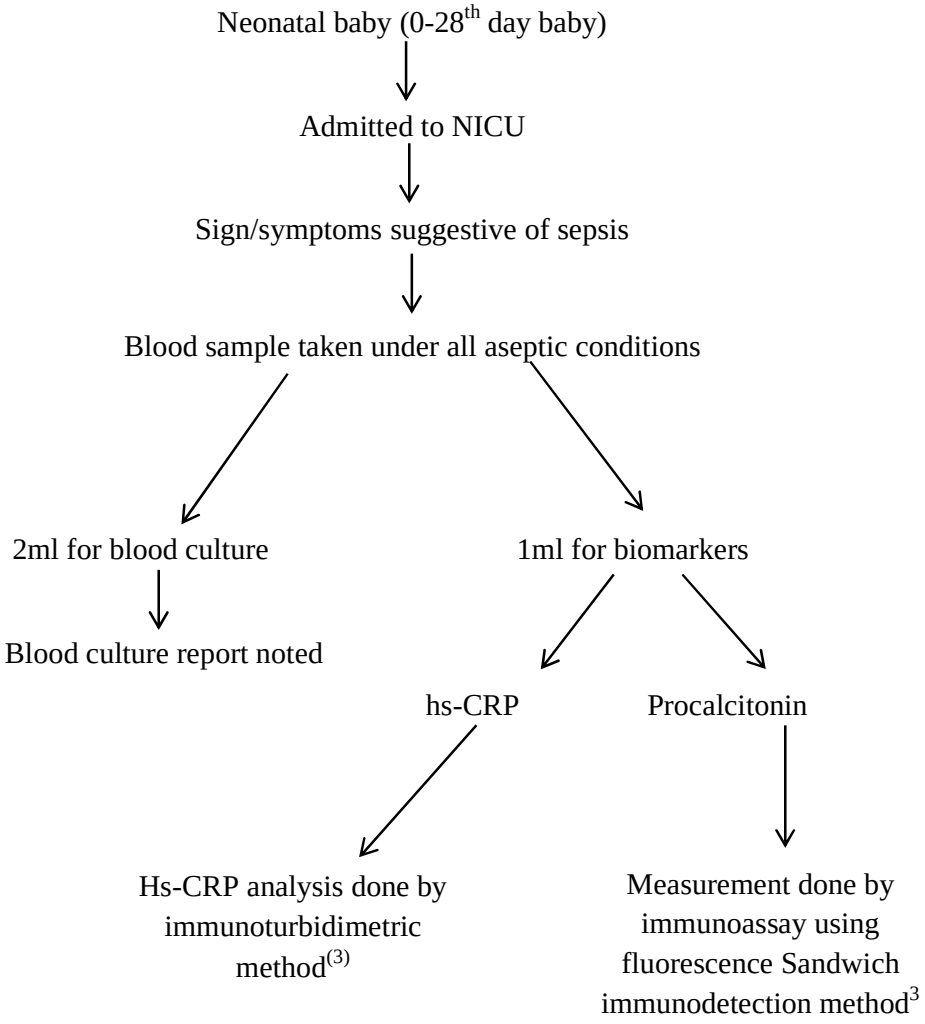
SAMPLE COLLECTION AND PROCESSING -

In this study, two separate blood samples were collected and processed as follows:

1. Whole blood for blood culture
2. Whole blood for PCT and hs-CRP

- Blood samples were collected from neonates in the neonatal intensive care units. The first blood sample was collected bedside aseptically in syringe (3 ml) while they secure IV line peripherally from the hand and 2ml was directly inserted in blood culture bottle (BACTEC vial) and was sent to the Microbiology Department for further processing and 1 mL of blood was drawn in the plain vacutainer (Red color) for biomarkers like PCT and hs-CRP.
- Blood culture media (20ml) containing the sample was incubated at 37 ℃ for 5-7 days. After 72 hours the blood culture material was sub-cultured for preliminary finding of the bacterial or fungal growth. Underlying mechanism is because of CO_2 release by the organism fluorescent dye shows alert. If positive growth was observed it was processed. It was further confirmed by standard microbiological tests. The isolates on the agar plates were identified by Colony characteristics, Gram staining and Motility test.

METHODOLOGY



Estimation of Procalcitonin (PCT) by Electrochemiluminescence Immunoassay "ECLIA"

Method- Estimation of Procalcitonin was done using Cobas e immunoassay analyser, which is based on Electrochemiluminescence Immunoassay "ECLIA" technique.

Principle: Electrochemiluminescence Immunoassay "ECLIA" technique.

Sandwich principle

Total duration: 18 minutes.

First Incubation:

Components: The sample comprises 18 µL of antigen, a monoclonal antibody specific to procalcitonin (PCT) that's undergone biotinylation, and another single-label antibody labelled with a ruthenium compound and is specific to PCT. Process: These components combine to create a sandwich arrangement.

Second Incubation:

Micro particles coated with the streptavidin are added to the mixture. Binding: The interaction between biotin and streptavidin enables the sandwich complex to adhere to the solid phase.

Setting Up Measurements: Aspiration: The reaction mixture is placed within a measuring cell. Capture: The microscopic particles adhere to the electrode surface via magnetic capture. Cleaning: ProCell or ProCell M are used to remove any loose materials.

Detection: - Voltage Application: The ruthenium complex emits light through chemiluminescence upon applying a voltage to the electrode. Measurement: A photomultiplier is used to measure the light that is emitted.

Finding the Results: - Calibration Curve: A calibration curve, created especially for the instrument using a master curve and a two-point calibration provided by the reagent's barcode, is used to find the results.

REGENTS:

1. M (Transparent-capped, streptavidin-coated microparticles): 1 6.5 mL container with 0.72 mg/mL of microparticles coated with streptavidin and a form of preservative.
2. R1 (biotin-anti-PCT-Ab, gray capped) : 1 9 mL bottle contains 2.0 µg/mL of biotinylated monoclonal anti-PCT antibody (mouse), mixed with 95 mmol/L of phosphate buffer (pH 7.5) and preservative.
3. R2 (Black cap, Anti-PCT-Ab~Ru(bpy)): 1 9 mL bottle labeled with a ruthenium complex at 5.6 µg/mL of mouse monoclonal anti-PCT antibody, pH 7.5 phosphate buffer at 95 mmol/L, and preservative

Specimen collection of PCT:

Use a sterile needle and syringe to draw 0.5-1 mL of blood, considering the small blood volume in neonates. Transfer the collected blood into an EDTA or serum tube. Gently invert the tube several times to mix if using an EDTA tube. Centrifuge the blood sample at 3000 rpm for 10 minutes to separate the plasma/serum. Load the separated plasma/serum samples, calibrators, and controls into the analyzer. Start the

assay run. The analyzer will automatically perform the steps of sample incubation, reagent addition, and measurement.

Procedure	
Sample size (serum or plasma)	20µL
Reagent 1 volume (Contains biotinylated monoclonal anti-procalcitonin antibody)	50µL
Reagent 2 volume (Contains monoclonal anti-procalcitonin antibody labelled with ruthenium complex)	50µL
First Incubation (After adding Reagent 1)	9 minutes
Second Incubation (After adding Reagent 2)	9 minutes
Addition (R1 and R2 to be added to the sample)	automatic pipetting system
Magnetic Separation and Washing	remove any unbound substances
Measurement (the intensity of the emitted light is proportional to the concentration of procalcitonin in the sample.)	Electrochemiluminescence is measured

Result interpretation- The analyzer provides PCT concentrations in ng/mL. Interpret the results according to the reference ranges and clinical context.

Estimation of high sensitivity C-reactive protein(hsCRP) by Particle enhanced turbidimetric immunoassay (PETIA) technique.[58]

Method- estimation of hsCRP was done using an autoanalyser (SIEMENS dimension clinical chemistry system), which is based on a particle enhanced turbidimetric immunoassay (PETIA) technique.

Principle : particle enhanced turbidimetric immunoassay (PETIA)

Synthetic particles coated with antibody to C-reactive protein (AbPR) aggregate in presence of C-reactive protein in the sample. The increase in turbidity which accompanies aggregation is proportional to the C-reactive protein concentration, which is determined turbidimetrically at 340nm wavelength

CRP + AbPR -------- aggregate (absorbs at 340nm)

Specimen collection:

Normal procedures for collecting and sorting serum were used.samples can be stored at 4 C for analysis within 72hours.samples are stable for upto 6 months if they are frozen at – 20 C within 24hours of collection.repeated freezing and thawing were avoided.very lipemic or frozen samples which become turbid after thawing were clarified by centrifugation before test.

Regents:

Reagent 1 : available in liquid form. It contains Anti-CRP coated particles (0.3mg/mL) , glycine ,SDS,Microbial inhibitors.

Reagent 2 : available in liquid form.it contains Buffer ,PEG and microbial inhibitors.

Reagent preparation : all reagents were liquid and ready to use.

Procedure ;

- -RCRP Flex reagent cartridge is required to perform RCRP test.
- -RCRP Assay using SIEMENS Dimension clinical chemistry system was performed after the method is calibrated using RCRP Calibrator.
- -Sampling, reagent delivery,mixing,processing and printing of results were automatically performed by the SIEMENS Dimension clinical chemistry system.

Test conditions of RCRP

	Cuvette 1	Cuvette 2
Sample size	2 µL	8 µL
Reagent 1 volume	80 µL	80 µL
Reagent 2 volume	168 µL	168 µL
Diluent volume	170 µL	164 µL
Total volume	420 µL	420 µL
Temperature	37 degree C	37 degree C
wavelength	340 and 700nm	
Type of measurement	Bichromatic rate	

RESULTS- the instrument automatically calculates and prints the concentration of hsCRP in **mg/dL.**

Analytical measurement range: 0.05-25.00mg/dL (0.5-250.0mg/dLResults and observation

STATISTICAL ANALYSIS

Data entry was done using Microsoft XL. Statistical analysis was performed using SPSS version 27. Mean, standard deviation, median, inter quartile range (IQR) was computed for numerical variable as well as frequency and percentage computed for categorical variable. Mann-Whitney test was used to check the difference between two medians of two different groups. Then, Kruskal Wallis test was used to compare the group means. Co-relation was also used to check the relationship between two variables. A receiver operating characteristics (ROC) curve was utilized. A p-value of less than 0.05 was considered statistically significant. The sensitivity, specificity, positive predictive value (PPV) ,negative predictive value (NPV) of PCT and hsCRP evaluated.

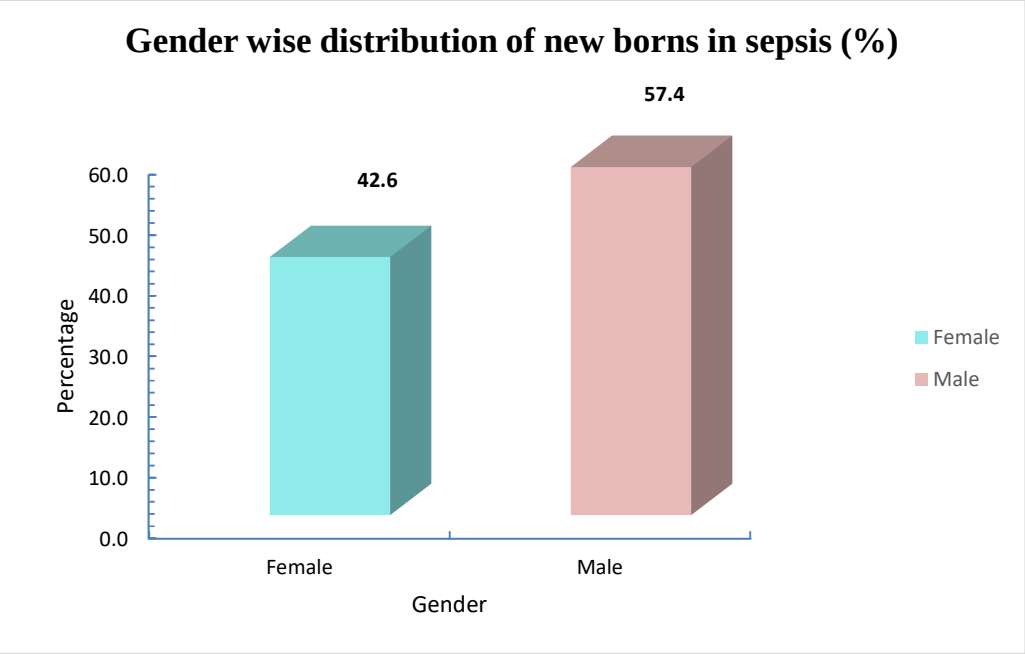
RESULT

The data obtained was tabulated and subjected to proper statistical analysis. The results obtained were systematically described here along with the tables and graphs wherever required.

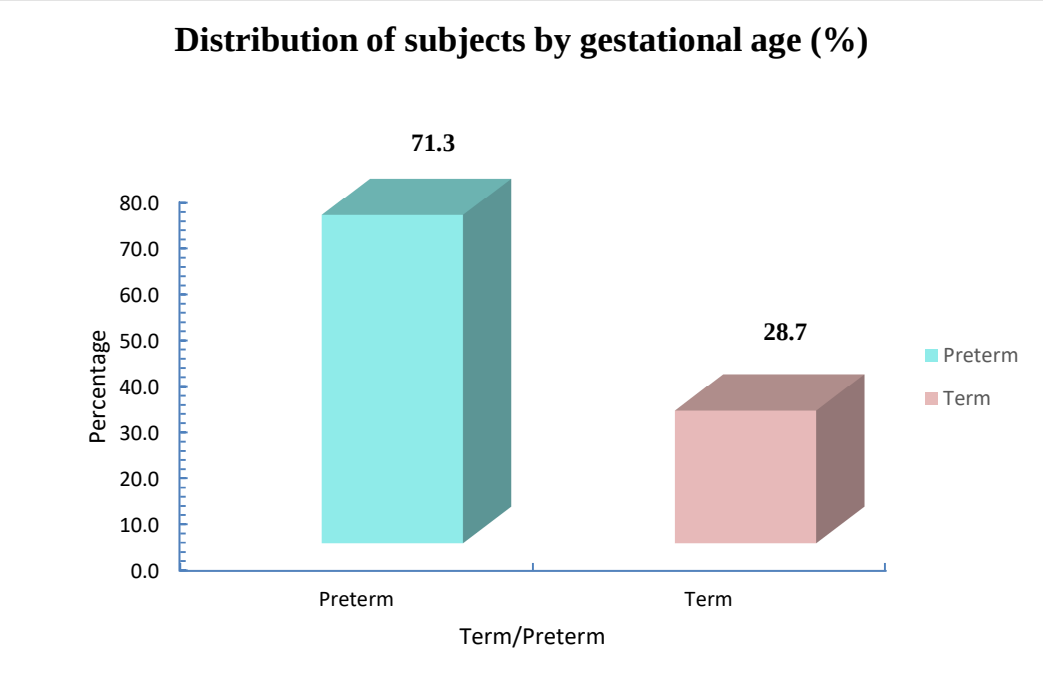
Table 1 Frequency distribution of variables

Variable (n= 101)	n(%)
GENDER DISTRIBUTION	
Female	43(42.60)
Male	58(57.40)
GESTATIONAL AGE	
Preterm	72(71.29)
Term	29(28.71)
MODE OF DELIVERY	
LSCS	67(66.34)
NVD	34(33.66)
BIRTH WEIGHT	
NBW	13(12.87)
LBW	64(63.37)
VLBW	22(21.78)
ELBW	2(1.98)
CULTURE	
K.PNEUMONIAE	65(64.36)
S.HAEMOLYTICS	26(25.74)
A.BAUMARII	5(4.95)
S.PNEUMONIAE	5(4.95)

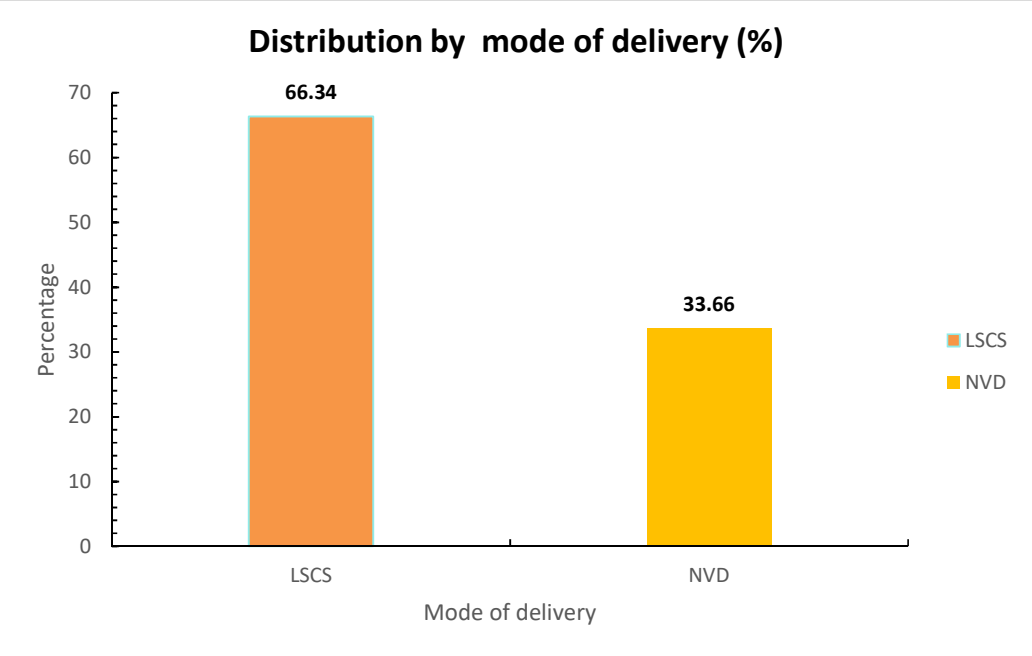
Graph1 : Gender wise distribution of new born in sepsis(%)



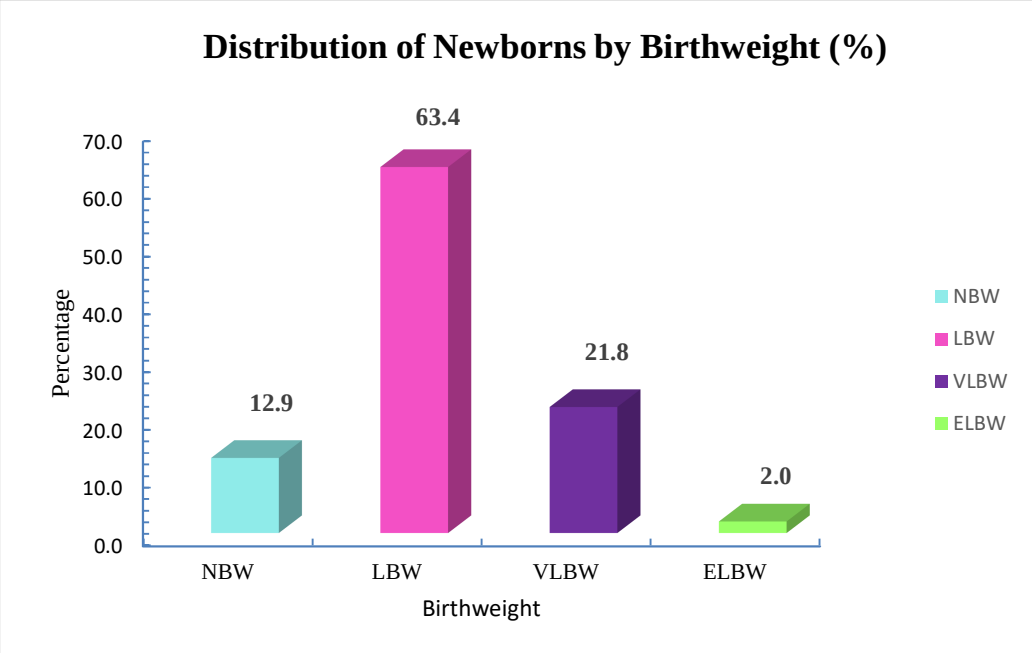
Graph 2 : Distribution of newborns in sepsis by gestational age (%)



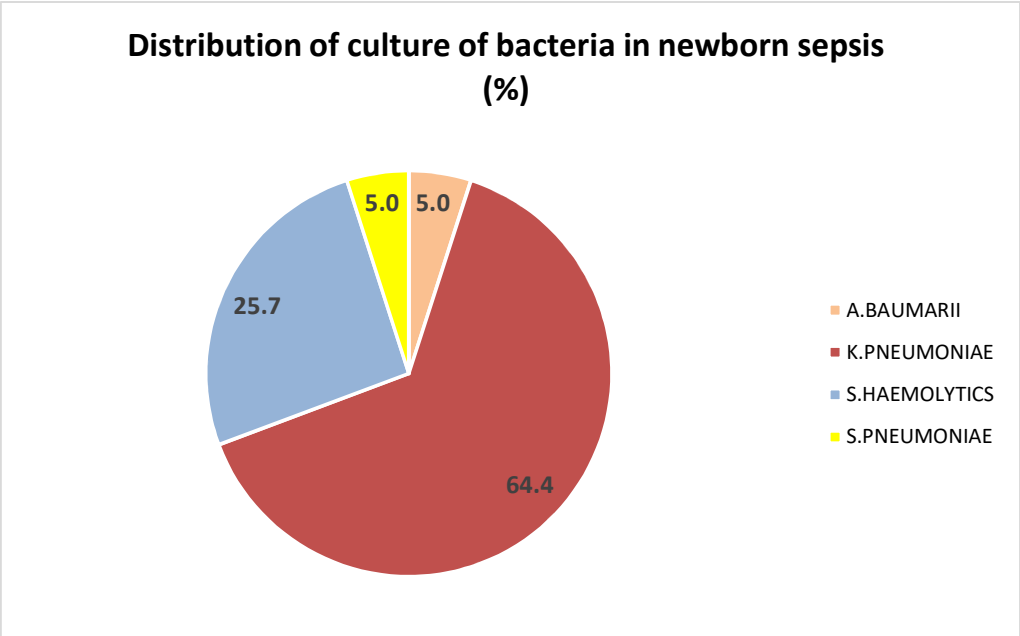
Graph 3 : Distribution by mode of delivery in neonatal sepsis (%)



Graph 4 : Distribution of neonates in sepsis by birthweight (%)



Graph 5 : Distribution of culture of bacteria in newborn sepsis (%)



From table 1 and Graph 1,2,3,4 and 5 shows

The study included 101 participants. The participants' distribution by sex showed that 43 (42.6%) were female and 58 (57.4%) were male. Regarding gestational age, 72 (71.29%) of the participants were preterm, while 29 (28.71%) were born at term. Regarding the mode of delivery, 67 infants (66.34%) were born via Lower Segment Caesarean Section (LSCS), while 34 infants (33.66%) were delivered via a typical vaginal birth (NVD). Regarding birth weight, 13 (12.87%) of the infants had Normal Birth Weight (NBW), 64 (63.37%) had Low Birth Weight (LBW), 22 (21.78%) had Very Low Birth Weight (VLBW), and 2 (1.98%) had Extremely Low Birth Weight (ELBW). The culture results indicated that infections were caused by different bacteria: Klebsiella pneumoniae in 65 (64.36%) cases, Streptococcus haemolyticus in 26 (25.74%) cases, and Streptococcus pneumoniae in 5 (4.95%) cases.

Table 2 Descriptive statistics of Neonatal, maternal parameters along with PCT and hs-CRP

	Median	IQR [1]	Minimum	Maximum
Mothers age	25.00	5	18.00	38.00
Birth Weight	2.3	1	1.00	3.40
1Min APGAR	6.0	2	3.00	8.00
5Min APGAR	8.0	2	2.00	9.00
PCT	1	1	0.00	3.90
hs-CRP	78.0	54	11.00	168.00

Table 2 shows in the present study the mothers' ages ranged from 18 to 38 years old, with a 25-year-old median age and a 5-year interquartile range (IQR). The birth weight of the neonates had a median value of 2.3 kg, with an IQR of 1 kg. The minimum birth weight recorded was 1.0 kg, and the maximum was 3.4 kg. The APGAR scores of 1 minute ranged from 3 to 8, and median score of 6 and an IQR of 2. The APGAR scores of 5minute ranged from 2 to 9, and a median score of 8 and an IQR of 2. Procalcitonin (PCT) values had an average of 0.00 to 3.90 ng/mL, with a median and an interquartile range of 1 ng/mL. The interquartile range for the High-sensitivity C-reactive protein (hsCRP) was 54 mg/L, and the range of results was 11.00 to 168.00 mg/L, with a median of 78 mg/L.

Table 3 Gender wise distribution by mode of delivery in neonatal sepsis

DELIVERY MODE	Female n(%)	Male n(%)	n
LSCS	32(47.76)	35(52.24)	67
NVD	11(32.35)	23(67.64)	34

Table 3 shows that Depending on the mode of delivery 32 new-born (47.76%) were female and 35 new-borns (52.24%) were male, making a total of 67 new-borns delivered by LSCS and 11 new-borns (32.35%) were female and 23 new-borns (67.64%) were male, with a total of 34 new-borns delivered by NVD.

Table 4 Comparison of PCT and hs-CRP levels by gender , gestational age and birth weight

GENDER	Female(n=43)	Male(n=58)	p-value
PCT(ng/ml)			
Median	0.52	1.20	
Range	0-2.90	0-3.90	<0.05*
hs-CRP(mg/dl)			
Median	60.00	84.00	
Range	28-155	11-168	0.052
GESTATIONAL AGE	**Preterm**	**Term**	**p-value**
PCT(ng/ml)			
Median	1.15	0.5	<0.05*
Range	0-3.90	0-3.30	
hs-CRP(mg/dl)			
Median	83.5	55	<0.05*
Range	28-168	11-156	
BIRTH WEIGHT	**LBW**	**VLBW**	**p-value**
PCT (ng/ml)			
Median	.9500	.9500	
Range	0-3.90	0-2.20	0.263
hs-CRP(mg/dl)			
Median	69.5	81.9	
Range	26.2-162.0	28.0-168.0	0.108

Table 4 Shows

Gender-Based Comparison:-The median PCT level was significantly higher in males (1.20 ng/ml) compared to females (0.52 ng/ml), with a range of 0-3.90 ng/ml for males and 0-2.90 ng/ml for females. The difference between genders was statistically significant ($p < 0.05$).The median hs-CRP level was higher in males (84.00 mg/dl) than in females (60.00 mg/dl), with a range of 11-168 mg/dl for males and 28-155 mg/dl for females. This difference was not statistical significant. ($p = 0.052$).

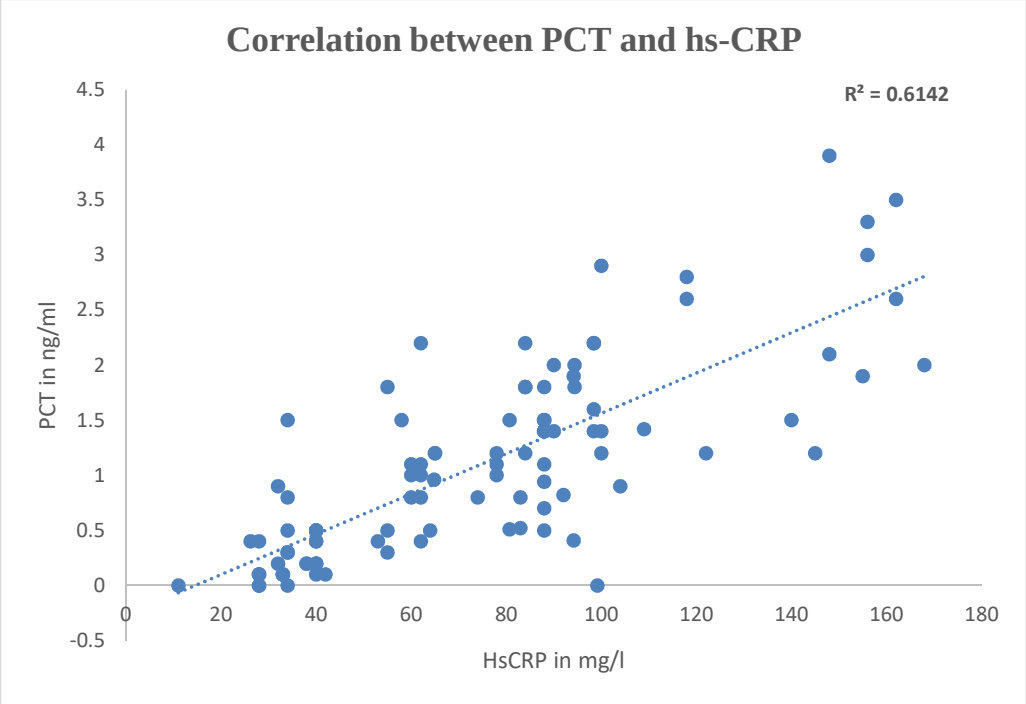
Gestational Age-Based Comparison:-Preterm infants had a significantly higher median PCT level (1.15 ng/ml) compared to term infants (0.50 ng/ml), with ranges of 0-3.90 ng/ml and 0-3.30 ng/ml, respectively. This difference was statistically significant ($p < 0.05$).Preterm infants also exhibited higher median hs-CRP levels (83.5 mg/dl) compared to term infants (55 mg/dl), with ranges of 28-168 mg/dl and 11-156 mg/dl, respectively. The difference was statistically significant ($p < 0.05$).

Birth Weight-Based Comparison:-The median PCT levels were identical for low birth weight (LBW) and very low birth weight (VLBW) infants at 0.9500 ng/ml.The range was 0-3.90 ng/ml for LBW infants and 0-2.20 ng/ml for VLBW infants. There was no statistically significant difference between the two groups ($p = 0.263$).The median hs-CRP levels were 69.5 mg/dl for LBW infants and 81.9 mg/dl for VLBW infants, with ranges of 26.2-162.0 mg/dl and 28.0-168.0 mg/dl, respectively. The difference was not statistically significant ($p = 0.108$).

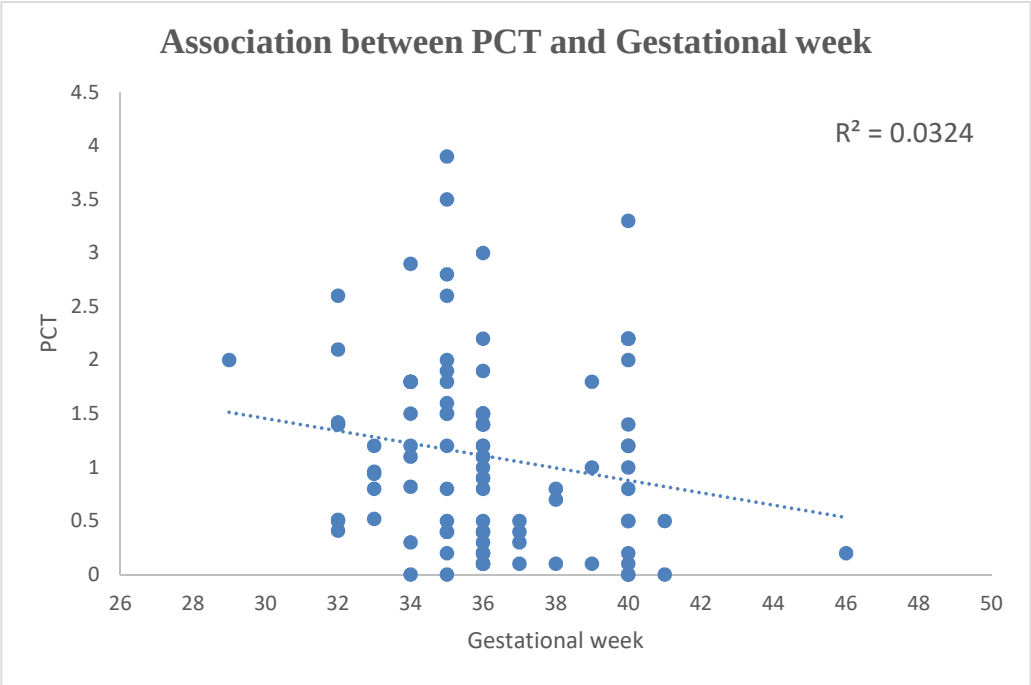
.Table 5 Correlation of PCT and hs-CRP with other parameters

Correlation		hs-CRP	Gestational age	Birthweight
PCT	r	0.784	0.771	-0.064
	p value	<0.05	<0.05	>0.05
hs-CRP	r	0.00	-0.307	-0.307
	p value	0.00	<0.05	<0.05

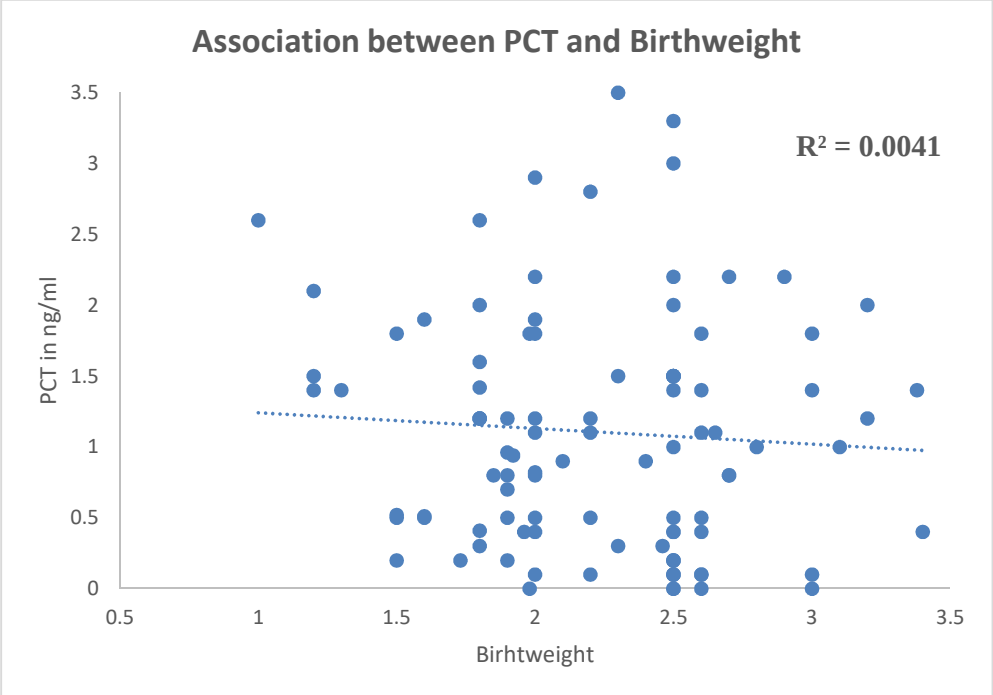
Graph 6 : correlation between PCT and hs-CRP



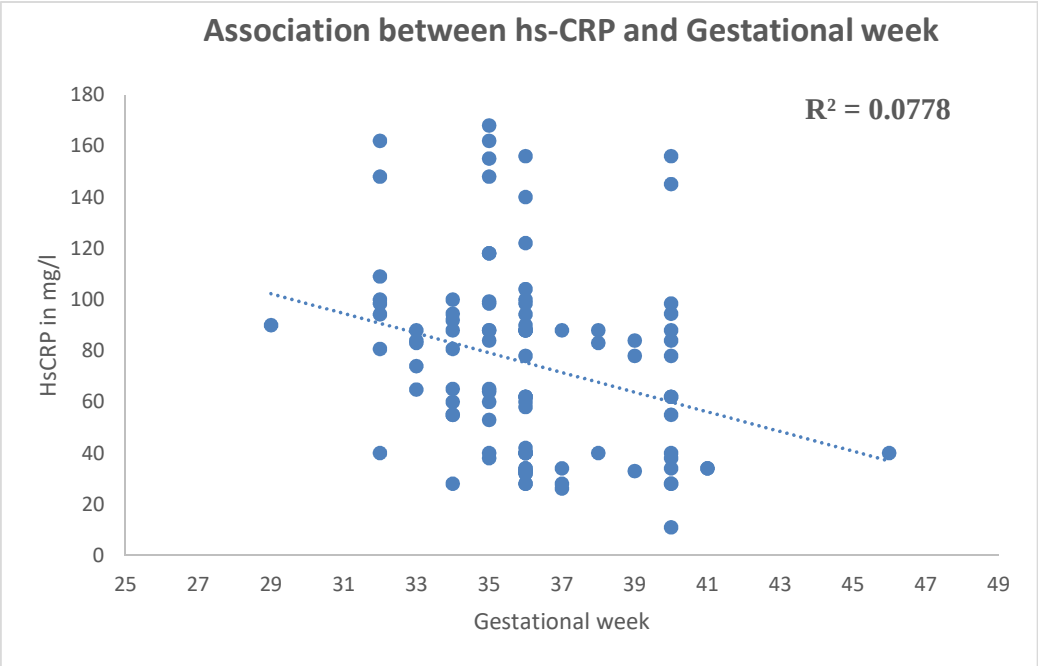
Graph 7 : Association between PCT and Gestational week



Graph 8 : Association between PCT and Birthweight



Graph 9 : Association between hs-CRP and Gestational week



Graph 10: Association between hs-CRP and Birthweight

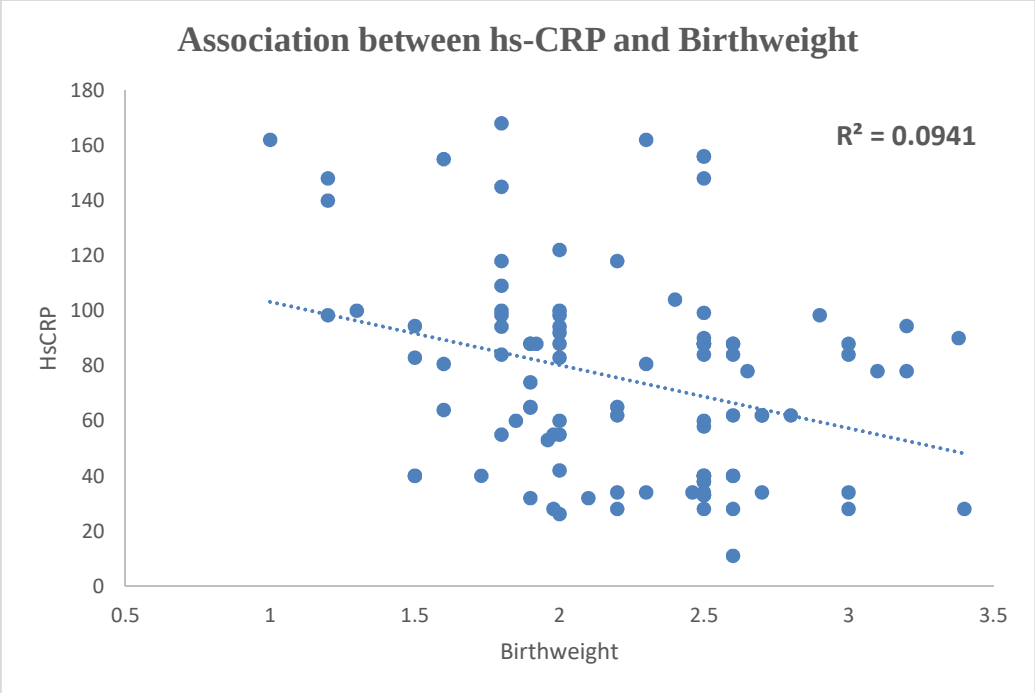


Table 5 and graph 6,7,8,9 and 10 shows the correlation of Procalcitonin (PCT) and high-sensitivity C-reactive protein (hs-CRP) with gestational age and birth weight.

These results indicate that PCT levels are strongly associated with both hs-CRP levels and gestational age, whereas hs-CRP levels show a moderate inverse relationship with both gestational age and birth weight.

Table 6: Distribution and Biomarker Levels in Different Bacterial Cultures

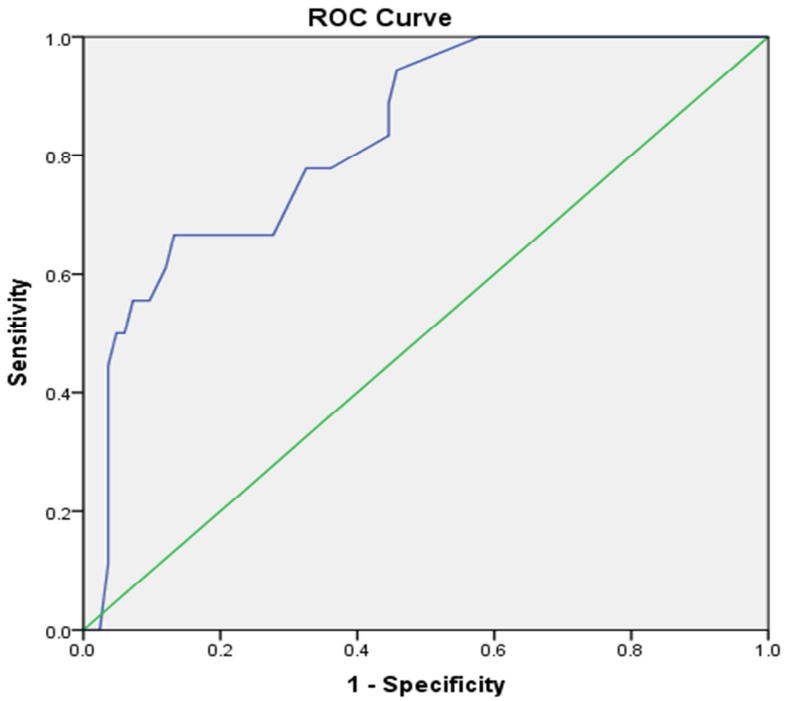
Bacterial Culture	n (%)	PCT (Median, IQR)	hs-CRP (Median, IQR)
K. pneumoniae	65 (64.4%)	1.10 (1.20)	80.70 (61.60)
S. haemolyticus	26 (25.7%)	1.05 (0.80)	70.00 (26.00)
S. pneumoniae	5 (5%)	0.80 (0.70)	65.00 (11.20)
A. baumannii	5 (5%)	0.40 (1.02)	62.00 (9.80)

These findings indicate that K. pneumoniae infections are the most prevalent and are associated with the highest median levels of both PCT and hs-CRP followed by S.Haemolyticus , S. pneumoniae, and A. baumannii.

Table 7: diagnostic performance of Procalcitonin (PCT) and high-sensitivity C-reactive protein (hs-CRP)

	Sensitivity	Specificity	AUC	95% C. I		Sig.	PPV	NPV
				LL	UL			
PCT	75	72.4	0.776	0.67	0.88	<0.05	73.1	74.3
hs-CRP	77.8	67.5	.831	0.74	0.93	<0.05	70.5	75.3

Graph 11: ROC curve for PCT



Graph 12: ROC curve for hs-CRP

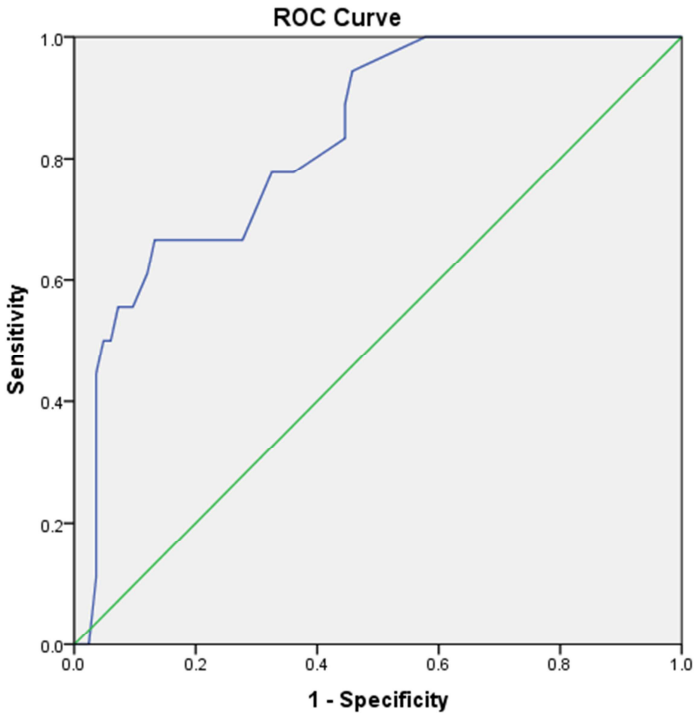


Table 7 and Graph 11,12 summarizes the diagnostic performance of Procalcitonin (PCT) and high-sensitivity C-reactive protein (hs-CRP) in terms of sensitivity, specificity, area under the curve (AUC), and predictive values.

Both biomarkers demonstrate good diagnostic performance, with hs-CRP showing a higher sensitivity and AUC compared to PCT. This indicates that hs-CRP may have a marginally better overall accuracy in detecting the condition under study. Both biomarkers have similar positive and negative predictive values, suggesting they are comparably reliable in predicting true positive and true negative cases. The significance values ($p < 0.05$) for both AUCs indicate that the results are statistically significant.

Table 8: PCT and hs-CRP levels in relation to microorganisms and death outcome.

Micro-organism	n (Death)	PCT (ng/ml)	hs-CRP (mg/l)
K. Pneumoniae	7	2.1	148
S. Haemolytics	4	2.6	162
		1.9	155
		3.9	148
		1.5	140
		3	156
		1.2	145
		1.4	98.4
S. Pneumoniae	1	1.4	100
		1.4	88
		2	168
		1.2	65
A. Baumarii	1	0	99.2

The data show varying levels of PCT and hs-CRP associated with each microorganism, reflecting the severity and inflammatory response in cases that resulted in death. Klebsiella pneumoniae and Staphylococcus haemolyticus are prominent, with multiple cases showing elevated hs-CRP and PCT levels prior to death. Streptococcus pneumoniae also exhibited elevated biomarker levels across cases. Notably, Acinetobacter baumannii, despite only one case, showed a detectable level of hs-CRP but no detectable PCT.

DISCUSSION

Neonatal sepsis possess greatest challenge, as its mortality, morbidity ratios are high and if its timely diagnosed it will decrease the death ratio and unauthorized use of antibiotics which will lead to bacterial antibiotic resistance. Microbial cultures do help us, but results take a long time. Biomarkers can aid in rapid diagnosis. hs-CRP, is a good marker but it lacks in specificity hence it has to be used in conjugation with another serum biomarker and PCT is one such marker.

The thyroid glands C cells produce procalcitonin (PCT), which is calcitonin's precursor and undergoes proteolysis to form the mature hormone calcitonin. It is a glycoprotein with a molecular weight of 14.5 kDa and a peptide containing 116 amino acids. In healthy individuals, PCT is present in very low concentrations but in the presence of bacterial infections its levels significantly increase in case of sepsis, meningitis, and urethritis, but severe rise during sepsis or septic shock. Serum PCT levels are raised during such illnesses as a result of the thyroid's C cells, macrophages, and monocytes producing it in reaction to bacterial endotoxins. Dandona et al.[61] discovered in healthy individuals that following the injection of a small amount of endotoxin, PCT can be detected as early as within 4 hours due to inflammation. Its levels rise quickly within 6 to 8 hours, and then reach a peak, and within 24 hours it returns to normal.

In this study with 101 neonates.57% male neonates had sepsis more compared to female which is correlating with Morad EA study found that neonatal sepsis was more common in male, possibly as a result of an X-linked immunoregulatory gene

factor that increases their vulnerability to infections[10]. However, some studies have found no correlation among gender distribution[69]

Preterm neonates were showing higher PCT and hs-CRP levels than term neonates (71.29%). This aligns with existing literature suggesting higher infection susceptibility in preterm infants due to their underdeveloped immune systems. Due to an inherent defective mechanism, they lack the protective maternal IgG antibodies. Infection risk was higher in preterm new-born's. Similarly, Khasawneh et al. observed a comparable trend in their research, with 76.27% of preterm infants diagnosed with sepsisa[62]. A total of 69.3% of pre term babies were affected with NS which was in concordance with the study conducted by Kumari D et al., 2021[70]

Our study found that most neonates with sepsis were born by Lower Segment Caesarean Section delivery (LSCS) (66.34%). Our study is in consistency with study done by Adatara P et al. (2018) found Sepsis was 16.6 times more common in neonates who delivered via LSCS .This is because firstly longer stay in hospital leading to nosocomial infections[75]. Secondly, the surgical procedure can introduce pathogens, and the neonates might be exposed to different microbial environments compared to vaginally delivered infants according to Boyle et al., (2015)[106]. Fetal laceration occurs in around 0.1% to 3.1% of LSCS births[76,77] and it may be a channel of entry for bacteria that cause newborn sepsis. However, some studies have found Neonatal sepsis was more common in babies born spontaneously by vaginal delivery, possibly due to birth canal bacteria.[14]

In our study Low Birth Weight (LBW) was prevalent (63.37%), indicating severe infection or inflammation in LBW new-born's babies, because Asphyxia, improper physical growth, respiratory and metabolic dysfunction, and other newborn

complications caused by low birth weight (LBW) can increase the likelihood that a child will suffer from infectious illnesses like sepsis or be undernourished during childhood [71,72,73] This significantly lowers the likelihood that an infant will survive. The results of this investigation are consistent with a meta-analysis by Belachew et al. on the link between gestational period, weight at birth, and neonatal septicemia. Their analysis revealed that infants weighing < 2.5 kg at birth were 1.42 times more probably develop neonatal septicemia and experience associated morbidity compared to infants born weighing 2.5 kg or more. Similarly, In a four-year cohort research on the risk factors for neonatal sepsis, Leal Y A et al. found that low birth weight, low Apgar scores, preterm, and prenatal hypoxia were all linked to early mortality from neonatal sepsis.[64] The prevalence of LBW by neglecting the absence of information may result in underestimating [74] as previously found by the study.

In our study, Klebsiella pneumoniae was identified as the predominant pathogen, accounting for 64.36% of isolates. This aligns with findings from various studies in developing nations that leading cause of neonatal sepsis was Klebsiella pneumoniae has consistently been reported. Zakariya BP et al. also reported a significant association between Klebsiella pneumoniae and sepsis, particularly among inborn infants and those having a birth weight of 2.5 kg or less.**[6]** Karthik R et al., who discovered that Staphylococcus aureus (78%) was the most frequently isolated Gram-positive bacterium, whereas Klebsiella species (50%) and E. coli (14.8%) were the most frequently isolated Gram-negative bacteria. Rath S et al. reported similar results, with Klebsiella pneumonia (32.0%) being the most commonly isolated pathogen in blood cultures followed by Staphylococcus aureus (21%).In this study, Gram-negative organisms were the predominant pathogens, consistent with previous research from India and Nigeria [62] The newborns admitted to hospitals are more prone to hospital-

acquired illnesses like Klebsiella pneumoniae. Prolonged hospital stays, invasive operations, and contact with infected medical equipment all raise the risk. Simonsen et al. (2014) examine the effect of hospital environments in the spread of Klebsiella infections in newborns.[78] Sheng tao yan et al.,found PCT levels caused by gram-negative bacteria and PCT can distinguish between gram-negative bacteria and gram-positive bacterial infection, as well as between bacterial species and infection sites.[79]

The median age of the mothers in the study is 25 years, with an interquartile range (IQR) of 5 years, suggesting that the middle 50% of the mothers are between 23 and 28 years old. The age range extends from a minimum of 18 to a maximum of 38 years. This age distribution is consistent with Bailey et al.(2013)[92]studies that show maternal age between 20 and 35 years is associated with the optimal outcomes for both mother and child However, the presence of younger and older mothers indicates a broader demographic, which could influence neonatal outcomes and complicates the interpretation of age-related risks.

The APGAR scores, recorded at 1 and 5 minutes after birth, are critical indicators of the newborn's immediate health status. The median APGAR scores of 6.0 (1 min) and 8.0 (5 min) suggest that most neonates were in relatively good condition shortly after birth, as scores of 7 and above are generally considered normal (American Academy of Pediatrics, 2015)[93]. The IQR of 2 indicates variability, with some neonates experiencing lower scores, which could reflect initial distress at birth requiring medical intervention.

Procalcitonin (PCT) and high-sensitivity C-reactive protein (hs-CRP) are biomarkers used to assess inflammation and infection. The median PCT level of 1 ng/mL with an IQR of 1 ng/mL and a range from 0.00 to 3.90 ng/mL suggests varied

levels of inflammation among the neonates. Assicot et al., (1993)[94] found elevated PCT levels are associated with bacterial infections, indicating that some neonates might have been exposed to significant infections at or after birth.

The hs-CRP levels, with a median of 78.0 mg/L and an IQR of 54 mg/L, range from 11.00 to 168.00 mg/L. Elevated hs-CRP levels are also indicative of inflammation or infection, reinforcing the findings from the PCT data. Pepys & Hirschfield,(2003)[95] studies showed high median and broad range highlight that a substantial proportion of neonates experienced significant inflammatory responses, which could be due to various factors such as infection, stress, or other perinatal complications.

The data indicates a higher incidence of neonatal sepsis in males compared to females across both delivery modes. Specifically, in LSCS, 52.24% of the cases were male, while 47.76% were female. According to Cloherty et al., (2018)[96] the disparity is more pronounced in NVD, where 67.64% of the cases were male, and only 32.35% were female. This male predominance in neonatal sepsis cases which suggests that male neonates are more susceptible to sepsis due to differences in immune system maturity and responses . Kent et al., (2012)[97] studies indicates that male neonates have higher morbidity and mortality rates compared to females, which may be attributed to genetic, hormonal, and immunological differences. This finding aligns with Makrigiannakis et al., (2011)[98] studies suggesting that male neonates are generally more susceptible to infections and adverse outcomes, possibly due to differences in immune system maturity and responses.

The study demonstrated that male infants had significantly higher median PCT and hs-CRP levels compared to females. This disparity suggests that male neonates

exhibit a stronger inflammatory response to sepsis, consistent with Casimir et al., (2010)[99] studies that identify higher susceptibility and severity of infections in male neonates. Uzzan et al., (2006)[100] studies indicated elevated PCT levels are a reliable marker for bacterial infections, highlighting the need for vigilant monitoring and potentially more aggressive treatment strategies in male neonates. Pepys & Hirschfield, (2003)[95] studies indicated that hs-CRP, an acute-phase protein and a measure of systemic inflammation, is raised in male new-borns, which supports the established pattern of greater sensitivity to infections. Further research is warranted to explore the underlying mechanisms driving these differences.

In addition, compared to term infants, preterm infants had significantly greater median levels of both PCT and hsCRP, indicating that prematurely born babies are more likely to develop sepsis. Faix at el., (2013)[101] found elevated PCT levels in preterm infants indicate a higher inflammatory response, which is associated with their greater sensitivity to infections due to an immature immune system as well as Ng et al., (2015)[102] studies showed the significant difference suggests that preterm babies have a stronger inflammatory response, which matches their higher risk of infections and inflammation.

The median PCT levels are similar between LBW (median 0.95 ng/mL) and VLBW (median 0.95 ng/mL) neonates, with a p-value of 0.263, indicating no statistically significant difference. Sullivan et al.,(2011)[103] research has highlighted that both LBW and VLBW neonates are at increased risk of infections due to their immature immune systems and other factors related to prematurity. Similarly, the median hs-CRP levels are slightly higher in VLBW neonates compared to LBW neonates, though this difference also does not reach statistical significance (p-value =

0.108). Shah et al., found that elevated hs-CRP levels reflect the presence of inflammation, which is common in both LBW and VLBW neonates due to their vulnerable health status and increased susceptibility to infections[104].

Correlation analysis

PCT shows a strong positive correlation with hs-CRP ($r = 0.784$, $p < 0.05$), indicating that higher PCT levels are closely associated with elevated hs-CRP levels. This strong correlation suggests that both biomarkers collectively reflect the severity of systemic inflammation in neonates, often indicative of bacterial infections according to Assicot et al., (1993)[94]. PCT also demonstrates a significant positive correlation with gestational age ($r = 0.771$, $p < 0.05$). Uzzan et al., (2006)[100] finding suggests that PCT levels tend to increase with advancing gestational age, potentially reflecting the maturation of the neonatal immune response and the likelihood of encountering infections .In contrast, PCT shows a negligible correlation with birth weight ($r = -0.064$, $p > 0.05$), indicating that birth weight does not significantly influence PCT levels in this study. Other factors such as gestational age and the presence of infection may have a more substantial impact on PCT levels in neonates.

hs-CRP exhibits a negative correlation with gestational age ($r = -0.307$, $p < 0.05$), indicating that lower gestational age is associated with higher hs-CRP levels. This finding aligns with Ng et al., (2015)[102] studies that preterm neonates are more susceptible to experiencing systemic inflammation and infections. Similar to gestational age, hs-CRP also shows a negative correlation with birth weight ($r = -0.307$, $p < 0.05$). Lower birth weight correlates with higher hs-CRP levels, reflecting the increased inflammatory responses seen in smaller and potentially more vulnerable neonates. Monitoring PCT and hs-CRP levels in neonatal care is crucial for early

detection and intervention in systemic inflammation and infections, especially for preterm and low birth weight infants.

The data highlights the distribution of various bacterial cultures and their associated biomarker levels, particularly focusing on Procalcitonin (PCT) and high-sensitivity C-reactive protein (hs-CRP). Klebsiella pneumoniae (K. pneumoniae) is the predominant bacterial culture, accounting for 64.4% of the cases followed by Staphylococcus haemolyticus, Streptococcus pneumoniae, and Acinetobacter baumannii.

The median PCT level for K. pneumoniae is 1.10 ng/mL with an interquartile range (IQR) of 1.20 ng/mL. Elevated PCT levels are known to be a marker for bacterial infections, particularly sepsis. The relatively high median PCT level in K. pneumoniae infections suggests a significant inflammatory response, indicative of severe infection or sepsis. This finding aligns with Assicot et al.,(1993)[94] studies that have demonstrated elevated PCT levels in patients with K. pneumoniae infections, reinforcing its utility as a diagnostic biomarker for bacterial sepsis. According to Uzzan et al., (2006)[100] Procalcitonin is a well-established biomarker for bacterial infections, and its increased levels in the presence of Klebsiella pneumoniae are consistent with prior research highlighting its significance in detecting severe bacterial infections.

The median hs-CRP level for K. pneumoniae is 80.70 mg/L with an IQR of 61.60 mg/L. Elevated hs-CRP levels are also indicative of inflammation and infection This finding is consistent with research by (Pepys & Hirschfield, 2003)[95] showing that hs-CRP levels rise in response to acute bacterial infections, making it a valuable

marker for assessing the severity of infection .In our study, we are not doing serial value of PCT .

Correlation Between PCT and hs-CRP in K. pneumoniae

The high median values of both PCT and hs-CRP in K. pneumoniae infections suggest a strong inflammatory response, characteristic of severe bacterial infections. The correlation between elevated PCT and hs-CRP levels highlights their combined utility in diagnosing and monitoring bacterial infections. Schuetz et al., (2011)[105] Studies have shown that while PCT is more specific to bacterial infections, hs-CRP is a broader marker of inflammation, and their concurrent elevation can provide a more comprehensive picture of the infection's severity. The findings support the use of PCT and hs-CRP as reliable markers for assessing the severity of infections caused by K. pneumoniae, thereby aiding in better clinical decision-making and improved patient outcomes

Diagnostic Utility of PCT and hs-CRP

Our study found both biomarkers showed significant diagnostic utility.PCT showing sensitivity (75%), specificity (72.4%) and hs-CRP showing sensitivity (77.8%), specificity (67.5%). Prior research has provided varying sensitivity and specificity values for PCT: Sakha et al.[107] reported 66.7% sensitivity and 50% specificity, Boo et al[108]. reported 88.9% sensitivity and 65.2% specificity, Hatherill et al.[109] and Chiesa et al[110.] Respectively, reported 92.6% and 97.5% sensitivity and specificity, respectively. PCT demonstrated superior efficacy as a clinical marker in the experiment compared to hs-CRP, although being more costly.

Per Ballot et al. (2013)[111], the PCT test's slightly lower sensitivity, specificity, positive predictive value, and AUC may make it insufficient for verifying newborn sepsis. Furthermore, Lapillonne et al[112.] showed that, despite PCT's usefulness as a diagnostic tool, premature birth may raise levels of the protein even in the absence of bacterial infection. This could result in decreased specificity. According to a study by Monneret et al. [113], increased PCT levels that are frequently a sign of bacterial infection on the second day after birth drop to normal physiological ranges (<0.5 mg/L) by the fourth day, a trend that is also observed in healthy infants. According to their findings, circumstances including transient hypoxia during labor and respiratory distress syndrome (RDS) may frequently result in elevated PCT levels.

Furthermore, we used a ROC curve study to evaluate the diagnostic performance of PCT versus hs-CRP in the diagnosis of newborn sepsis. Area Under the Curve (AUC) for both markers showed strong discriminating power. According to Blommendahl et al. [114], PCT is not a more accurate predictor than hsCRP levels. This is corroborated by another study conducted by Chiesa et al.[110] , prenatal antibiotic usage may result in false-negative PCT readings in cases with early neonatal sepsis. Additionally, noted that PCT levels will slightly exceed normal if prenatal antibiotics are given to babies within a day after delivery.

In our study PCT and hs-CRP values were recorded in neonates who died from different bacterial infections. Klebsiella pneumoniae caused seven deaths, In four cases of Staphylococcus haemolyticus-related deaths, One Streptococcus pneumoniae-related death. Sarita chawdhary et al observed At the 2.5 ng/ml cut-off value; PCT on the third day of suspected sepsis shows higher sensitivity, specificity, and reliability for prognosticating surgical newborn sepsis. PCT levels are trending

upward, which suggests a bad prognosis.[66] Prashant Singh et al observed higher hs-CRP levels have been demonstrated to be predictive of death in individuals and neonates with sepsis and/or bacteremia; however, this has not been well investigated in newborns with sepsis.[67]

PCT levels showed a strong positive correlation with hs-CRP levels and gestational age, but a weak negative correlation with birth weight. This suggests that while PCT is a reliable indicator of inflammation, its levels are less influenced by birth weight Highest median levels of both PCT and hs-CRP in case of Klebsiella pneumonia indicating PCT well correlated with it.

Clinical Implications

The results support PCT's usefulness as a biomarker for early detection of newborn sepsis. Elevated PCT levels were significantly associated with preterm birth and male sex, indicating that these groups may benefit from more rigorous monitoring. The strong correlation between PCT and hs-CRP also suggests that a combined biomarker approach could enhance diagnostic accuracy.

Limitations

Even with the encouraging outcomes, there are a few restrictions on the study. The sample size is sufficient, but it restricts how broadly the results can be applied. Furthermore, possible confounders that might affect neonatal outcomes, such as the mother's health state and intrapartum variables, were not taken into consideration in this study. Larger, more varied populations should be included in future studies, and longitudinal follow-ups should be taken into account to evaluate the long-term outcomes of newborns with sepsis utilizing PCT and hs-CRP.

CONCLUSION

Procalcitonin was found to be domineering indicator including high sensitivity C - reactive protein as a predictor of neonatal sepsis with sensitivity, specificity, and other indicator of good diagnostic marker were established. In addition Procalcitonin and high sensitivity C - reactive protein levels correlated well with Klebsiella Pneumoniae, indicating use of Procalcitonin as an alternative marker for culture. This has also paved way for further studies with large sample, multicenter studies to refine the biomarker, use and validate its efficacy across diverse neonatal populations for diagnosis of neonatal sepsis instead of culture.

The merits and demerits of these biomarkers in early recognition and management of neonatal sepsis must be readily available information to the neonatologist for immediate management of neonatal sepsis to prevent mortality and morbidity.

SUMMARY

Sepsis is a potentially fatal illness and Neonatal sepsis is one of the most frequent infections identified in new-born worldwide.

The present a cross-sectional study comprises of 101 Neonates [0-28 days] who were suspected of having sepsis admitted in NICU, Pediatrics Department of KLES Dr. Prabhakar Kore hospital and MRC, Belagavi, Karnataka

The study included 101 neonates, with a male predominance (57.4% male, 42.6% female) and 71.29%, were preterm, while 28.71% were term births. The mode of delivery was predominantly Lower Segment Caesarean Section (LSCS) at 66.34%, was observed in 32.67% of cases.

Maximum Birth weight distribution showed 63.37% had Low Birth Weight (LBW). Mechanical ventilation was required for 30.69% of the infants. The culture results revealed that maximum infections were caused by Klebsiella pneumoniae (64.36%).Neonatal Birth Weight: Median of 2.3 kg (IQR: 1 kg), ranging from 1.0 to 3.4 kg hsCRP done by using an immunoturbidimetric assay and PCT done by fluorescence immunoassay sandwich immunodetection method. Procalcitonin (PCT): Ranged from 0 to 3.90 ng/mL (median: 1 ng/mL, IQR: 1) .High-sensitivity C-reactive Protein (hsCRP)- Ranged from 11 to 168 mg/L (median: 78 mg/L, IQR: 54).

Biomarker Analysis

PCT Levels: Median PCT was significantly higher in males (1.20 ng/ml) than in females (0.52 ng/ml), with a p-value < 0.05.hsCRP Levels:- Males had higher

median hsCRP levels (84.00 mg/dl) compared to females (60.00 mg/dl), with a p-value of 0.052, indicating a non-significant difference.

Gestational Age and Birth Weight Correlation: Preterm infants had higher median PCT (1.15 ng/ml) and hsCRP levels (83.5 mg/dl) compared to term infants (PCT: 0.5 ng/ml, hsCRP: 55 mg/dl), with significant p-values < 0.05.

Correlation with Birth Weight: No significant differences in PCT and hsCRP levels were found across different birth weight categories (p-values of 0.263 and 0.108, respectively).

Correlation Analysis

PCT: Strong positive correlations were found with hsCRP levels (r = 0.784) and gestational age (r = 0.771), both with p-values < 0.05. Birth weight showed a weak negative correlation (r = -0.064) with a non-significant p-value.

hs-CRP Showed weak negative correlations with gestational age (r = -0.279) and birth weight (r = -0.307), both significant (p < 0.05).

ROC Curve Analysis-

hs-CRP: Sensitivity 77.8%, specificity 67.5%, AUC 0.831 (95% CI: 0.74-0.93).

PCT: Sensitivity 75%, specificity 72.4%, AUC 0.776 (95% CI: 0.67-0.88).

Biomarker Levels in Bacterial Cultures

K. pneumoniae infections are the most prevalent and are associated with the highest median levels of both PCT and hs-CRP and it reflecting the severity and inflammatory response in K.Pnemoniae cases that resulted in death.

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ANNEXURE I - CONSENT FORMS

Title: DIAGNOSTIC UTILITY OF PROCALCITONIN IN EARLY DIAGNOSIS OF NEONATAL SEPSIS IN A TERTIARY CARE CENTRE: A CROSS SECTIONAL STUDY

Study investigator : _____

Guide : _____

Purpose of the study:

- Estimation of Procalcitonin, hs-CRP and blood culture in neonatal sepsis patients.
- Correlation of Procalcitonin, hs-CRP with automated blood culture, gestational age and mode of delivery.

Procedure: 3ml of blood will be collected under aseptic precautions before starting of antibiotics treatment.2ml of blood will be inoculated immediately into the blood culture bottle. 1ml of blood will be collected in plain tubes for further PCT and hs-CRP measurement.PCT test will be done based on fluorescence immunoassay technology, using a sandwich immunodetection method and hs-CRP analysis will be done by using immunoturbidimetric method.

Risks and benefits: There is no risk

Privacy & Confidentiality: All the information regarding you during the course of this study will be kept confidential.

Institutional/sponsor policy: You will not receive any remuneration for participating in this study.

Financial incentives for participants: Your participation is voluntary & you will not be paid any remuneration.

Voluntary Participation/Withdrawal: Your participation in the study is completely voluntary.

Contact detail:

If you have any question about your rights as a study participant, you may contact **Dr. Harsha Hegde,** Chairperson, Institutional ethics Committee on Human Subjects Research, J. N. Medical College, Belagavi-590010, Mob. 9448113403.

Authorization to publish result: The researcher may use information gathered from this study for presentation or publication but your identity will not be disclosed.

Consent statement:

I mother/father of baby_____ making a voluntary decision to allow myself to participate. My signature below indicates that I have read the information provided above, that I have been given the opportunity to ask question and the said question have been answered to my satisfaction.

Signature or left thumbprint of participants or legally authorized Representative

Participant's name: _____Signature/thumb print of participant: _____

Name of the legally authorized Representative / guardian _____

Signature of Representative / guardian _____

Signature of co-guide/witness: _____

Signature of Researcher: _____ Date: _____

ANNEXURE II- PERFORMA

Neonatal Sepsis Data Collection Form

1. ID:
2. Date of Birth
3. Gender: Male / Female
4. Birth Weight:
5. Gestational Age
6. Mode of Delivery: Vaginal / Caesarean
7. Apgar Score at 1 minute:
8. Apgar Score at 5 minutes:

Maternal History

1. Maternal Age:
2. Gravidity:
3. Parity:
4. History of Maternal Infections: Yes / No (If Yes, specify)
5. Maternal Fever during Labor: Yes / No
6. Premature Rupture of Membranes (PROM): Yes / No (If Yes, duration in hours)
7. Maternal Antibiotics during Labor: Yes / No (If Yes, specify)

Clinical Presentation

1. Date and Time of Admission:
2. Presenting Symptoms:
   - Fever
   - Hypothermia
   - Respiratory Distress
   - Lethargy
   - Poor Feeding
   - Jaundice
   - Seizures
   - Others (specify)

3. Onset of Symptoms: Early-Onset (within 72 hours of birth) / Late-Onset (after 72 hours of birth)

Laboratory Investigations

1. Complete Blood Count (CBC):
    - WBC Count
    - Platelet Count
    - Haemoglobin
2. PCT /High sensitivity C - reactive protein (hs-CRP): Positive / Negative
3. Blood Culture: Positive / Negative (If Positive, specify organism)
4. Lumbar Puncture: Yes / No (If Yes, results)
5. Urine Culture: Yes / No (If Yes, results)
6. Chest X-Ray: Normal / Abnormal (If Abnormal, specify findings)

Treatment

1. Antibiotics Administered:
    - Type of Antibiotics
    - Dosage
    - Duration
2. Supportive Care:
    - Mechanical Ventilation: Yes / No
    - Oxygen Therapy: Yes / No
    - Intravenous Fluids: Yes / No
    - Phototherapy: Yes / No

Outcomes

1. Length of Hospital Stay:
2. Complications:
    - Respiratory Distress Syndrome (RDS)
    - Necrotizing Enterocolitis (NEC)
    - Intraventricular Haemorrhage (IVH)
    - Others (specify)
3. Discharge Status: Discharged / Referred / Expired
4. Follow-Up: Yes / No (If Yes, specify details)

Additional Notes

1. Observations:
2. Comments:

ANNEXURE III - PHOTOGRAPHS

Figure 12 : Cobas e 801 Autoanalytical Unit

ANNEXURE IV MASTER CHART

SL NO	M AGE	NAME	GENDER	WEIGHT	BIRTH WEIGHT	DELIVERY MODE	PROM	GESTATIONAL AGE	DAYS	TERM/PRETERM	DEPARTMENT	18	CULTURE	1MIN APGAR	5MIN APGAR	HsCRP		PCT		MECHANICAL VENTILATION	
1	32	B/O-JYOTI	FEMALE	1.2	VLBW	NVD	YES	32	3	PRETERM	NICU		K.PNEUMONIAE	6	8	148	mg/l	2.1	ng/ml	YES	
2	24	B/O-SAVITA	FEMALE	1.5	VLBW	NVD	NO	33	4	PRETERM	NICU		K.PNEUMONIAE	7	9	83	mg/l	0.52	ng/ml	NO	
3	31	B/0-BHARTI	MALE	1	ELBW	LSCS	YES	32	4	PRETERM	NICU		K.PNEUMONIAE	3	6	162	mg/l	2.6	ng/ml	YES	DEATH
4	24	B/O-AARTI	MALE	1.5	VLBW	LSCS	NO	34	3	PRETERM	NICU		K.PNEUMONIAE	7	8	94.4	mg/l	1.8	ng/ml	NO	
5	21	B/O-SUMITRA	MALE	1.92	VLBW	LSCS	NO	33	2	PRETERM	NICU		K.PNEUMONIAE	7	9	88	mg/l	0.94	ng/ml	NO	
6	21	B/O-NIKITA	MALE	1.6	VLBW	NVD	YES	32	3	PRETERM	NICU		K.PNEUMONIAE	6	9	80.7	mg/l	0.51	ng/ml	NO	
7	22	B/O-NIKITA	FEMALE	1.8	VLBW	NVD	NO	32	3	PRETERM	NICU		K.PNEUMONIAE	7	9	94.2	mg/l	0.41	ng/ml	NO	
8	25	B/O-GANGAVA	MALE	1.2	VLBW	NVD	YES	32	4	PRETERM	NICU		S.HAEMOLYTICS	5	8	98.4	mg/l	1.4	ng/ml	YES	**DEATH**
9	24	B/O-SAROJ	MALE	1.9	VLBW	NVD	YES	33	2	PRETERM	NICU		K.PNEUMONIAE	6	8	64.8	mg/l	0.96	ng/ml	NO	
10	31	B/O-SOMYA	MALE	2	LBW	LSCS	NO	37	2	TERM	NICU		S.HAEMOLYTICS	7	9	26.2	mg/l	0.4	ng/ml	NO	
11	25	B/O-LAXMI	MALE	1.8	LBW	LSCS	YES	32	3	PRETERM	NICU		A.BAUMARII	4	9	109	mg/l	1.42	ng/ml	YES	
12	34	B/O-JYOTI	FEMALE	2	LBW	LSCS	NO	36	2	PRETERM	NICU		K.PNEUMONIAE	6	9	42	mg/l	0.1	ng/ml	NO	
13	25	B/O-POOJA	MALE	1.8	LBW	LSCS	NO	35	3	PRETERM	NICU		K.PNEUMONIAE	3	7	98.4	mg/l	1.6	ng/ml	YES	

14	20	B/O-PRIYA	FEMALE	1.8	LBW	LSCS	NO	33	2	PRETERM	NICU		S.HAEMOLYTICS	5	8	84	mg/l	1.2	ng/ml	YES	
15	28	B/O-DEEPTI	FEMALE	1.9	LBW	NVD	YES	38	1	TERM	NICU		K.PNEUMONIAE	7	9	88	mg/l	0.7	ng/ml	NO	
16	32	B/O-SUNANDA	FEMALE	1.5	VLBW	LSCS	NO	36	2	PRETERM	NICU		K.PNEUMONIAE	8	9	40	mg/l	0.2	ng/ml	NO	
17	22	B/O-KOMAL	MALE	1.85	LBW	LSCS	NO	35	2	PRETERM	NICU		S.PNEUMONIAE	4	8	60	mg/l	0.8	ng/ml	NO	
18	27	B/O-ROOPALI	MALE	1.9	LBW	LSCS	NO	37	1	TERM	NICU		K.PNEUMONIAE	7	9	88	mg/l	0.5	ng/ml	NO	
19	24	B/O-CHANDNI	FEMALE	2.6	LBW	LSCS	NO	38	2	TERM	NICU		K.PNEUMONIAE	8	9	40	mg/l	0.1	ng/ml	NO	
20	25	B/O-NEHA	MALE	1.3	LBW	LSCS	YES	32	2	PRETERM	NICU		S.HAEMOLYTICS	5	7	100	mg/l	1.4	ng/ml	YES	**DEATH**
21	23	B/O-PARVATI	FEMALE	1.8	LBW	NVD	YES	34	4	PRETERM	NICU		K.PNEUMONIAE	6	9	55	mg/l	0.3	ng/ml	NO	
22	24	B/O-PRIYANKA	MALE	1.9	LBW	LSCS	NO	33	5	PRETERM	NICU		K.PNEUMONIAE	7	9	74	mg/l	0.8	ng/ml	NO	
23	28	B/O-SUJATA	MALE	2	LBW	LSCS	NO	34	3	PRETERM	NICU		S.HAEMOLYTICS	6	8	92	mg/l	0.82	ng/ml	NO	
24	31	B/O-ANKITA	FEMALE	1.6	VLBW	NVD	YES	35	2	PRETERM	NICU		K.PNEUMONIAE	6	8	64	mg/l	0.5	ng/ml	NO	
25	26	B/O-DIVYA	MALE	2	LBW	LSCS	NO	36	3	PRETERM	NICU		K.PNEUMONIAE	5	8	122	mg/l	1.2	ng/ml	NO	
26	36	B/O-ADITI	FEMALE	1.8	LBW	NVD	YES	35	2	PRETERM	NICU		S.HAEMOLYTICS	6	9	118	mg/l	2.6	ng/ml	NO	
27	22	B/O-KUMARI	FEMALE	1.96	LBW	LSCS	NO	35	3	PRETERM	NICU		K.PNEUMONIAE	6	7	53	mg/l	0.40	ng/ml	YES	
28	18	B/O-NISHA	FEMALE	2.65	LBW	LSCS	NO	36	4	PRETERM	NICU		K.PNEUMONIAE	7	8	78	mg/l	1.10	ng/ml	NO	
29	24	B//O-AARTI	FEMALE	3.38	NBW	LSCS	NO	36	4	PRETERM	NICU		S.HAEMOLYTICS	3	8	90	mg/l	1.4	ng/ml	YES	
30	27	B/0-DIVYA	FEMALE	1.6	LBW	LSCS	YES	35	3	PRETERM	NICU		K.PNEUMONIAE	5	7	155	mg/l	1.9	ng/ml	NO	**DEATH**
31	22	B/O-PUJA	MALE	2.5	LBW	LSCS	NO	46	2	TERM	NICU		S.HAEMOLYTICS	7	8	40	mg/l	0.2	ng/ml	NO	
32	23	B/0-ANUSHRI	FEMALE	2.3	LBW	LSCS	NO	37	3	TERM	NICU		A.BAUMARII	6	7	34	mg/l	0.3	ng/ml	NO	
33	25	B/0-PALAK	FEMALE	2.2	LBW	LSCS	NO	37	3	TERM	NICU		K.PNEUMONIAE	7	7	28	mg/l	0.1	ng/ml	NO	
34	23	B/O-SUNITA	FEMALE	2.5	LBW	VD	NO	39	4	TERM	NICU		K.PNEUMONIAE	5	7	33	mg/l	0.1	ng/ml	YES	
35	26	B/0-SUNAINA	MALE	2	LBW	LSCS	YES	40	2	TERM	NICU		S.HAEMOLYTICS	6	6	55	mg/l	0.5	ng/ml	NO	
36	20	B/0-DEVI	FEMALE	2.7	NBW	LSCS	YES	36	2	PRETERM	NICU		K.PNEUMONIAE	7	8	62	mg/l	0.8	ng/ml	NO	

37	26	B/O-PULKIT	MALE	2.5	LBW	VD	YES	40	3	TERM	NICU		K.PNEUMONIAE	4	2	156	mg/l	3.3	ng/ml	YES	
38	25	B/O-SASHI	MALE	1.9	LBW	VD	YES	35	2	PRETERM	NICU		S.PNEUMONIAE	6	8	65	mg/l	1.2	ng/ml	YES	**DEATH**
39	35	B/O-ADITI	FEMALE	3.2	NBW	VD	NO	40	3	TERM	NICU		K.PNEUMONIAE	3	7	78	mg/l	1.2	ng/ml	YES	
40	23	B/O-RIDDHI	MALE	1.73	VLBW	LSCS	YES	36	2	PRETERM	NICU		K.PNEUMONIAE	5	7	40	mg/l	0.2	ng/ml	YES	
41	27	B/O-KUSHI	FEMALE	2.46	LBW	LSCS	NO	36	1	PRETERM	NICU		S.HAEMOLYTICS	7	7	34	mg/l	0.3	ng/ml	NO	
42	24	B/O-JYOTI	MALE	3.4	NBW	LSCS	NO	36	2	PRETERM	NICU		K.PNEUMONIAE	8	8	28	mg/l	0.4	ng/ml	NO	
43	33	B/O-ANUKRITI	MALE	2.1	VLBW	LSCS	NO	36	2	PRETERM	NICU		K.PNEUMONIAE	4	8	32	mg/l	0.9	ng/ml	NO	
44	24	B/O-NIKITA	MALE	2.9	NBW	VD	YES	40	1	TERM	NICU		S.HAEMOLYTICS	7	8	98.4	mg/l	2.2	ng/ml	NO	
45	26	B/0-CHANDHNI	FEMALE	3	NBW	LSCS	NO	39	2	TERM	NICU		K.PNEUMONIAE	8	8	84	mg/l	1.8	ng/ml	NO	
46	27	B/O-PRACHI	MALE	2.5	LBW	VD	NO	36	2	PRETERM	NICU		S.HAEMOLYTICS	5	8	88	mg/l	1.4	ng/ml	YES	
47	23	B/O-SOMYA	FEMALE	2.5	LBW	LSCS	NO	36	4	PRETERM	NICU		A.BAUMARII	6	7	40	mg/l	0.4	ng/ml	NO	
48	38	B/O-TOSHITA	FEMALE	2.5	LBW	LSCS	NO	36	5	PRETERM	NICU		K.PNEUMONIAE	7	7	60	mg/l	1	ng/ml	NO	
49	22	B/0-NANDINI	MALE	2.6	LBW	VD	NO	36	3	PRETERM	NICU		K.PNEUMONIAE	6	6	88	mg/l	1.4	ng/ml	NO	
50	24	B/0-PRIYANKA	FEMALE	2.5	LBW	LSCS	NO	35	2	PRETERM	NICU		S.HAEMOLYTICS	6	8	40	mg/l	0.4	ng/ml	NO	
51	20	B/0-RAJINI	FEMALE	2	LBW	LSCS	NO	34	3	PRETERM	NICU		K.PNEUMONIAE	5	8	100	mg/l	2.9	ng/ml	NO	
52	27	B/O-NEETU	MALE	2.5	LBW	LSCS	YES	35	2	PRETERM	NICU		K.PNEUMONIAE	5	8	148	mg/l	3.9	ng/ml	NO	
53	29	B/O-PALAK	FEMALE	2	LBW	LSCS	NO	38	3	TERM	NICU		S.PNEUMONIAE	7	7	83	mg/l	0.8	ng/ml	YES	
54	20	B/O-SHRUTI	MALE	2.3	LBW	VD	NO	35	4	PRETERM	NICU		K.PNEUMONIAE	8	7	162	mg/l	3.5	ng/ml	NO	
55	24	B/O-PRERNA	MALE	3.2	NBW	LSCS	NO	40	4	TERM	NICU		K.PNEUMONIAE	4	8	94.4	mg/l	2	ng/ml	YES	
56	26	B/O-REPHALI	FEMALE	3	NBW	LSCS	YES	40	3	TERM	NICU		S.HAEMOLYTICS	7	7	88	mg/l	1.4	ng/ml	NO	
57	23	B/O-AARTI	MALE	2.3	LBW	LSCS	NO	34	2	PRETERM	NICU		K.PNEUMONIAE	8	7	80.7	mg/l	1.5	ng/ml	NO	
58	31	B/O-KOMAL	MALE	2	LBW	LSCS	NO	36	3	PRETERM	NICU		S.PNEUMONIAE	5	7	94.2	mg/l	1.9	ng/ml	NO	
59	21	B/O-NAINA	FEMALE	1.8	VLBW	LSCS	NO	36	3	PRETERM	NICU		K.PNEUMONIAE	5	7	100	mg/l	1.2	ng/ml	YES	

60	24	B/O-PHULWATI	FEMALE	2.4	LBW	VD	YES	36	4	PRETERM	NICU		S.HAEMOLYTICS	6	7	104	mg/l	0.9	ng/ml	YES	
61	36	B/0-RAJNESH	MALE	2.2	LBW	VD	NO	35	2	PRETERM	NICU		K.PNEUMONIAE	6	8	118	mg/l	2.8	ng/ml	NO	
62	23	B/O-PEEHU	MALE	2.5	LBW	LSCS	NO	35	2	PRETERM	NICU		K.PNEUMONIAE	6	8	88	mg/l	1.5	ng/ml	NO	
63	28	B/O-SHIKHA	MALE	2.6	NBW	VD	YES	40	3	TERM	NICU		K.PNEUMONIAE	5	9	11	mg/l	0	ng/ml	YES	**REFUSED**
64	32	B/O-HARSHA	FEMALE	2.5	LBW	LSCS	YES	29	2	PRETERM	NICU		K.PNEUMONIAE	7	8	90	mg/l	2	ng/ml	NO	
65	32	B/O-HARSHITA	MALE	2.2	LBW	VD	YES	36	3	PRETERM	NICU		K.PNEUMONIAE	7	9	62	mg/l	1.1	ng/ml	YES	
66	24	B/0-DEEPASHA	MALE	1.2	VLBW	LSCS	YES	36	2	PRETERM	NICU		K.PNEUMONIAE	5	7	140	mg/l	1.5	ng/ml	YES	DEATH
67	31	B/O-PRIANKA	FEMALE	2.2	LBW	LSCS	NO	41	1	TERM	NICU		K.PNEUMONIAE	7	9	34	mg/l	0.5	ng/ml	NO	
68	24	B/O-SACHI	MALE	3	NBW	LSCS	YES	40	2	TERM	NICU		S.HAEMOLYTICS	5	8	28	mg/l	0.1	ng/ml	NO	
69	21	B/O-AAKRITI	FEMALE	2.5	LBW	LSCS	NO	36	2	PRETERM	NICU		K.PNEUMONIAE	6	8	33	mg/l	0.1	ng/ml	NO	
70	21	B/O-PHULKUMARI	FEMALE	2.5	LBW	LSCS	NO	35	1	PRETERM	NICU		S.HAEMOLYTICS	7	9	38	mg/l	0.2	ng/ml	NO	
71	22	B/O-NISHA	MALE	2.6	LBW	LSCS	YES	36	2	PRETERM	NICU		A.BAUMARII	4	9	62	mg/l	0.4	ng/ml	NO	
72	25	B/O-DHRITHI	MALE	2.5	LBW	VD	NO	36	2	PRETERM	NICU		K.PNEUMONIAE	6	9	156	mg/l	3	ng/ml	YES	
73	24	B/O-BABY	MALE	2.2	LBW	VD	NO	34	4	PRETERM	NICU		K.PNEUMONIAE	3	7	65	mg/l	1.2	ng/ml	NO	
74	31	B/O-SONAM	MALE	3.1	NBW	VD	YES	39	5	TERM	NICU		S.HAEMOLYTICS	5	8	78	mg/l	1	ng/ml	NO	
75	25	B/0-VIDYA	MALE	1.5	VLBW	VD	NO	32	3	PRETERM	NICU		K.PNEUMONIAE	7	9	40	mg/l	0.5	ng/ml	NO	
76	34	B/O-NEELAM	FEMALE	3	NBW	VD	YES	41	2	TERM	NICU		K.PNEUMONIAE	8	9	34	mg/l	0	ng/ml	NO	**REFUSED**
77	25	B/0-USHA	FEMALE	2.6	LBW	LSCS	NO	36	3	PRETERM	NICU		S.PNEUMONIAE	4	8	28	mg/l	0.1	ng/ml	NO	
78	20	B/O-GURIYA	FEMALE	1.9	VLBW	LSCS	NO	36	2	PRETERM	NICU		K.PNEUMONIAE	7	9	32	mg/l	0.2	ng/ml	NO	
79	28	B/O-PRACHI	MALE	2	VLBW	VD	YES	36	8	PRETERM	NICU		K.PNEUMONIAE	8	9	98.4	mg/l	2.2	ng/ml	YES	
80	32	B/O-RANI	MALE	2.6	LBW	LSCS	NO	35	8	PRETERM	NICU		S.HAEMOLYTICS	5	7	84	mg/l	1.8	ng/ml	NO	
81	22	B/O-ROOPALI	MALE	2.5	LBW	LSCS	NO	35	8	PRETERM	NICU		K.PNEUMONIAE	6	9	88	mg/l	1.5	ng/ml	YES	
82	27	B/O-RAKSHA	MALE	2.5	LBW	VD	NO	40	7	TERM	NICU		K.PNEUMONIAE	7	9	40	mg/l	0.5	ng/ml	NO	

83	24	B/O-JYOTI	MALE	2	VLBW	LSCS	YES	34	8	PRETERM	NICU		S.HAEMOLYTICS	6	8	60	mg/l	1.1	ng/ml	NO	
84	25	B/O-ANUKRITI	FEMALE	2.5	LBW	LSCS	NO	36	8	PRETERM	NICU		K.PNEUMONIAE	6	8	88	mg/l	1.5	ng/ml	NO	
85	23	B/O-NIKITA	MALE	2.8	NBW	LSCS	NO	40	7	TERM	NICU		K.PNEUMONIAE	5	8	62	mg/l	1	ng/ml	NO	
86	24	B/0-CHANDHNI	FEMALE	2.6	LBW	LSCS	NO	36	8	PRETERM	NICU		S.HAEMOLYTICS	6	9	40	mg/l	0.5	ng/ml	YES	
87	28	B/O-KANIKA	MALE	2.7	LBW	VD	NO	40	8	TERM	NICU		K.PNEUMONIAE	6	7	34	mg/l	0.8	ng/ml	NO	
88	31	B/O-HARSHA	FEMALE	1.98	VLBW	LSCS	NO	34	7	PRETERM	NICU		K.PNEUMONIAE	7	8	28	mg/l	0	ng/ml	NO	**REFUSED**
89	26	B/O-PREETI	MALE	2.5	LBW	VD	NO	36	7	PRETERM	NICU		S.HAEMOLYTICS	3	8	33	mg/l	0.1	ng/ml	YES	
90	36	B/O-PIYUSHI	MALE	2.5	LBW	LSCS	NO	40	7	TERM	NICU		K.PNEUMONIAE	7	8	38	mg/l	0.2	ng/ml	NO	
91	22	B/O-PHULWATI	MALE	1.8	VLBW	LSCS	YES	35	8	PRETERM	NICU		S.HAEMOLYTICS	5	7	168	mg/l	2	ng/ml	YES	DEATH
92	18	B/0-RAJNESH	MALE	2.5	LBW	LSCS	NO	35	8	PRETERM	NICU		A.BAUMARII	5	7	99.2	mg/l		ng/ml	YES	REFUSED
93	24	B/O-PEEHU	MALE	2.5	LBW	VD	NO	40	7	TERM	NICU		K.PNEUMONIAE	6	9	84	mg/l	2.2	ng/ml	NO	
94	27	B/O-SHAMBHAVI	MALE	2	VLBW	LSCS	YES	34	8	PRETERM	NICU		K.PNEUMONIAE	7	9	88	mg/l	1.8	ng/ml	NO	
95	22	B/O-NEHA	FEMALE	2.5	LBW	LSCS	NO	36	8	PRETERM	NICU		S.HAEMOLYTICS	6	8	58	mg/l	1.5	ng/ml	NO	
96	23	B/O-ABHI	MALE	1.8	VLBW	LSCS	NO	40	7	TERM	NICU		K.PNEUMONIAE	5	7	145	mg/l	1.2	ng/ml	YES	DEATH
97	25	B/O-SAKSHI	FEMALE	2.6	LBW	LSCS	NO	36	8	PRETERM	NICU		K.PNEUMONIAE	5	8	88	mg/l	1.1	ng/ml	NO	
98	23	B/O-PRERNA	MALE	2.7	LBW	VD	NO	40	8	TERM	NICU		S.HAEMOLYTICS	6	9	62	mg/l	2.2	ng/ml	YES	
99	26		FEMALE	1.98	VLBW	LSCS	NO	34	7	PRETERM	NICU		K.PNEUMONIAE	6	7	55	mg/l	1.8	ng/ml	NO	
100	20		MALE	2.5	LBW	VD	NO	36	7	PRETERM	NICU		K.PNEUMONIAE	7	8	34	mg/l	1.5	ng/ml	NO	
101	26		MALE	2.5	LBW	LSCS	NO	40	7	TERM	NICU		S.HAEMOLYTICS	3	8	28	mg/l	0	ng/ml	NO	REFUSED