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Research Article

A Prospective Comparative Study of Umbilical Cord Blood Culture Versus Peripheral Venous Blood Culture in Diagnosis of Early Onset Neonatal Sepsis in Neonatal Intensive Care Unit of Tertiary Care Hospital in Bhavnagar, Gujarat, India

Pratyush Jain*, Mehul Gosai

Department of Pediatrics, Govt. Medical College and Sir T. Hospital, Bhavnagar, Gujarat, India

ABSTRACT

Background: Neonatal sepsis is a major cause of neonatal morbidity and mortality. Blood culture and sensitivity is gold standard for the diagnosis of neonatal sepsis. Low sensitivity of blood culture especially in newborn is due to small volume of blood sample collected from neonates and antibiotics given before sampling. The aim was to evaluate the use of umbilical cord blood culture in the diagnosis of early onset neonatal sepsis as compared to Peripheral vein blood culture.

Aims and Objective: To compare the sensitivity and specificity of umbilical cord blood culture and peripheral vein blood culture in diagnosis of early onset neonatal sepsis. also, to compare the organisms identified by umbilical cord blood culture and venous blood culture.

Research methodology: A prospective, analytical, cross sectional study where comparison of umbilical cord blood culture and peripheral venous blood culture was done in 100 inborn neonates who fulfilled the inclusion exclusion criteria. Sensitivity and specificity, positive and negative predictive values were calculated. The results were evaluated and comparison of two methods was done. P value was calculated, Chi Square test was applied and association was quantified.

Discussion and Conclusion: The association between both methods was found to be significant in our study. The higher sensitivity (81.0%) and accuracy (87%) for predicting disease outcome of patients by UCBC method against PVBC Method conclude that UCBC can be used as reliable and alternate tool to predict the final outcome. Similarly, the high specificity of 88.6% and moderate NPV of 94.59% shows a higher diagnosis capacity of negative outcome by UCBC method as compared to gold standard PVBC method.

Keywords: Umbilical cord blood culture; Sepsis; Peripheral venous blood culture

INTRODUCTION

Neonates constitute the nation's foundation and mothers are its pillars and no one can afford to neglect their needs and rights"- UNICEF. Neonatal period is considered the most important age group at all times as new borns are most susceptible to diseases and death. Historically the probability of death during neonatal period was so high that many traditional practices were postponed until after first week of life, ensuring the probability of child's survival. Also, the quality of life and health as the child grows to adult life is partly determined at this stage. Neonatal sepsis is the most common cause of neonatal mortality. It accounts for nearly 3 million neonatal deaths per year and an estimated neonatal mortality rate of 23. 9 per 1000 live births globally [1]. About 2% of foetuses are infected in utero and up to 10% of infants have infections in the 1st month of life [2]. Sepsis related mortality is largely preventable with prevention of sepsis itself, timely recognition, rational antimicrobial therapy and aggressive supportive care. The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database is 30 per 1000 live births. The NNPD network comprising of 18 tertiary care neonatal units across India found sepsis to be one of the commonest causes

Correspondence to: Pratyush Jain, Department of Pediatrics, Govt. Medical College and Sir T. Hospital, Bhavnagar, Gujarat, India, Tel: +09993352124; E-mail: jainpratyush@gmail.com

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of neonatal mortality contributing to 19% of all neonatal deaths [3]. Among intramural births, *Klebsiella pneumoniae* was the most frequently isolated pathogen (32.5%), followed by *Staphylococcus aureus* (13.6%). Among extramural neonates (referred from community/other hospitals), *Klebsiella pneumoniae* was again the commonest organism (27%), followed by *Staphylococcus aureus* (15%) and *Pseudomonas* (13%).

Neonatal sepsis is defined as a blood stream infection which develops within 28 days after birth [4]. Early onset neonatal sepsis is defined as an infection within the 1st three days of life and is associated with transmission of organism from birth canal. The mortality associated with early onset neonatal sepsis is higher than that of late onset sepsis [5]. Early recognition of sepsis is required for prompt initiation of antibiotics to prevent neonatal morbidity and mortality [6]. Gold standard for the diagnosis of neonatal sepsis is blood culture collected from peripheral veins. Blood cultures remain the mainstay of laboratory diagnosis of Bloodstream Infections (BSIs) in infants and children. Recovery of a pathogen is advantageous, as it confirms the diagnosis of bacteraemia and allows for identification and susceptibility testing on the organism to optimize antimicrobial therapy and duration. A negative blood culture is just as important, as it rules out cases of bacteraemia and prompts continued investigation of other infectious or non-infectious etiologies or cessation of unnecessary empirical antimicrobial therapy [7]. Variable sensitivity of blood culture is mainly due to inadequate sample volume, intrapartum antibiotics, and administration of antibiotics prior to sample collection [8]. Other sites of blood collection for blood culture are heel prick collection, blood from arterial and central venous lines, and umbilical (neonatal end) vein [5]. Umbilical cord (placental end) is a less commonly used site for collection of blood culture. There are some studies on Umbilical Cord Blood Culture (UCBC) for diagnosis of neonatal sepsis, which suggest umbilical cord blood can be collected for blood culture safely and without contamination. Umbilical cord blood collection procedure for culture is painless and it ensures adequate volume of blood for culture with less contamination [9]. Serum proteins like C-Reactive Protein (CRP), haptoglobin and fibrinogen, can be used as non- specific indicators of bacterial sepsis. However, the utility of CRP for the diagnosis of neonatal infection has been the subject of controversy because of its low sensitivity [10]. There are less published data to support umbilical cord blood culture's routine use in early onset neonatal sepsis. Need of research on this topic is we have to move from invasive to non-invasive procedure in diagnosis of early onset sepsis. The early identification of septic neonates is difficult because subtle initial signs are not seen or not present. This study was carried out to evaluate the utility of umbilical cord blood culture in neonates at high risk for early onset neonatal sepsis in comparison to peripheral vein blood culture.

AIMS & OBJECTIVES

The aim is to compare umbilical cord blood culture in the diagnosis of early onset neonatal sepsis as compared to Peripheral vein blood culture.

The objectives are:

- To determine the sensitivity and specificity of umbilical cord blood culture vs peripheral vein blood culture in diagnosis of sepsis.
- To correlate Umbilical cord blood culture with septic screen results.
- To compare organisms identified by umbilical cord blood culture and venous blood culture.

REVIEW OF LITERATURE

Neonatal mortality is increasingly recognized as an important global public health challenge that must be addressed if we are to reduce child health disparities between rich and poor countries. Most of the estimated 4 million neonatal deaths per year occur in low and middle income countries. Three conditions: infection, birth asphyxia, and consequences of premature birth/low birth weight, are responsible for majority of these deaths. More than one-third are estimated to be due to severe infections, and a quarter are due to the clinical syndrome of neonatal sepsis/ pneumonia. Case fatality rates for neonatal infections remain high among both hospitalized new borns and those in the community [11,12]. Globally, of the three million annual neonatal sepsis cases (2202/1,00,000 live births), India has the highest incidence of clinical sepsis (17,000/ 1,00,000 live births) [13]. The case fatality rate of sepsis among neonates ranges between 25% to 65% in India [14,15]. In general, the identification and treatment of new borns with infection is unsatisfactory in many developing country settings. Because sick new borns present with nonspecific signs and symptoms, a clinical diagnosis of neonatal sepsis is difficult in even the most sophisticated settings. Many factors contribute to the high mortality due to infections, including under-recognition of illness, delay in care seeking at the household level, and lack of access to both appropriately trained health workers and to high quality services to manage sepsis. Even if quality services are available, the cost of treatment is beyond the reach of many families. It is particularly poignant that many neonatal deaths occur in the community, without the new born ever having contact with the appropriate health services. Over the past several years, attempts have been made to raise awareness of the contribution of neonatal problems to overall infant mortality and to promote strategies to reduce mortality at the country level [16,17]. Early-onset sepsis is defined as the onset of symptoms before 7 days of age, although some experts limit the definition to infections occurring within the 1st 72 hr of life. Late-onset sepsis is generally defined as the onset of symptoms at ≥ 7 days of age. Similar to early-onset sepsis, there is variability in the definition, ranging from an onset at >72 h of life to ≥ 7 days of age. Early onset sepsis is conventionally regarded as maternally-acquired, with causative organisms, such as *Escherichia coli* and *Group B Streptococcus* (GBS) usually found in the maternal genital tract, whereas late onset sepsis is considered environmental in origin-either hospital or community acquired. Commonly implicated organisms in hospital acquired infections are *coagulase-negative Staphylococci*, *Staphylococcus aureus* and Gram-negative organisms such as *Klebsiella* and *Pseudomonas species*.

Infections in the new born are often classified by their timing relative to birth and include congenital, perinatal, early-onset, and late-onset disease. These are clinically useful designations because the mechanisms of infection, etiologies, and outcomes are distinct at each stage. Congenital infection denotes infection acquired in utero. Such infections are generally caused by viral or other non-bacterial organisms and are often associated with injury to developing organs. Perinatal infection indicates acquisition around the time of delivery. Perinatally acquired organisms include both bacteria and viruses, some of which are the same as those causing congenital

infection but often manifest with different features. Hospital-acquired infections typically occur beyond the1st week of life. Neonates are uniquely prone to invasive disease because of their lack of fully responsive innate immunity. Attenuated immune responses often result in minimal or nonspecific clinical manifestations, and effective treatment requires attention to subtle signs of infection. Compared to older infants, new borns are often treated empirically while awaiting results of laboratory investigations. Preterm infants are particularly susceptible to infection because of their decreased innate immune and barrier defences and their prolonged stay in hospital settings. A number of bacterial and nonbacterial agents may infect new borns in the intra partum or postpartum period. Although herpes Simplex Virus (HSV), Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and Tuberculosis (TB) can each result in transplacental infection, the most common mode of transmission for these agents is intra partum, during labor and delivery with passage through an infected birth canal (HIV, HSV, HBV), or postpartum, from contact with an infected mother or caretaker (TB) or with infected breast milk (HIV). Any microorganism inhabiting the genitourinary or lower gastrointestinal tract may cause intra partum and postpartum infection.

Early-onset infections are acquired before or during delivery (vertical mother-to-child transmission). Late-onset infections develop after delivery from organisms acquired in the hospital or the community. The age at onset depends on the timing of exposure and virulence of the infecting organism. Very-late- onset infections (onset after age 1 month) may also occur, particularly in Very- Low-Birth Weight (VLBW) preterm infants or term infants requiring prolonged neonatal intensive care. Studies suggest that term male infants have a higher incidence of sepsis than term females. This sex difference is less clear in preterm Low-Birth Weight (LBW) infants. Attack rates of neonatal sepsis increase significantly in LBW infants in the presence of maternal chorioamnionitis, congenital immune defects, mutations of genes involved in the innate immune system, asplenia, galactosemia (*E. coli),* and malformations leading to high inocula of bacteria (e.g., obstructive uropathy). The overall rate of early-onset sepsis was 0.98 cases per 1,000 live births, with rates inversely related to birth weight: 401-1,500 g, 10.96 per 1,000 births; 1,501-2,500 g, 1.38/1,000; and >2,500 g, 0.57/1,000.

In most cases, the fetus or neonate is not exposed to potentially pathogenic bacteria until the membranes rupture and the infant passes through the birth canal and/or enters the extra uterine environment. The human birth canal is colonized with aerobic and anaerobic organisms that may result in ascending amniotic infection and/or colonization of the neonate at birth. Vertical transmission of bacterial agents that infect the amniotic fluid and vaginal canal may occur in utero or, more often, during labor and delivery. Chorio-amnionitis results from microbial invasion of amniotic fluid, often as a result of prolonged rupture of the chorioamniotic membrane. Amniotic infection may also occur with apparently intact membranes or with a relatively brief duration of membrane rupture. The term chorioamnionitis refers to the clinical syndrome of intrauterine infection, which includes maternal fever, with or without local or systemic signs of chorioamnionitis (uterine tenderness, foul-smelling vaginal discharge/amniotic fluid, maternal leukocytosis, maternal and/or fetal tachycardia). Chorioamnionitis may also be asymptomatic, diagnosed only by amniotic fluid analysis or pathologic examination of the placenta.

Chorioamnionitis was thought to result from infection of the amniotic fluid but is now better defined by the term intrauterine inflammation or infection at birth (Triple I). This is defined by fetal tachycardia, maternal leukocytosis (>15,000 cells in the absence of corticosteroids), purulent fluid from the cervical OS, biochemical or microbiologic amniotic fluid changes consistent with infection, and fever (≥ 39.0°C/102°F). Rupture of membranes for >24 hour was once considered prolonged because microscopic evidence of inflammation of the membranes is uniformly present when the duration of rupture exceeds 24 hours. At 18 hour of membrane rupture, however, the incidence of early-onset disease increases significantly; 18 hours is the appropriate cut-off for increased risk of neonatal infection. Aspiration or ingestion of bacteria in amniotic fluid may lead to congenital pneumonia or systemic infection, with manifestations becoming apparent before delivery (fetal distress, tachycardia), at delivery (failure to breathe, respiratory distress, shock) or after a latent period of a few hours (respiratory distress, shock). Aspiration or ingestion of bacteria during the birth process may lead to infection after an interval of 1-2 days. Resuscitation at birth, particularly if it involves endotracheal intubation, insertion of an umbilical vessel catheter, or both, is associated with an increased risk of bacterial infection. Explanations include the presence of infection at the time of birth or acquisition of infection during the invasive procedures associated with resuscitation. The most important neonatal factor predisposing to infection is prematurity or LBW. Preterm LBW infants have a 3-10-fold higher incidence of infection than full-term normal-birth weight infants. Possible explanations include: -

- Maternal genital tract infection is considered to be an important cause of preterm labor, with an increased risk of vertical transmission to the new born
- The frequency of intra amniotic infection is inversely related to gestational age
- Premature infants have documented immune dysfunction
- Premature infants often require prolonged intravenous access, endotracheal intubation, or other invasive procedures that provide a portal of entry or impair barrier and clearance mechanisms, putting them at continued risk for hospital-acquired infections.

Signs and symptoms in the neonate are often subtle and nonspecific. Temperature instability, tachypnoea, lethargy, and poor feeding are common initial signs and should raise suspicion for systemic or focal infection [18]. Definition of perinatal risk factors is explained in Table 1.

Preterm Infants

Preterm infants will often have apnea, bradycardia, and cyanosis (104/158; 65.8%) as the first sign of infection Additionally, Lim et al. reported a high incidence of poor activity, presumably lethargy (77/158; 48.7%) and increased respiratory effort (68/158; 43.0%). In general, symptoms are more severe with Gram-negative and fungal infections than with Gram-positive infections.

Term Infants

Signs of EOS in term infants typically present by the first 6 h, and the majority present usually within the first 24 h of life. Most infants will present with respiratory distress, which can masquerade as other diagnoses such as Congenital Heart Disease,

Respiratory Distress Syndrome (RDS), pneumothorax, transient tachypnoea of new borns, Congenital Diaphragmatic Hernia, and other congenital masses in the chest. Sepsis should be the initial differential diagnosis for each of these. In mildly symptomatic new borns, it is acceptable to monitor the new born for 6 h before performing a Complete Blood Count (CBC) and starting antibiotics. If the infant clinically improves, sepsis is very unlikely; if symptoms progress, blood culture and LP with CSF culture and studies should be obtained prior to initiation of antibiotics, and antibiotics should be started promptly. Most cases (80%-90%) of EOS will present in the first 24-48 hour of life. When evaluating a new born for suspected sepsis/meningitis, a thorough review of antenatal risk factors should be performed, as this may help guide therapy. Such factors include documentation of maternal colonization status with pathogen, gestational age of the infant, prolonged rupture of membranes, intra-amniotic infection, younger maternal age, and previous delivery of an infant with invasive infective diseases. Frequent evaluation of the new born is critical in order to recognize the signs and symptoms of disease during the neonatal period, which can range from nonspecific to multiorgan failure.

LABORATORY FINDINGS AND DIAGNOSTICS

The typical complete sepsis workup in a neonate consists of obtaining a complete white blood cell count with differential, a single blood culture, urine cultures, and a lumbar puncture for cell count and culture. In addition, there may be a role for culture and Gram staining of tracheal aspirates in incubated neonates shortly after birth. Acute-phase reactants, such as C-Reactive Protein (CRP) and Procalcitonin (PCT), along with hematologic scoring systems are increasingly being used to assist in the diagnosis of infants with suspected sepsis. The need for a chest radiograph is usually determined by the presence of respiratory symptoms [19]. Components of a Septic Screen (Table 2).

Blood testing for neonatal sepsis

White blood count and differentials: Many investigators have analysed subcomponents of the white blood cell count (neutrophil indices)-absolute neutrophil count, absolute band count, and immature to total neutrophil (I/T) ratio-to identify infected infants. Like most diagnostic tests for neonatal sepsis, neutrophil indices have proven most useful for excluding infants without infection rather than identifying infected neonates. Neutropenia may be a better marker for neonatal sepsis and has better speci city than an elevated neutrophil count, because few conditions besides sepsis (maternal pregnancy-induced hypertension, asphyxia, and haemolytic disease) depress the neutrophil count of neonates. The definitions for neutropenia vary with gestational age, type of delivery (infants born by caesarean delivery without labor have lower counts than infants delivered vaginally), site of sampling (neutrophil counts are lower in samples from arterial blood), and altitude (infants born at elevated altitudes have higher total neutrophil counts). In late preterm and term infants, the definition for neutropenia most commonly used is that suggested by Manroe et al. (<1800/mm^3 at birth and <7800/mm^3 at 12-14 hours of age). The absolute immature neutrophil count follows a similar pattern to the absolute neutrophil count and peaks at approximately 12 hours of life. The number of immature neutrophils increases from a maximal value of 1100 cells/mm^3 at birth to 1500 cells/mm^3 at 12 hours of age. Absolute immature counts have a poor sensitivity and positive predictive accuracy for early-onset sepsis. Furthermore, if exhaustion of bone marrow reserves occurs, the number of immature forms will remain depressed. The I/T ratio has the best sensitivity of any of the neutrophil indices. A single determination of the I/T ratio has a poor positive predictive accuracy (approximately 25%) but a very high negative predictive accuracy (99%). The I/T ratio may be elevated in 25%-50% of uninfected infants. Exhaustion of bone marrow reserves will result in low band counts and lead to falsely low ratios. The timing of the white blood cell count is critical. Counts obtained 6-12 hours after birth are more likely to be abnormal than are counts obtained at birth, because alterations in the numbers (and ratios) of mature and immature neutrophils require an established inflammatory response. Therefore, once the decision is made to start antimicrobial therapy soon after birth, it is worth waiting 6-12 hours before ordering a white blood cell count and differential count [20-22].

Platelet counts: Platelet counts are not very sensitive or specific for the diagnosis of neonatal sepsis and not are very helpful in monitoring the response to therapy.

Table 1: Definition of perinatal risk factors.

Prolonged rupture of membranes (PROM)	Interval between rupture of membranes and birth of baby; > 18 hours.
History of Maternal Fever	Fever in mother > 38°C before delivery with evidence of bacterial infection in the mother in hemogramor culture. Exclusion of malaria byperipheral blood film examination.
Prematurity and Low birth weight (LBW)	<37 week, <2500 gm
Foul smelling liquor	Abnormal smell of liquor asperceived by attending obstetrician oneonatologist. Frankly purulent liquor.
Birth asphyxia (BA)	Apgar score at 5 minutes and or requirement of Intermittent positive pressure respiration for resuscitation or intubation

Table 2: Components of a septic screen.

Components	Abnormal Value
Total leukocyte count	<5000/mm^3
Absolute neutrophil count	Low counts As Per Monroe[20] and
Immature/total neutrophil	Mouzinho[21] Charts (Term and VLBW infants)
Immature/total neutrophil	>0.2
Micro-Erythrocyte sedimentation	>15mm in 1st hour

Acute-phase reactants: CRP and procalcitonin are the two most commonly studied acute-phase reactants in neonatal sepsis. CRP levels rise within 6-8 h of infection and peak at 24 h. Inflammation triggers the release of IL-6, which stimulates an increase in CRP concentrations. Depending on the study, individual CRP values of 0.2 to 95 mg/litre (mean, 1.7 mg/litre; median, 10 mg/litre) have a sensitivity range of 41-96% and a specificity range of 72 to 100% for neonatal sepsis. A value of 10 mg/litre is the most commonly used cut-off in most published studies. Viral infections are not usually associated with an elevated CRP level, and if the CRP level is elevated, it is usually <5 mg/litre. CRP has its best predictive value if measured within 24-48 h of onset of infection. An increasing CRP level is a better predictor than individual values. Two normal CRP determinations (8 to 24 h after birth and 24 h later) have been shown to have a negative predictive value of 99.7% and a negative likelihood ratio of for proven neonatal sepsis. Thus, repeatedly normal CRP values are strong evidence against bacterial sepsis and can enable antibiotics to be safely discontinued.

Procalcitonin is a pro-peptide of calcitonin produced mainly by monocytes and hepatocytes that is significantly elevated during infections in neonates, children, and adults. The half-life is about 24 h in peripheral blood. The normal level for neonates >72 h of age is usually <0.1 ng/ml. While procalcitonin has been used primarily in research settings, it is increasingly being used as a guide in managing infections in real time by clinical laboratories and generally takes about 90 min-2 h to process. In general, procalcitonin is more sensitive for earlier detection of sepsis than is CRP. The procalcitonin level is more likely to be elevated during bacterial infections than during viral ones and declines rapidly with appropriate therapy. However, a physiologic increase in the procalcitonin concentration occurs within the first 24 h of birth, and elevated levels in serum can occur under non-infectious conditions (e.g., infants with respiratory distress syndrome, hemodynamic instability, and diabetic mothers). Procalcitonin is also useful for detecting neonatal nosocomial sepsis. The probability of nosocomial sepsis is doubled with a PCT of > 0.5 ng/ml for VLBW infants (<1,501 g).

Other biomarkers

Cytokines, including interleukin 6 (IL-6), interleukin 8 (IL-8), gamma interferon (IFN-γ), and tumor necrosis factor alpha (TNF-α), and cell surface antigens, including soluble intercellular adhesion molecule (sICAM) and CD-64, have also all been studied as measures for neonatal sepsis, but none are currently in routine clinical use.

They all generally have very similar sensitivities and specificities. Based on a systematic review of the global literature have classified biomarkers for detection neonatal sepsis into three categories: -

- Early phase (IL-6, IL-8, CD64, sICAM, TNF- α, and IFN- γ)
- Mid phase (PCT)
- Late phase (CRP)

IFN- γ levels are particularly responsive early in detection of viral infections.

Molecular testing

Molecular methods for detection of neonatal sepsis in blood include PCR and DNA microarray-based methods. Most of these tests hold the promise of rapid detection directly from blood without prior culture combined with high sensitivity and specificity in relation to cultures. Of these tests, PCR-based methods have been the most studied for neonates.

PCR techniques are increasingly being used for the diagnosis of neonatal sepsis in research and some clinical laboratories. They have a high sensitivity in relation to culture when positive organisms identified are considered the gold standard as a result of detection of bacterial DNA, and pathogens can be detected at much lower concentrations. There is promise of faster diagnosis (as quickly as 30 min) and quicker time to begin appropriate targeted therapy with the use of real-time PCR that utilizes the detection of fluorescent signals generated during each amplification cycle and is able to give some measure of bacterial load. This approach holds much promise but is currently still limited primarily to research laboratories due to the relatively high cost and the detection limit of the organisms targeted in the kit. Furthermore, false-negative results may still occur if the etiologic organism is not included in the kit. In addition, the use of sterile venipuncture to collect the specimen may prove tricky for some neonates.

Urine testing

Neonates with suspected sepsis in the first few days of life (<72 h) do not need urine obtained for chemical and microscopic analysis because most infections of the urinary tract in this population are secondary to hematogenous seeding of the kidney by bacteraemia. However, subsequent workups for sepsis should include careful consideration of a urinalysis and urine culture, especially in symptomatic neonates. Only specimens obtained by suprapubic aspiration or urethral catheterization are appropriate for urine cultures due to the risk of bacterial contamination. Catheter-obtained urine cultures have a sensitivity of ≥95% and a specificity of ≥99% compared to suprapubic tap specimens when >1,000 CFU/ml of bacteria of a single colony are identified.

In contrast, bag urine specimens have a sensitivity of 100% but low specificity (14% to 84%). Urine analysis may be helpful in providing adjunctive information to support or rule out the diagnosis of urinary tract infection (UTI). The dipstick reagent tests related to possible UTI are leukocyte esterase (LE), blood, nitrite, and protein tests.

Cerebrospinal fluid testing

The incidence of meningitis in neonatal sepsis has varied from 0.3-3% in various studies [23]. While Lumbar Puncture (LP) is an important means of obtaining Cerebrospinal Fluid (CSF) to rule out the presence of meningitis in infants with suspected sepsis, its routine use in neonates is controversial. The risk of concomitant meningitis in high-risk neonates who appear healthy or those whose clinical signs appear to be due to non-infectious conditions such as Respiratory distress syndrome is very low. For this reason, there should be a very low threshold for obtaining CSF through LP in neonates who have a strong clinical picture suggestive of neonatal sepsis or who end up with a positive blood culture and who have not previously had an LP. Furthermore, up to 38% of those with meningitis will have a negative blood culture; hence, lumbar puncture should be a component of every neonatal sepsis evaluation and not just performed if cultures return positive. It

should be noted that an LP done in the setting of previous receipt of antibiotics by the neonate could lead to falsely negative CSF cultures. Conditions that may lead to a delay or cancellation of lumbar puncture include severely ill infants with either cardiovascular or respiratory distress, tense or bulging anterior fontanelle, the presence of severe thrombocytopenia, or infection around the lumbosacral region [19]. Normal cerebrospinal fluid examination in neonates (Tables 3 and 4) [24].

Blood cultures

Gold standard for isolation and verification of microbial pathogens of sepsis are blood cultures. Correct diagnosis of sepsis is based on a combination of clinical and laboratory findings. Blood cultures findings (positive or negative) should be taken with a reserve due to low bacterial load, presence of biofilms or antibiotic treatment. It is known that new borns with sepsis often have negative blood culture findings. For the diagnosis of sepsis, in premature infants, blood culture should be taken, but the diagnosis should be set on the basis of other parameters, in particular regarding the values of procalcitonin and C-reactive protein.

All neonates suspected of having sepsis should have a blood sample sent for cultures. It is presumed that bacteraemia's with high concentrations of organisms require less blood to be sampled than low density bacteraemia's. The concentration of a variety of common pathogens in neonatal and paediatric bacteraemia's has been documented in numerous studies using quantitative culture systems. Despite many organisms occurring in high concentrations, low density bacteraemia is also recorded for most pathogens [25].

The volume of blood needed for cultures for neonates is substantially lower than that needed for adults because neonates tend to have a 1-log-higher concentration of bacteria in their bloodstream than adults. As a result, 0.5 ml was traditionally considered the standard volume of blood adequate to detect bacteraemia in neonates. However, some recent studies have shown that up to one-quarter of all neonates with sepsis have bacteraemia involving low colony counts (≤ 4 CFU/ml), and two-thirds of those <2 months old have colony counts of <10 CFU/ml. A 0.5 ml volume of blood has been shown to be insufficient to detect most infants with these levels of bacteraemia, while 1.0 ml doubles the likelihood of a positive yield for these reasons, several experts now recommend that 1.0 ml of blood should be the minimum volume to be inserted into a single paediatric blood culture bottle. The blood is most frequently drawn from a peripheral vein, but samples obtained from an umbilical catheter shortly after insertion are also acceptable. Blood drawn from the umbilical vein has a much greater risk of being contaminated unless obtained during delivery from a carefully cleaned segment of a doubly clamped cord [23]. In the absence of other comorbidities, both EONS and LONS respond adequately to appropriate antibiotics when introduced in the early stage of sepsis. Hence, early recognition of sepsis is imperative for prompt initiation of proper antibiotic therapy to prevent adverse clinical outcomes [26]. It is well accepted that the volume of blood collected is the single most important factor. Evidence from both adult and paediatric studies show that the probability of recovering a pathogen from blood culture increases with the volume of blood obtained. In addition, the time to detection inversely correlates with the volume of blood cultured. Optimal collection of blood volume is particularly relevant in the paediatric population, as collecting a sufficient volume can be difficult due to the diminutive size of the patients and the risk of requiring blood transfusion to compensate for repeated phlebotomy [27].

The important factors in the ability of blood cultures to detect significant organisms include:

1. Volume of blood drawn
2. Dilution: - the ratio of blood to culture medium in the blood culture bottle
3. Number of cultures taken

Table 3: Normal cerebrospinal fluid examination in neonates.

CSF Components	Normal Range
Cells/mm^3	8 (0-30)
PMN (%)	60%
CSF Protein (mg/dl)	90 (20-170)
CSF Glucose (mg/dl)	52 (34-119)
CSF /Blood Glucose (%)	51

Table 4: Normal cerebrospinal fluid examination in neonates.

Symptoms	Frequency	Percent
Abdominal Distension	12	12
Bleeding	1	1
Feed intolerance	14	14
Hypoglycemia	6	6
Hypothermia	6	6
Jaundice	12	12
No symptoms	25	25
Sclerema	1	1
Respiratory Distress	10	10
Vomiting	13	13
Total	100	100

4. Blood culture technique, including skin preparation and choice of culture site
5. Timing of culture
6. Choice of blood culture bottle and system

(including whether it preferentially detects aerobic or anaerobic organisms) [28]. Increasing the volume of blood inoculated into blood culture bottles improves the timely detection of bacteraemia in paediatric patients and spares the patients the cost and pain of an additional venipuncture [29].

Despite the uniform use of blood cultures for the diagnosis of sepsis, the optimal volume of blood required to detect bacteraemia in new borns has not been established. Investigations using quantitative blood cultures for new borns with septicaemia have stimulated questions concerning the sensitivity of cultures utilizing small blood volumes. Although dissimilar techniques of blood collection and methodology make it difficult to compare published reports, at least 1.0 ml of blood has been recommended for cultures of new borns. However, in critically ill very low birth weight infants with delicate peripheral veins, volumes of 1 ml or more may be difficult to obtain. Furthermore, blood sampling for diagnostic studies may increase the need for blood transfusions [30]. The Umbilical Cord Blood Culture (UCBC) has been suggested as an alternative method for detecting neonatal bacteraemia, but has not come into general clinical use because of reported high incidence of false positive results as compared to those of postnatal peripheral venous blood cultures. Lack of precision in technique of sampling and inoculation of culture media in these studies may account for high incidence of positive UCBCs [31]. An inadequate amount of blood samples, faulty collection techniques, and antibiotic exposure (both intra-partum and post-partum) are hindering factors that can reduce the sensitivity of blood culture. Peripheral veins are used most commonly to collect blood from neonates. Heel, arterial, or venous line and the umbilical cord are the other sites accessed for blood collection. Although the umbilical cord is infrequently used for blood collection, the procedure is painless and technically less challenging when compared to peripheral veins. Moreover, it ensures adequate volume of blood for culture with less contamination. Currently, there is inadequate published data to support its routine use in neonatal sepsis [32].

Radiology

Chest x-ray should be considered in the presence of respiratory distress or apnea. An abdominal x-ray is indicated in the presence of abdominal signs suggestive of Necrotizing Enterocolitis (NEC). Neurosonogram and Computed Tomography (CT scan) should be performed in all patients diagnosed to have meningitis.

Management of neonatal sepsis

Adequate and proper supportive care is crucial in a sick neonate with sepsis. He/she should be nursed in a thermo-neutral environment taking care to avoid hypo/hyperthermia. Oxygen saturation should be maintained in the normal range; mechanical ventilation may have to be initiated if necessary. If the infant is hemodynamically unstable, intravenous fluids should be administered and the infant is to be monitored for hypo/hyperglycemia. Volume expansion with crystalloids/colloids and judicious use of inotropes are essential to maintain normal tissue perfusion and blood pressure. Packed red cells and fresh frozen plasma might have to be used in the event of anaemia or bleeding diathesis.

Prophylactic antibiotics

There is no use of prophylactic antibiotics in the following circumstances:

- Infants on IV fluids/TPN
- Meconium aspiration syndrome
- After exchange transfusion(s).

An exchange transfusion conducted under strict asepsis (single use catheter, sterile gloves, removal of catheter after the procedure) does not increase the risk of sepsis and hence does not merit antibiotics. However, a messy exchange transfusion could be treated with prophylactic antibiotics.

Choice of antibiotics

Empirical antibiotic therapy should be unit-specific and determined by the prevalent spectrum of etiological agents and their antibiotic sensitivity pattern. Antibiotics once started should be modified according to the sensitivity reports. Consider Vancomycin if MRSA is suspected. The empirical choice of antibiotics is dependent upon the probable source of infection. For infections that are acquired during hospital stay, resistant pathogens are likely and a combination of ampicillin or cloxacillin with gentamicin or amikacin may be instituted. 3rd generation cephalosporins have very good CSF penetration and are traditionally thought to have excellent antimicrobial activity against gram negative organisms.

A combination of piperacillin-tazobactam with amikacin should be considered if *pseudomonas sepsis* is suspected. Penicillin resistant *staphylococcus aureus* should be treated with cloxacillin, nafcillin or methicillin. Addition of an aminoglycoside is useful in therapy against *staphylococcus*. Methicillin Resistant *Staphylococcus aureus* (MRSA) should be treated with a combination of ciprofloxacin or vancomycin with amikacin. Ciprofloxacin has excellent activity against gram negative organisms also; however, it does not have good CSF penetration. It may be used for the treatment of resistant gram-negative bacteraemia after excluding meningitis.

For sepsis due to enterococcus, a combination of ampicillin and gentamicin is a good choice for initial therapy. Vancomycin should be used for the treatment of enterococcus resistant to the first line of therapy.

Reserve antibiotics

Newer antibiotics like aztreonam, meropenem and imipenem are also now available in the market. Aztreonam has excellent activity against gram-negative organisms while meropenem is effective against most bacterial pathogens except methicillin resistant *staphylococcus aureus* (MRSA) and enterococcus. Imipenem is generally avoided in neonates because of the reported increase in the incidence of seizures following its use. Empirical use of these antibiotics should be avoided; they should be reserved for situations where sensitivity of the isolated organism warrants its use [33].

Neurological sequleae of neonatal sepsis

Infection is an important problem among VLBW preterm infants.

Previous studies from the NICHD Neonatal Research Network have demonstrated that as many as 25% of these infants have 1 or more positive blood cultures and about 5% have a positive cerebrospinal fluid culture over the course of their neonatal hospitalization. Rates of infection increase with decreasing birth weight and gestational age. Moreover, postnatal infection is associated with an increased risk of neonatal complications, prolonged hospitalization, and death.

Possible interventions to reduce brain injury associated with infection might include earlier diagnosis and improved therapies, including efforts to stabilize blood pressure and maintain adequate oxygenation, reduction of systemic inflammation and generation of proinflammatory cytokines, and pharmacologic interventions to reduce the impact of reactive oxygen species on vulnerable oligodendroglial precursors. Ultimately, efforts to reduce the high rates of infection in ELBW infants are the most important interventions [34].

Neonatal immunisation for sepsis control

Neonates have a functionally immature immune system. They have extremely low Immunoglobulin (Ig) levels except for IgG to specific maternal antigens transferred passively across the placenta during the last trimester of pregnancy. T cell function is relatively unimpaired but complement activity is half that of healthy adults. Neonates have a low neutrophil storage pool, and their existing neutrophils have impaired capacity to migrate from the blood to sites of infection. These immunological problems are reflected in the clinical presentation of neonatal sepsis. Neonates have a rapid and fulminant progression of septicaemic disease, nonspecific clinical signs of infection, and difficult-to-interpret laboratory results including haematological and immunological biomarkers of infection and inflammation. Low birthweight (preterm and small for gestational age) infants have even poorer functional immunity, and are especially at risk of sepsis.

Neonatal immunisation has long been considered an important method of reducing neonatal infections. However, response varies according to the antigen. BCG, polio, and hepatitis B vaccines are highly immunogenic when given at birth. However, maternal antibodies interfere with a neonate's response to measles vaccine when administered under six months. Protein antigen vaccines (e.g., pertussis and tetanus toxoid) given at birth have been shown to produce poor responses compared to the same antigen given at two months of age and are associated with later tolerance. Studies also indicate that *S. agalactiae* and *Streptococcus pneumoniae* vaccines are both likely to be ineffective when given in the neonatal period [35].

MATERIALS AND METHODS

- A prospective, analytical, study was conducted in Neonatal Intensive Care Unit, Sir T. Hospital, Bhavnagar for a period of 12 months from 1st August 2019 to 31st July 2020.
- Participants of the study were newborns who were attended at birth by paediatric resident at the time of delivery in Labor room and Obstetric Operation theatre, Sir T. Hospital, Bhavnagar. Those newborns were included who were at risk of developing sepsis based on presence of two or more risk factors.

Inclusion criteria

1. Prematurity (>or = 28 weeks ~<38 completed weeks)
2. Premature rupture of membranes(rupture 1hr before onset of labor)
3. Prolonged rupture of membranes (>18 h of membrane rupture)
4. Foul smelling liquor
5. Maternal fever (>100.4 F)
6. Birth asphyxia
7. Low Birth Weight (<2.5 kg)
8. Frequent Vaginal Examination (≥ 3 examination)

Exclusion criteria

1. Congenital Anomaly
2. Birth Trauma
3. Full term infant
4. Still Birth

Those newborns being admitted in other NICU's after birth, Still births, Failed to resuscitate after birth were excluded. There were many instances where sample couldn't be acquired in a proper method and time these also were excluded from the study.

PROCEDURE

Pre-delivery the parents were explained about the purpose of the study and procedure in detail. Written consent was taken beforehand. Those neonates fulfilling the criteria were given appropriate care after birth and before cutting the umbilical cord, blood was collected. Post-delivery the umbilical cord was clamped on both placental and umbilical end and was cut between each pair of clamps. The placental end was wiped with isopropyl alcohol and with a 22-gauge syringe, 2-3ml blood was collected from the placental end of umbilical vein. The blood was immediately transferred to blood culture bottle. The blood culture vials containing 25 ml of brain heart infusion, yeast extract, SPS and other stabilisers were used. The principle of the test is that each type of organism needs a certain time to grow and multiply. Plating on Agar medium and subsequent antibiotic susceptibility testing was done according to microbiology laboratory protocol of the hospital. Since the neonate was at risk of sepsis, patient was admitted in Neonatal Intensive Care Unit (NICU)for further care. After Admission in NICU, similarly peripheral venous blood culture and test for sepsis (CBC, CRP, ESR, Blood culture) were done. Since the study involved preterm with less blood volume, the amount of blood in culture was approximately 0.5 ml to 1 ml. Empirical Antibiotics were started according to NICU protocol till culture sensitivity reports came.

Specific Antibiotics were started after sensitivity reports came for the organism isolated. The newborns were routinely monitored, vitals were charted in case sheet and case record forms. (temperature, respiratory rate, heartrate, Capillary refill time, Oxygen saturation). Patients were routinely reviewed for any signs of septicaemia-feed intolerance, vomiting, abdominal distension, temperature instability, jaundice, sclerema, bleeding etc. Appropriate steps were

taken if any of these were noted. The patient was followed up till the stay in NICU. The entire process was done with full aseptic precautions to avoid any inadvertent error.

Equipment needed for the study

Cord Clamp Scissor

Syringe with needle

Isopropyl alcohol, Cotton swabs 26 G Intra catheter

EDTA and Plain Vacutainers Blood Culture Bottles.

This study has no role of controls.

Statistical analysis

Comparison of both methods were done together and in comparison, with septic screen. Frequency and type of organisms isolated from both the techniques were charted.

Sensitivity and specificity, positive and negative predictive values were calculated. Statistical tests in form of Chi Square tests were applied and results were generated.

- How to collect umbilical cord blood for culture (Figure 1).
- How to collect blood for umbilical cord-blood culture (Figure 2).

RESULTS

Distribution on basis of symptoms

Majority of neonates in study (25%) were asymptomatic,14% had Feed Intolerance, 13% had Vomiting, 12% had Jaundice and Abdominal Distension followed by 10% of neonates who had Respiratory distress, 6% had Hypoglycaemia and Hypothermia, while, least i.e. only 1% had Bleeding and Sclerema and symptoms shown in Figure 3. The above table shows the distribution of respondents based on UCBC and PVBC results. The highest percentage of respondents i.e., 74% were having negative while, 26% were having positive UCBC. 21% had positive PVBC culture reports (Table 5 and Figure 4).

Distribution on basis of UCBC & PVBC results

9 females were PVBC positive, while 12 out of 21 positives were male neonates in PVBC group (Table 6).

Similarly, 12 out 26 positive UCBC neonates were female, rest were male.

18 out of 21 PVBC positive neonates belonged to 1-2 kg weight group. None of the patients less than 1 kg came PVBC positive, while 1 out of 26 in UCBC positive group was less than 1 kg (Table 7).

The above table shows the association between PVBC outcome and mode of delivery which was found to be non-significant (P>0.05) (Table 8).

Patients having Positive PVBC Outcome show the higher percentage 52.4% for NVD mode while, the lower percentage 47.6% had LSCS mode of delivery.

The above table shows the association between PVBC outcome and Apgar score of the neonates which was found to be non-significant

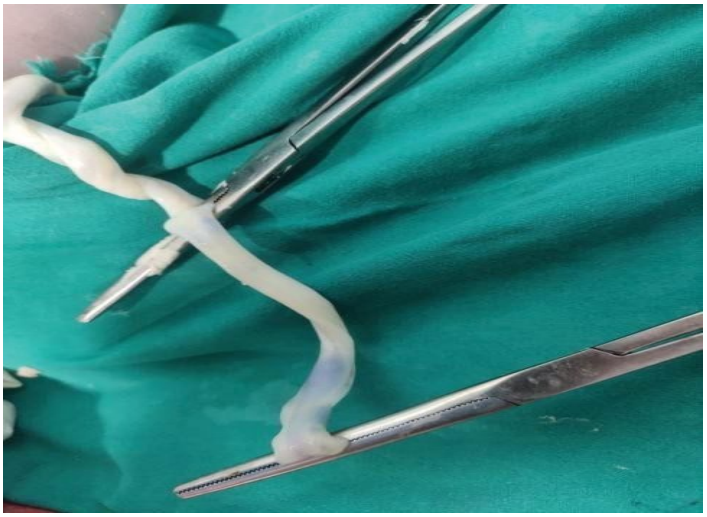

Figure 1: Collect umbilical cord blood for culture.

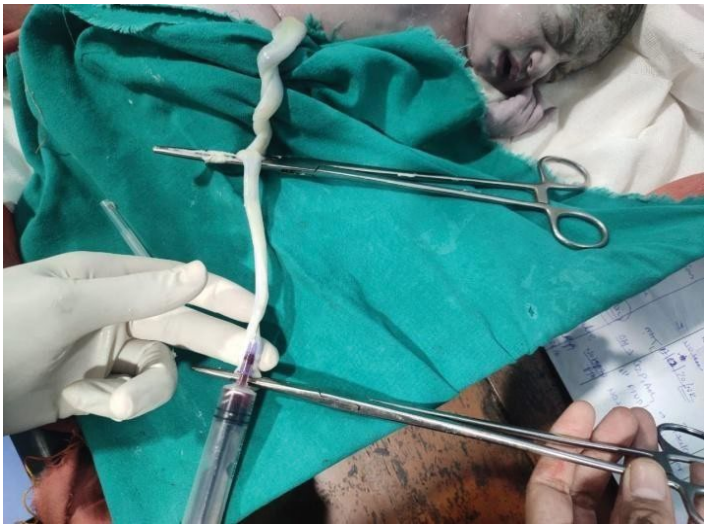

Figure 2: Collect blood for umbilical cord, blood culture.

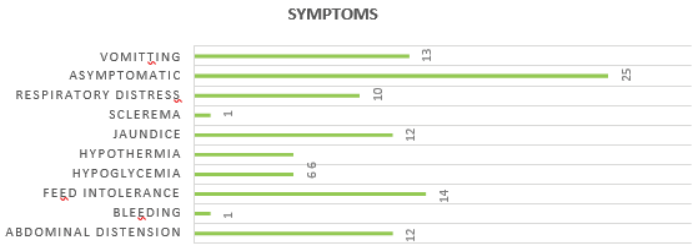

Figure 3: Symptoms.

Table 5: **Distribution on basis of UCBC& PVBC results.**

Results	UCBC Frequency	PVBC Frequency
Negative	74	79
Positive	26	21
Total	100	100.0

(P>0.05) (Table 9).

Patients having Negative PVBC Outcome show the higher percentage 86.1% for >7 score while; the lower percentage 13.9% had ≤ 7 Apgar score.

Similarly, patients having Positive PVBC Outcome show the higher percentage 85.7% for >7 score while; the lower percentage 14.3% had ≤ 7 Apgar score.

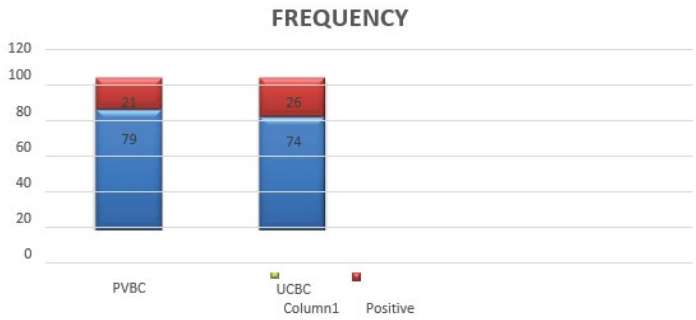

Figure 4: Frequency.

Table 6: Association of PVBC outcome and sex group.

Sex		PVBC Outcome		UCBC Outcome	
		Negative	Positive	Negative	Positive
Female	Count	36	9	33	12
Male	Count	43	12	41	14

Table 7: Association of PVBC method outcome and birth weight.

Birth Weight		PVBC Outcome		UCBC Outcome	
		Negative	Positive	Negative	Positive
<1 Kg	Count	7	0	6	1
1-2 Kg	Count	58	18	55	21
2-2.5 Kg	Count	14	3	13	4

Table 8: Association of PVBC method outcome and mode of delivery.

Mode of Delivery		PVBC Outcome		Total
		Negative	Positive	
LSCS	Count	26	10	36
	%	32.9%	47.6%	36.0%
NVD	Count	53	11	64
	%	67.1%	52.4%	64.0%
	Count	79	21	100
	%	100.0%	100.0%	100.0%
Chi square test = 1.558, df = 1, P value =0.212 non-significant				

Table 9: Association of PVBC method outcome and Apgar score.

APGAR Score		PVBC Outcome		Total
		Negative	Positive	
<=7	Count	11	3	14
	%	13.9%	14.3%	14.0%
>7	Count	68	18	86
	%	86.1%	85.7%	86.0%
	Count	79	21	100
	%	100.0%	100.0%	100.0%

The above table shows the association between PVBC outcome and frequent vaginal examination status of the respondents which found to be non-significant (P>0.05) (Table 10).

The above table shows the association between PVBC Outcome and Maternal Fever Status of the neonates which was found to be non-significant (P>0.05).

The above table shows the association between PVBC outcome and symptoms of the respondents which found to be significant (P>0.05) (Table 11).

Maximum neonates with Negative UCBC and Negative PVBC reports were Asymptomatic.

Most neonates with Positive PVBC outcome had respiratory distress (28.6%) followed by 23.8% having abdominal distension while none had sclerema, bleeding and hypoglycaemia. Bleeding, Feed intolerance, hypothermia, jaundice and vomiting do not show any major difference in positive and negative patients. Presence of Respiratory Distress, abdominal distension shows significant count of positive patients. Vomiting has less number of positive patients and patients not having any symptoms have higher count for negative outcome (Figure 5 and Figure 6).

The above table shows the association between PVBC outcome and septic screen which was found to be significant (P<0.05) (Table 12).

Chi square test for association, sensitivity and PPV for correct diagnosis of positive, specificity and NPV for correct diagnosis of negative and accuracy for overall correctness in diagnosis of outcome was calculated to compare the outcome of septic screen

Table 10: Association of PVBC method outcome and frequent vaginal examinationstatus.

Frequent Examination Vaginal		PVBC Outcome		Total
		Negative	Positive	
No	Count	66	16	82
	%	83.5%	76.2%	82.0%
Yes	Count	13	5	18
	%	16.5%	23.8%	18.0%
Total	Count	79	21	100
	%	100.0%	100.0%	100.0%
Chi Square Test = 0.608, df =1, P Value =0.436 Non-Significant				

Table 11: Association of PVBC method outcome and maternal fever status.

Maternal Fever		PVBC Outcome		Total
		Negative	Positive	
No	Count	62	18	80
	%	78.5%	85.7%	80.0%
Yes	Count	17	3	20
	%	21.5%	14.3%	20.0%
Total	Count	79	21	100
	%	100.0%	100.0%	100.0%
Chi Square Test = 0.542, df =1, P Value =0.461 Non-Significant				

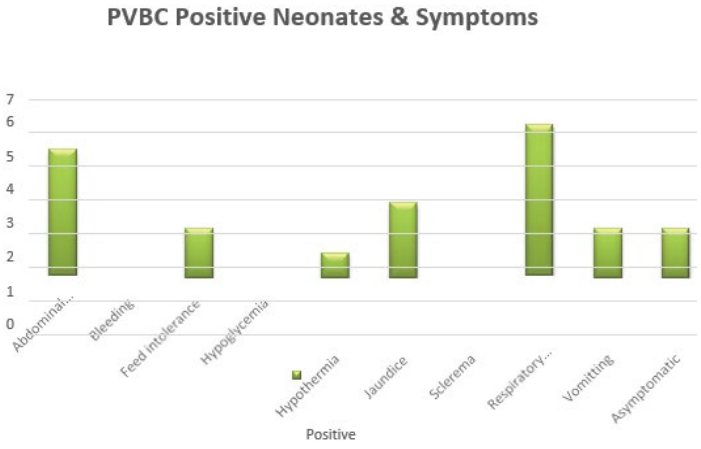

Figure 5: PVBC positive neonates & symptoms.

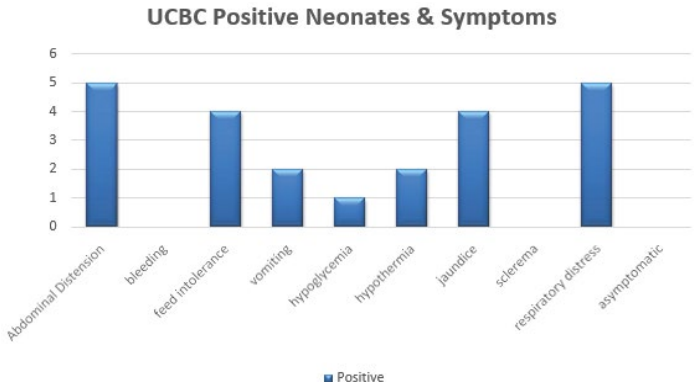

Figure 6: UCBC positive neonates & symptoms.

method with outcome of gold standard PVBC Method. 68.4% PVBC Negatives had Negative Septic Screen, while only 31.6% had positive septic screen. On the other hand, among patients having Positive PVBC outcome 95.2% had positive septic screen, while 4.8% of the positive neonates had Negative Septic Screen Results. The high sensitivity of 95.2% shows that the septic screen method is a quite reliable tool for predicting the positive patients as per the final outcome of gold standard PVBC method. Also, PPV 66.0% shows that patients who were diagnosed as positive were also finally diagnosed same as positive by PVBC method. Similarly the high specificity of 68.4% and high NPV of 86% shows a significant diagnostic capacity of negative outcome by septic screen method as compared to gold standard PVBC method (Table 13). Accuracy of 74% justify that the Septic screen method is highly reliable tool for correct prediction of grades as compare to final grades of gold standard PVBC method. Hence high sensitivity, high specificity and high accuracy for predicting disease positive outcome of patients by septic screen method against PVBC Method conclude that septic screen can be used as reliable tool to predict the final outcome. The above table shows the association between UCBC outcome and Septic Screen Results of the neonates which was found to be significant (P<0.05) (Table 14). Chi square test for association, sensitivity and PPV for correct diagnosis of positive, specificity and NPV for correct diagnosis of negative and accuracy for overall correctness in diagnosis of outcome was calculated to compare the outcome of septic screen method with outcome of gold standard UCBC Method.72.97% of Neonates having negative UCBC outcome had negative septic screen, while 27.03% had positive septic screen. On the other hand, 96.15% patients having Positive UCBC outcome had positive septic screen while, 3.85% of them had Negative Septic Screen Results. The high sensitivity of 96.15% shows that the septic screen method is a quite reliable tool for predicting the positive patients as per the final outcome of UCBC method. However low 55.55% PPV shows that patients who were diagnosed as positive may not be finally diagnosed same as positive by UCBC method. Similarly, the moderate specificity of 72.97% and high NPV of 98.18% shows a lesser diagnosis capacity of negative outcome by septic screen method as compare to UCBC method. Accuracy of 79% justify that the Septic screen method is highly reliable tool for correct prediction of grades as compare to final grades of gold standard UCBC method. Hence high sensitivity and specificity but moderate accuracy for predicting disease positive outcome of patients by septic screen method against UCBC Method conclude that septic screen may be used as a tool to predict the same outcome as of UCBC method.

Organism comparison chart (Table 15 and Figure 7).

The above table shows the association between PVBC outcome and UCBC Results of the respondents which was found to be significant (P<0.05) (Table 16). Chi square test for association, sensitivity and PPV for correct diagnosis of positive, specificity and NPV for correct diagnosis of negative and accuracy for overall correctness in diagnosis of outcome were performed to compare the outcome of UCBC method with outcome of gold standard PVBC Method. 88.6% of neonates with negative PVBC outcome had negative UCBC result, while 11.4% had positive results by UCBC method. Similarly, 81% of neonates having Positive PVBC outcome had positive UCBC result while 19.0% had negative UCBC Results. The high sensitivity of 81.0% shows that the UCBC method is a reliable tool for predicting the positive patients as per the final outcome of gold standard PVBC method. Also, PPV 65.38% shows that out of patients who were diagnosed as positive, majority were finally diagnosed same as positive by PVBC method also. Similarly, the high specificity of 88.6% and moderate NPV of 94.59% shows a higher diagnosis capacity of negative outcome by UCBC method as compared to Gold Standard PVBC method. Accuracy of 87% justify that the UCBC method is strong tool for correct prediction of grades as compared to final grades of gold standard PVBC method. Hence higher sensitivity, specificity and accuracy for predicting disease outcome of patients by UCBC method against PVBC Method conclude that UCBC can be used as reliable and alternate tool to diagnose early onset neonatal sepsis flow chart (Figure 8).

DISCUSSION

Sex wise distribution

- Out of the total 21 culture positive results by PVBC, 42.9% were female and rest 57.1% were male.
- Similarly, out of 26 culture positive results by UCBC method, 46.2% were female and rest 53.8% were male.

A study by Bhale et al. found the ratio to be around 1.46:1 (Male: Female) in a study on culture positive neonatal sepsis [36]. The male preponderance in neonatal septicaemia may be linked to X linked Immune regulatory gene factor resulting in host's susceptibility to infections in males [37]. Genetic basis of this phenomenon is that females possess 2X chromosomes. The factor regulating immunoglobulin synthesis is situated on X chromosome. Thus, Males are more predisposed to sepsis.

Distribution on the basis of mode of delivery

- 64% neonates had normal vaginal delivery and rest were delivered by Lower segment Caesarean Section.
- 52.4% PVBC culture positive were born through NVD, rest 47.6% PVBC culture positive were delivered through LSCS.
- Similarly, in UCBC outcome neonates (42.3%) were born through LSCS, rest 15 neonates (57.7.%) were born through normal vaginal delivery.

The mode of delivery and blood culture outcome was found to be non-significant.

Distribution on the basis of birth weight

Extremely Low birth weight new borns are more susceptible

Table 12: Association of PVBC method outcome and symptoms.

Symptoms		PVBC Outcome		UCBC Outcome	
		Negative	Positive	Negative	Positive
AbdominalDistension	Count	7	5	7	5
	%	8.9%	23.8%	9.5%	19.2%
Bleeding	Count	1	0	1	0
	%	1.3%	0.0%	1.4%	0
Feed intolerance	Count	12	2	10	4
	%	15.2%	9.5%	13.5%	15.4%
Hypoglycemia	Count	6	0	5	1
	%	7.6%	0.0%	6.8%	3.8%
Hypothermia	Count	5	1	4	2
	%	6.3%	4.8%	5.4%	7.7%
Jaundice	Count	9	3	8	4
	%	11.4%	14.3%	10.4%	15.2%
Sclerema	Count	1	0	1	0
	%	1.3%	0.0%	1.4%	0
Respiratorydistress	Count	4	6	5	5
	%	5.1%	28.6%	6.8%	19.2%
Vomiting	Count	11	2	11	2
	%	13.9%	9.5%	14.9%	7.7%
No symptoms	Count	23	2	22	3
	%	29.1%	9.5%	29.7%	11.5%
Total	Count	79	21	74	26
	%	100.0%	100.0%	100.0%	
Chi Square Test = 17.742, df =9, P Value =0.038* Significant					

Table 13: Association of PVBC method outcome and septic screen method results.

Septic Screen Results		PVBC Outcome		Total
		Negative	Positive	
Negative	Count	54	1	55
	%	68.4%	4.8%	55.0%
Positive	Count	25	20	45
	%	31.6%	95.2%	45.0%
Total	Count	79	21	100
	%	100.0%	100.0%	100.0%
Calculation		**Value**	**Df**	**P Value**
Pearson Chi-Square		27.107	1	0.000
Sensitivity			95.2%	
Specificity			68.4%	
PPV			66.00%	
NPV			86.00%	
Accuracy			76.00%	

to infections because of numerous factors including reduced immunity, immature skin, and exposure to invasive procedures like intubation at birth among others.

- In our study, 85.7% peripheral vein positive cultures were in neonates between 1-2 kg birth weight, while less than 1 kg there were no positive cultures.
- Similarly, In UCBC results, less than 1 kg there was a single positive culture. 84.6% positive neonates were less than 2 kg.

In a study by Hornik et al. In LBW babies EOS positive less than 1 kg were 57.4% and rest were more than 1 kg [38]. Birth weight determines a major susceptibility to EOS; preterm neonates, especially VLBW, showed incidence rates >10 times higher than those born at term with a total mortality of about one-third [39]. When classified by birth weight, the rate of early-onset neonatal sepsis in 1000 live births was reported to be 0.57 in babies over 2500 grams, and 10.96 in babies with a birth weight of between 401-1500 grams, according to Stoll et al. The variation in statistics between this study and others could be because of small sample size

Table 14: Association of UCBC method outcome and septic screen method results.

Septic Screen Results		UCBC Outcome		Total
		Negative	Positive	
Negative	Count	54	1	55
	%	72.97 %	3.8 %	55.0 %
Positive	Count	20	25	45
	%	27.03 %	96.2 %	45.0 %
Total	Count	74	26	100
	%	100.0 %	100.0 %	100.0 %
Calculation		**Value**	**Df**	**P Value**
Pearson Chi-Square		37.147	1	0.000
Sensitivity			96.15%	
Specificity			72.97%	
PPV			55.55%	
NPV			98.18%	
Accuracy			79.00%	

Table 15: Organism comparison chart.

Organism	UCBC Method		PVBC Method	
	Frequency	Percent	Frequency	Percent
E. coli	4	4.00%	3	3.00%
Klebsiella	15	15.00%	11	11.00%
Pseudomonas	1	1.00%	2	2.00%
Staph Aureus	6	6.00%	5	5.00%
No Growth	74	74.00%	79	79.00%
Total	100	100.00%	100	100.00%

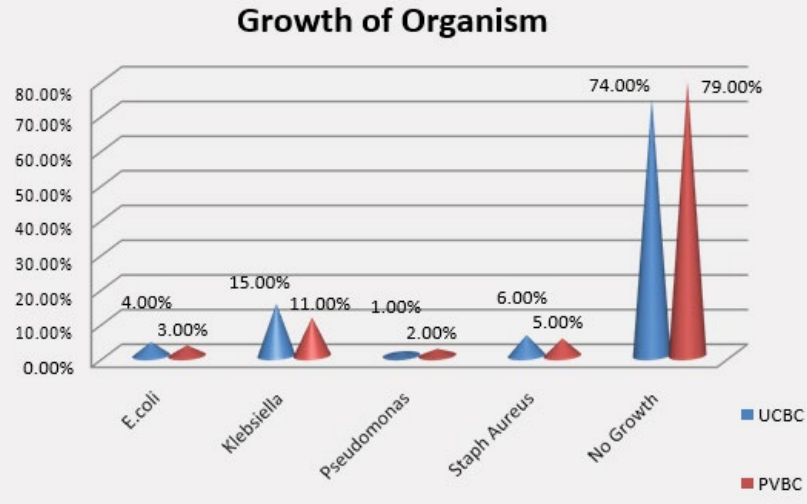

Figure 7: Growth of organisms.

of the study and inadequate sampling from ELBWs or because of low bacteraemia in these neonates.

Distribution on the basis of symptoms

- In PVBC positive cultures, the most common symptom in septic babies was respiratory distress (28.6%) followed by abdominal distension (23.8%). Around 9.5% were asymptomatic.
- In UCBC positive cultures, also the most common symptom was Respiratory distress and abdominal distension (19.2%), followed by feed intolerance and jaundice (15.4%) each. 11.5% subjects were asymptomatic.

In a study by Lakhey et al. also respiratory distress was most common symptom of septicaemia (56%) [40]. The study by Fanaroff et al. reported apnea (54.8 per cent) to be the commonest clinical sign in very low birthweight babies with suspected neonatal septicaemia and this was followed by gastrointestinal problems (46.3 per cent) and lethargy (22.7 per cent) [41]. Whereas, Khatua et al. reported refusal to feed, lethargy, and diarrhoea as common clinical features of suspected neonatal septicemia [42].

Bhakoo et al. concluded that lethargy, abdominal distension, and vomiting were the common signs among those who died of

Table 16: Association of PVBC method outcome and UCBC method results.

UCBC Results		PVBC Outcome		Total
		Negative	Positive	
Negative	Count	70	4	74
	%	88.6%	19.0%	74.0%
Positive	Count	9	17	26
	%	11.4%	81.0%	26.0%
Total	Count	79	21	100
	%	100.0%	100.0%	100.0%
Calculation		**Value**	**Df**	**P Value**
Pearson Chi-Square		41.722	1	0.000*
Sensitivity			81.0%	
Specificity			88.6%	
PPV			65.38%	
NPV			94.59%	
Accuracy			87.00%	

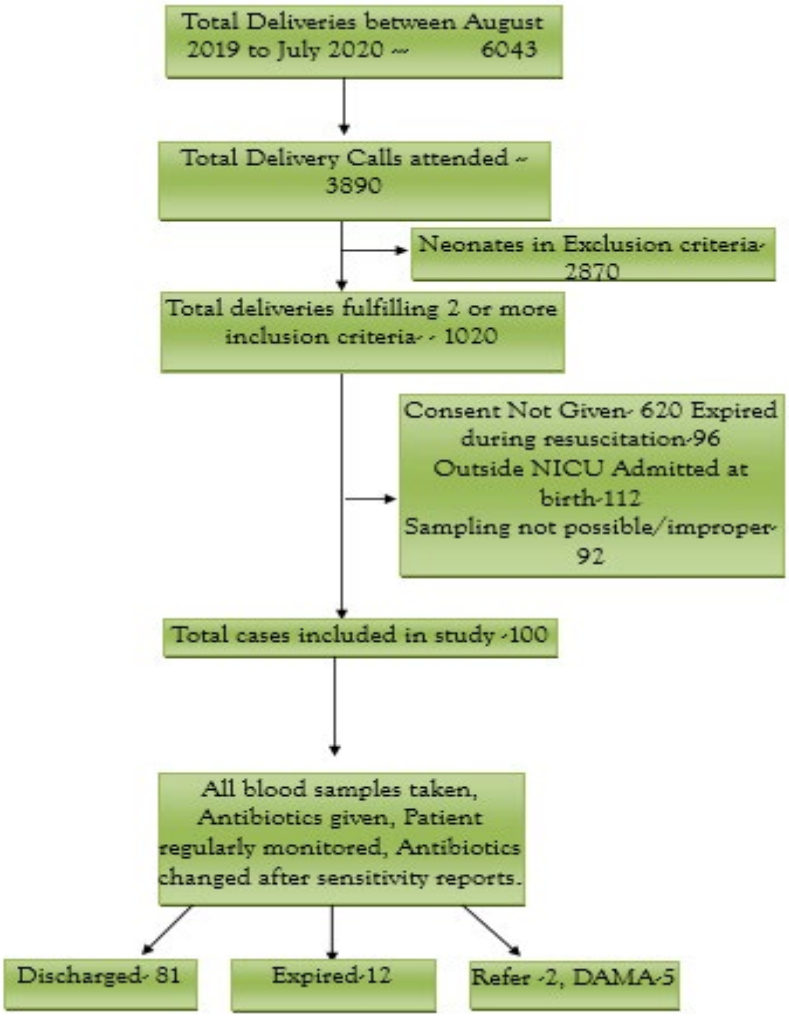

Figure 8: Flow chart of total deliveries.

neonatal septicaemia, whereas jaundice, lethargy, and fever were common among survivors [43].

Distribution on the basis of risk factors

Asphyxia causes an immunological insult and resuscitation procedures following birth asphyxia tend to explore newborns to pathogenic microbes. Neonates with low Apgar score tend to have poor adaptation to extra uterine life due to the stress experienced during labour and therefore are more prone to infection.

- 27% of UCBC positive subjects were having Apgar <7 at birth, while in PVBC positive subjects 14.3% were having Apgar <7 at birth.

Neonates whose Apgar score was less than 7 were 2.69 times more likely to have early onset neonatal sepsis as compared to those

whose Apgar scores were greater than or equal to 7, according to a study by Adatara et al. [44].

- 5 out of 18 babies whose mother underwent >3 vaginal examinations were positive by PVBC method. This result was consistent with UCBC Outcome where 3 out of 18 neonates came out culture positive.
- 6 out of 20 babies whose mother had PROM were positive by UCBC method. 4 out of 20 babies whose mother had PROM were positive by PVBC method.

In a study by Lawama et al. Of the total PROM affected Neonates, 5% had culture positive sepsis and 13% had culture negative sepsis [45].

Discussion on the basis of septic screen

The neonates were subjected to septic screen where on basis of blood parameters, neonates were labelled as septic screen positive and negative. Usually if two parameters are abnormal, it should be considered as a positive septic screen and it is reasonable to start antibiotic therapy.

- In 26 UCBC positive neonates, 25 had positive septic screen.
- Out of 21 PVBC positive neonates, 95% (20) had positive septic screen.

In our study, significant association was found between UCBC outcome and septic screen. Sensitivity was 96.15%, Specificity 72.9%, Positive predictive value 55.55% and Negative Predictive Value 98.18%. Similarly, significant association was seen between PVBC outcome & septic screen. Sensitivity of 95.2% with negative predictive value of 86%. While specificity was 68.4% and positive predictive value of 66%. These results correlate well with previous studies done by other authors. In a study by Heena et al., After Considering any two combination tests (haematological parameters and CRP) being positive as a screening tool for sepsis, the sensitivity and the negative predictive values were around 81% and 74.5%, respectively, while the specificity and the positive predictive values increased to 94.6% and 96.2%, respectively, and was found to be statistically significant in detecting septicaemia [46].

Distribution on the basis of organism

- In our study the most common isolated organism among UCBC positive neonates was Klebsiella (57.6%), followed by *Staphylococcus aureus* (23%), *Escherichia coli* (15.3%).
- Similarly, In PVBC positive neonates *Klebsiella* (52.3%) was most commonly isolated organism. *Staphylococcus* (23.8%) was the next most common followed by *Escherichia coli* (14.2%).

The principal route of neonatal early onset infection is vertical transmission from colonized mothers during passage through the vagina during labor and delivery. Isolation of an organism depends on colonisation status of mother's genitourinary tract, local hospital environment pattern, Antibiotic resistance pattern and varies among different institutions. In a study by Bambala et al. *Klebsiella pneumoniae* was isolated from 66% of culture positive cases followed by *Coagulase-negative Staphylococci* in 12% of cases [47].

Klebsiella pneumoniae (36%), *Staphylococcus aureus* (21%), and *Escherichia coli* (14%) were common organisms in a similar study by Jajoo et al. [48]. Umbilical cord blood culture has been suggested as an alternative method for detecting neonatal bacteraemia but has not come into general clinical use because of reported high incidence of false positive results as compared to those of postnatal peripheral venous blood culture. In our study, out of 100 at risk newborns, 21 were PVBC positive and 26 were UCBC positive. Among 21 PVBC positive, 17 had both UCBC and PVBC positive reports and all 17 had similar organism isolated from these 2 methods.

The association between both methods was found to be significant in our study.

The higher sensitivity (81.0%), specificity (88.6%) and accuracy (87%) for predicting disease outcome of patients by UCBC method against PVBC Method conclude that UCBC can be used as reliable and alternate tool to predict the final outcome. Albers and Tyler had 9% (13 out of 319), and Polin et al., had 3% (6 out of 200) positive UCBC [49,50]. These studies were screening studies without any focus on risk factors. In a study of Pryles et al. [51]. umbilical cord culture positive rate was 47% in high-risk newborns. Herson et al., in their study had UCBC positivity in 20% (7 out of 35) in high-risk newborns [52]. In a study by Fos et al. [53]. 43% (13 out of 30) UCBC were positive in high risk for sepsis newborns. In a study by Kalathia et al. A total of 11 out of 45 (24.44%) patients had positive UCBC growth and 8 (17.8%) had positive PVBC. All positive culture reports were from the patient having clinical diagnosis of sepsis, and septic screen was positive in all these new borns [54]. In a similar study by Mandot et al. sepsis screen was positive in 23 babies [55]. Among them, 6 babies had grown organism on blood culture (four on umbilical cord blood culture only and two on Umbilical cord blood culture and peripheral vein blood culture both).

LIMITATIONS OF THE STUDY

- This was a hospital-based time bound study.
- The sample size was small.
- More sophisticated septic screen tests couldn't be done due to non-availability in the hospital setup.

CONCLUSION

In our study, significant association was found between PVBC and UCBC outcome. 88.6% of Neonates negative by PVBC method were also negative by UCBC method, while only 11.4% PVBC negative neonates were positive by UCBC Results. The high sensitivity of 81.0% shows that the UCBC method is a reliable tool for predicting the positive patients as per the final outcome of gold standard PVBC method. Also, Positive predictive value of 65.38% shows that out of patients who were diagnosed as positive, majority were finally diagnosed same as positive by PVBC method also. 17 out of total 26 cultures positive neonates had same organism isolated from both the methods, which signify the usefulness of UCBC method. Also, out of 26 positive neonates by UCBC method, 25 had positive septic screen which indicates reliability of the method.

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