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Utility of cord blood culture in early onset neonatal sepsis

Jothi Meena[1], Marie Victor Pravin Charles[1], Arunava Kali[1], Siva Ramakrishnan[2], Seetesh Gosh[3], and Kunigal S Seetha[1]

1. Department of Microbiology, Mahatma Gandhi Medical College and Research Institute, Pondicherry, India
2. Department of Paediatrics, Mahatma Gandhi Medical College and Research Institute, Pondicherry, India
3. Department of Obstetrics and Gynaecology, Mahatma Gandhi Medical College and Research Institute, Pondicherry, India

RESEARCH

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Corresponding Author:
Marie Victor Pravin Charles
Dept. of Microbiology
Mahatma Gandhi Medical College and Research Institute
Pondicherry, India
Email: dr_mvpravincharles@yahoo.com

ABSTRACT

Background
Neonatal sepsis is a major cause of neonatal mortality. The clinical outcome mainly depends on the early diagnosis and peripheral venous blood culture (PVBC) is the most widely practiced diagnostic method.

Aims
This study aimed to evaluate the utility of umbilical cord blood culture (UCBC) in neonates at high risk of early onset neonatal sepsis (EONS) in comparison to peripheral venous blood culture.

Methods
A total of 40 neonates with two or more risk factors for EONS were included in the study. Umbilical cord blood was collected aseptically during delivery for blood culture and C-reactive protein (CRP). Peripheral venous blood was collected within 24 hours of birth for sepsis screen and PVBC.

Results
Although 11 babies were sepsis screen positive, cord blood CRP was negative in all cases. In comparison to PVBC, UCBC had 100 per cent sensitivity and 94.9 per cent specificity. The results of UCBC were consistent with PVBC. One neonate who was both UCBC and PVBC positive also had isolation of the same pathogen in both the cultures and 33 per cent PPV for UCBC.

Conclusion
UCBC is a simple convenient method, which ensures culture of adequate volume of blood from newborns allowing effective early isolation of bacterial pathogens, especially in EONS.

Key Words
Neonatal sepsis, umbilical cord blood culture, sepsis screen

What this study adds:

1. What is known about this subject?
Cord venous blood taken at delivery can be a reliable alternative to peripheral venous culture. Without proper aseptic technique, there is a higher risk of contamination from the cord blood than the peripheral venous blood.

2. What new information is offered in this study?
There is a higher risk of contamination in the UCBC group, and UVBC is more accurate than PVBC as there is a larger sample volume. Inadequate samples of PVBC can lead to a blood culture positive specimen being undiagnosed (lower bacteria count).

3. What are the implications for research, policy, or practice?
UCBC can be a useful diagnostic test for EONS. It is especially important for neonates who were given prophylactic antibiotic before collection of blood for culture.

Background

The neonatal period represents the period of highest risk to newborns. Among the major infective ailments, sepsis is the foremost cause of neonatal mortality, accounting for three million neonatal deaths per year worldwide and an estimated neonatal mortality rate of 23.9 per 1,000 live births.[1] Neonatal sepsis is defined as a blood stream infection which develops within 28 days after birth or up to four weeks after the expected due date for preterm infants.[2] Based on the time of onset, it is categorised into early onset neonatal sepsis (EONS) and late onset neonatal sepsis (LONS). EONS appears within the first three days of life and is associated with vertical transmission of organisms through the birth canal due to amniotic membrane rupture or leak. In contrast, LONS develops after the third day of birth and the source of infection is usually the caregiving environment (horizontal transmission).[3] The mortality associated with EONS is considerably higher than that of LONS.[4] 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.[5]

Although laboratory markers of sepsis complement the diagnosis, demonstration of organisms from patients' blood remains the gold standard for diagnosing neonatal sepsis. 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.[6] 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. This study was carried out to evaluate the utility of umbilical cord blood culture in neonates at high risk for EONS in comparison to peripheral venous blood culture.

Method

Type of study and study design

A prospective, analytical study was conducted over a period of six months from May to October 2014 in a tertiary care hospital in south India. Neonates of both sexes that crossed 28 weeks of gestation, had birth weight of more than 1500gm and two or more risk factors of EONS [i.e., prematurity, prolonged and/or premature rupture of membranes, prolonged labor (>24h), foul smelling liquor, maternal fever, frequent vaginal examination, birth asphyxia, and low birth weight < 2500gm] were included in the study irrespective of the mode of delivery (Caesarean section or normal vaginal delivery). Neonates without any risk factors at birth were excluded from the study. The study was conducted at Mahatma Gandhi Medical College and Research institute. The Institutional Human Ethics Committee, Mahatma Gandhi Medical College and Research Institute, Pondicherry, India approved the study. Informed consent in written format was obtained from both parents when the risk factors were noted.

Sample collection

All mothers who underwent normal delivery were given injection ampicillin and gentamicin and those who underwent Caesarean section were given injection cefotaxime. Umbilical cord blood was collected at birth. Post-delivery the umbilical cord was clamped on both the placental and the umbilical end and was cut between each pair of clamps. The placental end was wiped with 70 per cent isopropyl alcohol and with a 22 gauge syringe 4ml blood was collected and 2ml blood was immediately transferred to Bactec PEDS PLUS/F culture vials (Becton, Dickinson and Company, USA) and sent to the lab for culture.[7] Remaining cord blood was used for estimating C-reactive protein using commercial latex agglutination kit (Agappe, Mumbai, India).

The neonates were further evaluated for septic screen and assessed for lethargy, hyptonia, fever, tachycardia, abdominal distension, retractions, grunting, increased aspirates, hypotension, delayed capillary refill, hypoglycemia, pallor, hepatomegaly, apnea, abnormal skin colour, bradycardia, sclerema, shock, and features of disseminated intravascular coagulation in the NICU. Peripheral venous blood culture and test for sepsis screen (Total leucocyte count, absolute neutrophil count, I: T ratio, C-reactive protein) were done within 24 hours post-partum following standard procedure. Neonates showing two or more abnormal parameters were considered sepsis screen positive and were given antibiotic therapy empirically, which was later modified as per blood culture results.

Processing

The blood culture vials were placed in BACTEC-2 FX automated blood culture system. On identification of positivity, the vials were removed from the machine and were subcultured on blood agar and Mac-Conkey agar

plates. The organisms isolated on culture plates were identified biochemically. Antibiotic susceptibility test was carried out as per standard laboratory procedure.

Data collection and statistical analysis

Clinical information and medical treatments history were entered in data collection sheet. Sensitivity, specificity, and positive and negative predictive values of diagnostic tests were calculated.

Results

Forty neonates (11 males and 29 females) with two or more risk factors of EONS were included in the study and evaluated for sepsis by umbilical cord blood culture, umbilical cord CRP, peripheral venous blood culture, and sepsis screen. The mean gestational age was 36.6±0.7 weeks. Table 1 shows the distribution of male and female neonates in sepsis screen positive and sepsis screen negative groups. Cord blood CRP was negative in all cases, whereas 11 babies (four males and seven females) showed positive sepsis screen. We have analysed the clinical information and medical treatments history to identify the common risk factors associated with EONS. Prolonged rupture of membrane, frequent vaginal examinations, and prematurity were the common risk factors in both the groups (Table 1).

Table 1: Sex, gestational age, and risk factor distribution in newborns

Parameters	Sepsis screen positive (n=11)	Sepsis screen negative (n=29)	Total (n=40)
Gestational age (weeks)	36.4 ± 1	36.7 ± 0.5	36.6 ± 0.7
Male	4 (36.3%)	7 (24%)	11 (27.5%)
Female	7 (63.6%)	22 (75%)	29 (72.5%)
Prolonged rupture of membrane	5 (45.5%)	10 (34%)	15 (37.5%)
Premature rupture of membrane	5 (45%)	4 (14%)	9 (22.5%)
Prematurity	1 (9%)	3 (10%)	4 (10%)

Out of 40 neonates, two had positive growth only in UCBC (*Enterococcus faecalis* in both cases) and one baby recovered bacterial pathogen (*E. coli*) in both UCBC and PVBC. The three culture-positive cases had clinical diagnosis of sepsis and were also positive for sepsis screen. Table 2 shows the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of UCBC, sepsis screen and cord blood CRP for diagnosing neonatal sepsis.

Table 2: Diagnostic parameters of UCBC, sepsis screen, and cord blood CRP

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
UCBC	100% (2.5–100%)	94.9% (82.7–99.4%)	33.3% (0.8–90.6%)	100% (90.5–100%)
Sepsis screen	100% (2.5–100%)	74.4% (57.9–87%)	9% (0.3–41.3%)	100% (88.1–100%)
Cord blood CRP	0% (0– 97.5%)	100% (91–100%)	–	97.5% (86.8–100%)

The mean time to flag positive results in BACTEC was 9.6 hours for UCBC and 15.2 hours for PVBC. Although cord blood CRP was negative in all newborns, 11 babies (27.5 per cent) had positive sepsis screen, three (7.5 per cent) had positive UCBC, and one (2.5 per cent) had positive PVBC (Table 3). Empirical antibiotics were administered in positive sepsis screen cases. Premature rupture of membrane, prolonged rupture of membranes, preterm delivery, and frequent vaginal examinations were the most common risk factors. Male sex had a slightly higher incidence of positive septic screen, which is a risk factor for early onset sepsis.

Table 3: Results of diagnostic tests for neonatal sepsis

	Sepsis screen	Cord blood CRP	Umbilical cord blood culture	Peripheral venous blood culture
Positive	11	0	3	1
Negative	29	40	37	39

Discussion

The diagnosis of neonatal sepsis essentially depends on isolation of pathogenic organisms on blood culture.[8] It is also imperative for selection of rational antibiotic therapy according to the resistance pattern of the pathogen. While repeated isolation of the same pathogen confirms its causative association in sepsis, a sterile culture often indicates the therapeutic response to antibiotics. However, blood culture positivity usually accounts for a smaller proportion of clinically suspected neonatal sepsis cases.[7,9] Culture positivity has been reported more frequently in neonates with multiple risk factors.[10] Both neonatal (i.e., prematurity, low birth weight and birth asphyxia) and maternal (i.e., prolonged and/or premature rupture of

membranes, foul smelling or meconium stained of liquor, prolonged labor, maternal fever, and frequent vaginal examination) attributes have been found to be associated with a higher risk of developing EONS.

Premature rupture of membrane refers to rupture of amniotic membrane one hour before the onset of labour. Prolonged rupture membrane was considered if there was further delay more than 18 hours after rupture of the membrane. Risk factor analysis showed that prolonged rupture of membrane was seen in five out of 11 (45.4 per cent) cases with positive septic screen and 10 out of 29 (34.5 per cent) cases with negative septic screen. Male sex had a slightly higher incidence of positive septic screen.

Sepsis screen provide a rapid presumptive identification of neonates with clinical signs of sepsis with a battery of non-culture-based adjunctive investigations; i.e., total leucocyte count, absolute neutrophil count (ANC), I: T ratio, C-reactive protein.[8] These parameters effectively reflect the pathological changes in sepsis and can be used for diagnostic tools individually.[11] These tests achieve greater sensitivity and specificity when the results are combined. Swarnkar et al. evaluated the diagnostic utility of sepsis screen parameters individually and in combination.[10] It was found that CRP had only 52.3 per cent sensitivity and 56 per cent specificity. However, sepsis screen had higher sensitivity (66.7 per cent) and specificity (79 per cent) when three or more test results were considered in combination.[8] Leucopenia and ANC <1,800 neutrophils/cubic have been reported to be better predictors of neonatal sepsis.[10] We found 100 per cent sensitivity and 744 per cent specificity of sepsis screen. The difference from reported rates may be due to the fact that our study involved only neonates with two or more risk factors.

The volume of blood required to give a positive result is a critical consideration. More than 1ml of blood is required for optimum recovery of pathogenic organisms from blood.[12] Given the small and delicate nature of peripheral veins, it is often difficult to obtain 1ml of blood from preterm neonates. In the presence of several risk factors and signs of sepsis, it is often not feasible to withhold antibiotics. Consequently, the antimicrobial action of empirical antibiotics reduces the chance of recovery of causative pathogens in culture if empirical antibiotic therapy is initiated before collection of blood for PVBC.[6]

Umbilical cord is an easily accessible source of adequate neonatal blood. Since cord blood is collected at the time of delivery, it avoids the effect of antibiotic, which has to be given prophylactically in early post-partum.[6] In comparison to venous blood, a smaller volume of cord blood has been found to yield a positive result, especially in cases of EONS associated with intrauterine sepsis.[13] UCBC provides a painless alternative to traditional PVBC to overcome these drawbacks of PVBC.[14]

There are several studies that have demonstrated the advantages of UCBC over PVBC. Fos et al. evaluated 30 neonates and determined that UCBC as an easier alternative to PVBC.[15] Herson et al. concluded that UCBC could be a useful addition or substitute to PVBC in neonates with maternal risk factors.[16] In a study conducted by Kalathia et al., UCBC was reported as a useful method to increase aetiological diagnosis of blood stream infection in high-risk neonates. In comparison to PVBC (which recovered eight bacterial isolates), UCBC recovered 11 isolates and had a sensitivity of 80 per cent and specificity of 91.4 per cent.[17] They also found six out of the 11 UCBC positive neonates had similar organisms recovered in PVBC. Among the bacterial isolates *Pseudomonas, Acinetobacter, E. coli,* and *Klebsiella* were most common.[17] In our study, PVBC was positive in one neonate who also had cord culture positive and both cultures isolated same pathogen (*Escherichia coli*). Another two neonates who showed positive cord blood cultures, had no growth of pathogen on PVBC. The reason could be lower volume of blood obtained for culture in PVBC in comparison to UCBC. This may also explain the shorter mean time to flag positive results for UCBC in BACTEC.

Despite the advantages of UCBC, it is also documented that culture results had excess of contamination lacking clinical correlation. In a study conducted by Pollin et al., six UCBC cultures were positive out of 200 samples of which only one culture had clinical correlation and was considered significant.[18] The technique of collection of cord blood is critical to ensure meaningful results without contamination. Collection of blood from the umbilical cord on perineum before delivery of the placenta has been reported to have higher contamination.[11]

The major limitation of this study was small sample size. The risk factors responsible for direct intra-amniotic infection like maternal fever, foul smelling liquor, and prolonged labour were not present in our study. In a study conducted by Pollin et al. the positivity rate of UCBC was 0.5 per cent.[18] Though the number of neonates evaluated are less in our study, the positivity rate for UCBC was 7.5 per cent in comparison to a positive septic screen for neonatal sepsis. In a similar study from India by Kalathia et al., a total of 45

high-risk neonates were investigated for EONS by UCBC and PVBC. They found 24.4 per cent positivity of UCBC, which is higher in comparison to our study.[17] Multicentric studies should be conducted to improve knowledge on UCBC and thereby curb the mortality among this group of patients.

Conclusion

Neonatal sepsis is an infection occurring in early life associated with high morbidity and mortality. Umbilical cord blood cultures may be a good alternative to peripheral venous blood cultures for enhanced detection of early onset neonatal sepsis in high-risk neonates. However, its potential in replacing peripheral venous blood culture needs to be evaluated in large multicentric studies.

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PEER REVIEW

Not commissioned. Externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

ETHICS COMMITTEE APPROVAL

This submission is compliant with the requirements of Institutional Human Ethics Committee, Mahatma Gandhi Medical College & Research Institute, Pondicherry, India. IHEC No: ICMR/2014/12.