International Journal of Medical and Health Research
ISSN: 2454-9142
www.medicalsciencejournal.com
Volume 4; Issue 1; January 2018; Page No. 41-46

Study of umbilical cord blood culture in diagnosis of early-onset sepsis among newborns with high-risk factors

[1] CD Aundhakar, [2] Harshada Tatiya, [3] Geeta Karande, [4] Akhila S, [5] Madhura K
[1] Professor & HOD, Department of Pediatrics, Krishna Institute of Medical Sciences, Karad, Maharashtra, India
[2, 4, 5] Resident, Krishna Institute of Medical Sciences, Karad, Maharashtra, India
[3] Professor & HOD, Department of Microbiology, Krishna Institute of Medical Sciences, Karad, Maharashtra, India

Abstract
Introduction: Neonatal sepsis is most common cause of neonatal mortality. Umbilical cord is a less commonly used site for collection of blood culture.
Objectives: To analyze correlation of UCBC (Umbilical Cord Blood Culture) with Sepsis Screen and PVBC (Peripheral Vein Blood Culture) in Early Onset Neonatal Sepsis and also to compare organisms identified by UCBC and PVBC in neonatal sepsis.
Methodology: A cohort of 75 neonates with perinatal infection risk factors was followed up prospectively. In the delivery room a blood sample from the umbilical vein was collected for culture. Peripheral venous blood was also collected for culture. All enrolled neonates were followed till their hospital stay. Analysis: Sepsis screen and UCBC were compared with PVBC to find out their sensitivity and specificity.
Results: Among the 75 high risk neonates, sepsis screen was found positive in 24 (32%) while UCBC and PVBC were found positive in 13 (17.3%) and 4 (5.3%) respectively. *Pseudomonas* was the most commonly found organism in culture. Sepsis screen and UCBC had sensitivity of 100% and 75% respectively while specificity was 71.83% and 85.92% respectively when compared to PVBC.
Conclusion: UCBC has good diagnostic validity for etiological diagnosis of bacterial sepsis in high risk neonates. Organisms grown in umbilical cord blood samples are comparable with venous blood culture samples. UCBC could be a painless, kinder, gentle approach instead of painful collection of blood by pricking the neonate.

Keywords: sepsis Screen, Umbilical cord blood culture, perinatal infection risk, high risk neonate, UCBC, PVBC, early onset neonatal sepsis

Introduction

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 fetuses are infected in utero and up to 10% of infants have infections in the 1st month of life [2].

Neonatal sepsis is defined as a blood stream infection which develops within 28 days after birth [3]. 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 [4]. Early recognition of sepsis is required for prompt initiation of antibiotics to prevent neonatal morbidity and mortality [5].

Gold standard for the diagnosis of neonatal sepsis is blood culture collected from peripheral veins [6]. Identification of organism responsible for neonatal sepsis is important as decisions on antibiotics selection and duration of treatment are dependent on it [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].

Additionally, a positive blood culture is costly and time consuming. As a result, early diagnosis of neonatal sepsis has been a frustrating experience in developing and developed countries. 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]. Serial measurement of CRP is considered more useful than single titer in diagnosis of sepsis. Such tests could be of special importance in a newborn that is asymptomatic or has only equivocal signs at birth and risk factors for infection [11].

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.

Aim

To study UCBC in diagnosis of early-onset neonatal bacterial sepsis in high risk neonates.

Objectives

To study correlation of UCBC with Sepsis Screen and PVBC in Early Onset Neonatal Sepsis (EONS), to compare organisms identified by UCBC & PVBC and to compare EONS among primigravida and multigravida mothers.

Methodology

This prospective cohort study was conducted in Krishna Institute of Medical Sciences, Neonatal Intensive Care Unit (NICU) from December 2015 to May 2017, for 18 month duration. Sample size was calculated by using following formula:

$$Sample\ size\ (n) = \frac{1.96^2 \times p \times (1-p)}{l^2}$$

Taking prevalence (p) for positive umbilical cord blood cultute (UCBC) as 24.44% (Kalathia MB *et al.* 2013) [12] and allowable error at 10% the calculated sample size was 71 at 95% Confidence interval and design effect of 1. An additional sample of 10% was added to adjust sample loss due to attrision or contamination or any other unforseen events. So the final sample size was 78. In the present study 78 cases were recruited, however 2 blood samples were discarded due to contamination & 1 neonate expired due to DIC (Disseminated Intravascular Coagulation). So, finally 75 cases were included in the final analysis.

Newborns admitted to our hospital at a risk of developing sepsis based on presence of two or more risk factors were considered for inclusion in the study. Risk factors of EONS were prematurity, prolonged and/or premature rupture of membranes, prolonged labor (>24h), foul smelling liquor, maternal fever, frequent vaginal examination, instrumental delivery, and low birth weight < 2500gm.

Healthy newborns with no antenatal risk factors for EONS, very low birth weight babies (<1500gm), outborn babies, babies with congenital anomalies or babies who expired before collection of data were excluded from the study.

The study was approved by institutional ethics committee before initiation. Written informed consent was obtained from the parents of newborns prior to the enrolment of subjects in the study.

Methods

All newborns enrolled in the study were admitted to neonatal intensive care unit for observation. Clinical data for these neonates were recorded prospectively and in the delivery room a blood sample from the umbilical vein was sent for culture.

Method of umbilical cord blood collection

Umbilical cord was clamped at the placenta side and the infant side. Cord was wiped with 70% isopropyl alcohol using sterile technique. Using a sterile 22-guage needle and syringe, approximately 3.5 to 4 ml of blood was drawn into the syringe from umbilical vein. Needle was replaced from syringe with new sterile needle and culture bottle top was cleaned with alcohol. 1.5 ml blood was injected in aerobic blood culture bottle & remaining blood sample was sent to laboratory for sepsis screen.

Fig 1: Blood culture bottle

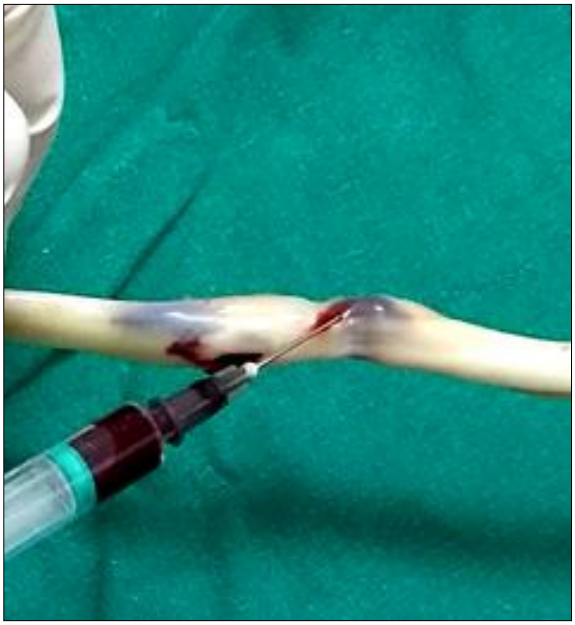

Fig 2: Umbilical cord blood collection

Peripheral Vein blood collection

After providing routine care, collection of peripheral vein blood was done using sterile technique in a separate culture

bottle and labeled. Both culture samples were immediately taken to microbiology laboratory for further processing.

Clinical Diagnosis of Sepsis

Newborns were examined for any clinical feature of sepsis such as lethargy, tachycardia, abdominal distension, intercostal retractions, grunting, increased aspirates, hypotension, hypoglycaemia, pallor, apnoea, abnormal skin color, bradycardia, sclerema, shock, and features of disseminated intravascular coagulation.

Sepsis Screen

Newborns were subjected to a sepsis screen (Total leucocyte count, absolute neutrophil count, I: T ratio, C-reactive protein, mESR) on admission. Sepsis screen was considered positive if two or more parameters were abnormal. All newborns were followed till the end of their hospital stay. Baseline characteristics such as sex, gestational age, weight, risk factors for sepsis, clinical features, and sepsis screen reports, UCBC and PVBC reports were recorded.

Statistical analysis

Qualitative variable are presented as frequency and percentage while quantitative variables are presented in term of mean and standard deviations. Statistical significance was calculated by chisquare test for categorial variables and by t test for continuous variable. P value less than 0.05 was considered as statistically significant.

The validity of UCBC for diagnosis of early neonatal sepsis was measured by mean of sensitivity, specificity, positive predictive value and negative predictive value.

Results

Table 1: Distribution of cases

	Frequency	Percentage
Gestational Age		
<34 weeks	13	17.3
34-36 weeks	35	46.7
37-38 weeks	24	32.0
>38 weeks	3	4.0
Sex		
Male	48	64.0
Female	27	36.0
Birth Weight (gms)		
1500-1999	42	56.0
2000-2500	28	37.3
>2500	05	6.7
Maternal Age (yrs.)		
<=20 years	5	6.7
21-25 years	27	36.0
26-30 years	31	41.3
>30 years	12	16.0
Mode of delivery		
Caesarean section	44	58.7
Vaginal Delivery	31	41.3

Among the 75 neonates, mean gestational age was 36.6 weeks with standard deviation of 0.7 weeks, male to female ratio was 1.8:1, mean birth weight was 1956 grams with standard deviation of 667 grams, mean maternal age was 26.1 year with standard deviation of 5.8 years.

Among the 75 neonates, 37 (49.3%) were premature, 63 (84%) were having low birth weight, 27 (36%) were having Prolonged rupture of membrane, 28 (37.3%) were having premature rupture of membrane, 6 (8%) had birth asphyxia and in 8 (10.7%) cases mother had fever. All of them had more than one risk factor.

Table 2: Distribution of cases according to sepsis screen

	Sepsis Screen	Percentage	UCBC	Percentage	PVBC	Percentage
Positive	24	32.00	13	17.3	4	5.3
Negative	51	68.00	62	82.7	71	94.7

As shown in table 3, 24 (32%) cases were having positive sepsis screen, 13 (17.3%) found to have UCBC result positive and 4 (5.3%) neonates found to have PVBC result positive. In 3 cases both, UCBC and PVBC were positive. All positive samples in UCBC and/or PVBC had shown sepsis screen positive.

Table 3: Diagnostic efficiency of Sepsis screen compare to UCBC

Sepsis Screen	Umbilical Cord Blood Culture			
	Positive		Negative	
	Frequency	%	Frequency	%
Positive	8	61.53	16	25.8
Negative	5	38.46	46	74.2
Total	13	100.0	62	100.0

Comparison of sepsis screen results with PVBC results found sensitivity of sepsis screen 61.54% and specificity 74.19%. Positive and negative predictive values were 33.33% and 72% respectively.

Table 4: Diagnostic efficiency of Sepsis screen compare to PVBC

Sepsis Screen	Peripheral Venous Blood Culture			
	Positive		Negative	
	Frequency	%	Frequency	%
Positive	4	100.0	20	28.2
Negative	0	0.0	51	71.8
Total	4	100.0	71	100.0

In comparison to PVBC, sensitivity of sepsis screen was found to be 100% and specificity was 71.83%. Positive and negative predictive values were 16.67% and 100% respectively. All PVBC positive had sepsis screen positive.

Table 5: Diagnostic efficiency of UCBC compare to PVBC

Umbilical Cord Blood Culture	Peripheral Venous Blood Culture			
	Positive		Negative	
	Frequency	%	Frequency	%
Positive	3	75.0	10	14.1
Negative	1	25.0	61	85.9
Total	4	100.0	71	100.0

In comparison to PVCB, the sensitivity of UCBC was found to be 75% and specificity was 85.92%. Positive and negative predictive values were 23.08% and 98.39% respectively.

Table 6: Organism found in UCBC& PVBC

Organism	UCBC		PVBC	
	Frequency	Percentage	Frequency	Percentage
Group B *Streptococcus*	2	2.7	1	1.3
E. coli	3	4.0	1	1.3
Staphylococcus aureus	1	1.3	0	0.0
Klebsiella	2	2.7	0	0.0
Acinetobacter	1	1.3	0	0.0
Pseudomonas	4	5.3	2*	2.7
No Growth (sterile)	62	82.7	71	94.7
Total	75	100.0	75	100.0

*One sample was positive in PVBC only, it was negative in UCBC.

Culture isolate in UCBC samples showed Group B *Streptococcus* growth in 2 (2.7%) samples. Out of these one was also found positive in PVBC. *E. coli* was found in 3 (4%) samples in UCBC but found in only 1 sample in PVCB. *Pseudomonas* grows in 4 (5.3%) samples in UCBC and was found positive in 2 (2.7%) sample in PVCB. *Klebsiella* (2.7%), *Acinetobacter* (1.3%) and *Staphylococcus* (1.3%) were found positive only in UCBC. Out of four samples found positive in PVBC, three had shown same results in UCBC. One sample was negative in UCBC but found positive in PVBC (*Pseudomonas*). In UCBC 62 (82.7%) samples did not show any growth while in PVBC 71 (94.7%) samples did not show any growth.

Table 7: Bivariate analysis of risk factors with UCBC results

Risk factor	UCBC		P value
	Positive (n=13)	Negative (n=59)	
Prematurity (<35 weeks)	10 (76.9%)	27 (45.8%)	0.03
Low birth weight (<2500)*	13 (100.0%)	50 (84.7%)	-
Maternal fever (>100.4F)	4 (30.8%)	4 (6.8%)	0.009
Prolonged rupture of membrane (>18hrs)	9 (69.2%)	18 (30.5%)	0.006
Premature rupture of membrane	8 (61.5%)	20 (33.9%)	0.047
Birth asphyxia	3 (23.1%)	3 (5.1%)	0.02

*One of the cell value was zero (BW>2500gm and UCBC positive) p value was not calculated

All risk factors like Prematurity, Low birth weight, Premature rupture of membrane, and birth asphyxia was significantly ($p<0.05$) associated with UCBC growth / positivity. Similarly maternal fever and prolonged rupture of membrane was highly significantly ($p<0.01$) associated with UCBC positivity.

Discussion

The present prospective cohort study was conducted among

75 neonates having risk of developing early onset neonatal sepsis. Sepsis is one of the most common infectious conditions in the neonatal period, and remains a major source of morbidity and mortality despite extraordinary progress in the field of neonatology in recent years [13].

Early onset sepsis typically manifests as a fulminant, multisystem illness usually acquired by vertical transmission from the mother. Late-onset sepsis may occur as early as postnatal day 3, but is more common after the first week of life and is usually a progressive illness characterized by focal infection. The case fatality rate is higher in Early onset neonatal sepsis (EONS) as compared with Late onset sepsis [14].

In our study among the 75 neonates, 13 (17.3%) found to have Umbilical Cord Blood Culture (UCBC) result positive while remaining 62 (82.7%) did not show any growth. Thus, in high risk neonates umbilical blood culture positivity was 17.3%. In a study by Kalathia MB *et al.* [12] 24.44% patient had positive UCBC growth. In a study of Pryles *et al.* [15], 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 [16]. In a study by Fos *et al.* [17], 43% (13 out of 30) UCBC were positive in high risk for sepsis newborns.

Collecting UCBC is studied years back by a few researchers. In 1963, Pryles *et al.* [15], reported effect of chorioamniotic infection on newborns by using UCBC in 150 patients. In 1966, Albers and Tyler [18]. studied umbilical cultures for diagnosis of neonatal sepsis. In 1981, Polin *et al.* [19], reported use of UCBC for diagnosis of neonatal sepsis by collecting 200 UCBC. In their study, Herson *et al.* [16], used blood collected from umblical vein from placental surface from 81 newborns and concluded it to be an useful addition in newborn at-risk for sepsis.

Growth of microorganism in a PVBC sample is the gold standard for diagnosis of neonatal sepsis [20-23]. Volume of blood sample collected is important factor in blood culture positivity. A major problem of PVBC is difficulty in collecting adequate volume of blood [8]. Antibiotics administration before collecting blood sample is a common reason for no growth in PVBC [24]. Health care providers with increased skills are needed to perform venipuncture of a neonate, and these highly skilled providers must spare so much time to obtain a newborn blood sample [20].

In 2005, Hansen *et al.* [9], assessed paired results of cord blood and venous blood for complete blood counts for analysis in 113 newborns. The conclusion was cord blood could be safely substituted for infant blood in sepsis evaluations of asymptomatic term infants.

In 2006, Costakos *et al.* [25], has substituted conventional blood culture collection with umbilical cord blood sample as part of universal screening for early-onset sepsis based on maternal risk factors and reported about the process of collecting UCBC and showed the method is reliable and less painful.

In 2010, Fos *et al.* [17]. had collected UCBCs of 30 newborns samples and concluded that UCBC represents more easier and sensible way of diagnosis of neonatal sepsis.

We analyzed samples of 75 newborns. Sample size of above-mentioned studies ranged from 30 to 319. Studies by Herson *et al.*, and Fos *et al.*, closely resemble our study, which focused on perinatal risk factors of neonatal sepsis. Herson *et al.*, had a sample size of 81 and Fos *et al.*, had an effective sample size of 30 UCBCs.

Diagnosis of sepsis was made based on positive PVBC. In our study among the 75 neonates, 4 (5.3%) neonates found to have Peripheral Venous Blood culture (PVBC) result positive while remaining 71 (94.7%) did not show any growth. Thus, in high risk neonates Peripheral Venous blood culture positivity rate was 5.3%.

Chacko and Sohi [13] have shown sepsis rate of 20.6% in high-risk newborns and 0.5% of no risk newborns. Fos *et al.* [17], has shown a sepsis rate of 28% in high-risk newborns. Pryles *et al.* [15], have shown a sepsis rate of 31% in neonates with high risk of sepsis.

In present study, culture isolate in UCBC samples showed Group B *Streptococcus* growth in 2 (2.7%) samples. Out of these one was also found positive in PVBC. *E. coli* was found in 3 (4%) samples in UCBC but found in only 1 sample in PVCB. *Pseudomonas* growth in 4 (5.3%) samples in UCBC and was found positive in 2 (2.7%) sample in PVCB. *Klebsiella* (2.7%), *Acinetobacter* (1.3%) and *Staphylococcus aureus* (1.3%) were found positive only in UCBC. Out of four samples found positive in PVBC, three had shown same results in UCBC. One sample was negative in UCBC but found positive in PVBC (*Pseudomonas*) In UCBC, 62 (82.7%) samples did not show any growth while in PVBC 71 (94.7%) samples did not show any growth.

In the study by Fos *Et al.* [17], gram-positive and gram-negative organisms constituted 50% each. According to National neonatal perinatal database of India of 2002-03, organisms causing sepsis in intramural babies were *Klebsiella* (32.5%), *Staphylococcus aureus* (13.6%), *E. coli* (10.6), *Pseudomonas* (5.6%), and *Acinetobacter* (2.7%) [26]. A study of Sundaram *et al.* [27], showed, nonfermenting gram-negative bacilli found in 30% of cultures, with *S. aureus* (20%), *Klebsiella* (12%), *Acinetobacter* (5%), and *Pseudomonas* in (4%). In these studies, gram-negative organisms were predominant, but organism profiles were different from our study. However, recent studies from India showed similar organism profile as in our study. A study by Bhat *et al.* [28], showed 90.8% organisms were gram-negative and commonest organism were *Pseudomonas* (33.2%), *Klebsiella* (31.2%), *Acinetobacter* (14.4%), and *E. coli* (4.4%). In a study by Chacko and Sohi[13] *Pseudomonas* was found in 60% culture positive sepsis, followed by *Klebsiella* (13%) *S. aureus* (13%), and *E. coli* (7%). Other study of Pais *et al.* [29], suggested that commonest organism growth was of *Pseudomonas* in early-onset sepsis (11.46%).

In our study out of 24 positive sepsis screen, 4 were showed growth on PVBC. The sensitivity of sepsis screen was found to be 100% and specificity was 71.83%. Positive and negative predictive values were 16.67% and 100% respectively. In a study by Kalathia MB *et al.* [12] all positive PVCB culture reports were from the patient having clinical diagnosis of sepsis, and sepsis screen was positive in all these newborns.

Conclusion

From our study we conclude that UCBC has good diagnostic validity for etiological diagnosis of bacterial sepsis in high-risk neonates as compared with PVBC. Meticulous &

fastidious collection of UCBC can prevent contamination. Organisms grown in umbilical cord blood samples are comparable with venous blood culture. It definitely has an additional value for diagnosis of neonatal sepsis. UCBC may be a painless, kinder & gentle approach instead of painful collection of blood by pricking the neonate. Suitability of this method of blood culture may require a larger study to document the accurate sensitivity & specificity.

References

1. Qazi SA, Stoll BJ. Neonatal sepsis: a major global public health challenge. Pediatr Infect Dis J, 2009; 28:1-2.
2. Barbara J, Stoll Shane AL. Nelson textbook of paediatrics. 20th edition. Infection of the Neonatal Infant. 1999, 909.
3. Liu L, Johnson HL, Cousens S. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012; 379:2151-61.
4. Stoll BJ, Hansen NI, Sanchez PJ. Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. Pediatr. 2011; 127:817-26.
5. Dutta S, Kadam S, Saini SS. Management of neonatal sepsis. evidenced based clinical practice guidelines. National Neonatology Forum of India, 2010, 155-72.
6. Chawla D, Agarwal R. Rational approach to diagnosis of neonatal sepsis. J Neonatol. 2006; 20:4-7.
7. Saini SS, Dutta S, Ray P, Narang A. Short course versus 7day course of intravenous antibiotics for probably neonatal septicemia: A pilot openlabel randomized controlled trial. Indian Pediatr. 2011; 48:19-24.
8. Brown DR, Kutler D, Rai B, Chan T, Cohen M. Bacterial concentration and blood volume required for a positive blood culture. J Perinatol. 1995; 15:157-9.[PubMed: 7595778]
9. Hansen A, Forbes P, Buck R. Potential substitution of cord blood for infant blood in the neonatal sepsis evaluation. Neonatology. 2005; 88(1):12-8.
10. Chiesa C, Pellegrini G, Panero A, Osborn JF, Signore F, Assumma *et al.* C-reactive protein, interleukin 6 and procalcitonin in immediate post natal period: influence of illness severity, risk status, antenatal and perinatal complications and infection. Clin Chem. 2003; 49:60-8.
11. Escobar GJ. Effect of systemic inflammatory response on biochemical markers of neonatal bacterial infection: A fresh look at old confounders. Clini Chem. 2003; 49:21-22.
12. Kalathia MB, Shingala PA, Parmar PN, Parikh YN, Kalathia IM. Study of Umbilical Cord Blood Culture in Diagnosis of Early-onset Sepsis Among Newborns with High-risk Factors. J Clin Neonatol. 2013; 2(4):169-72. Doi: 10.4103/2249- 4847.123092.
13. Chacko B, Sohi I. Early onset neonatal sepsis. Indian J Pediatr. 2005; 72:23-26.
14. Haque KN. Definitions of bloodstream infection in the newborn. PediatrCrit Care Med. 2005; 6:S45-49.
15. Pryles CV, Steg LN, Nair S, Gellis SS, Tenney B. A controlled study of the influence on the newborn of prolonged premature rupture of the amniotic membranes and/or infection in the mother. Pediatrics. 1963; 31:608-22.
16. Herson VC, Block C, McLaughlin JC, Tetreault J, Eisenfeld LI, Krause PJ *et al.* Placental blood sampling: An aid to the diagnosis of neonatal sepsis. J Perinatol. 1998; 18:135-7.
17. Fos NI, Gomis RV, Gomis CV, Rubio J, Justich P, Valera JC *et al.* Blood culture from the umbilical vein in the diagnosis of neonatal sepsis. Internet J PediatrNeonatol. 2010; 12:1.
18. Tyler CW, Jr Albers WH. Obstetric factors related to bacteremia in the newborn infant. Am J Obstet Gynecol. 1966; 94:970-6.
19. Polin JI, Knox I, Baumgart S, Campman E, Mennuti MT, Polin RA *et al.* Use of umbilical cord blood culture for detection of neonatal bacteremia. Obstet Gynecol. 1981; 57:233-7.
20. Polin RA Committee on Fetus and Newborn. Management of neonates with suspected or proven early-onset bacterial sepsis. [Last accessed on 2017 May 10]; Pediatrics. 2012 129:1006-15. Available from: http://pediatrics.aappublications.org/content/129/5/1006.full.html. [PubMed: 22547779]
21. Deorari AK, Paul VK. Investigators of National neonatal perinatal database NNPD. Report 2002-2003. NNPD Network. 2005. Jan, [Last accessed on 2017 May 10]. Available from: http://www.newbornwhocc.org/pdf/nnpdreport2002-03.PDF
22. Chawla D, Agarwal R. Rational approach to diagnosis of neonatal sepsis. J Neonatol. 2006; 20:4-7.
23. Dutta S, Kadam S, Saini SS, Bhakoo ON, Mathur NB Writing group. National neonatalogy forum of India: Evidenced based clinical practice guidelines; Management of Neonatal Sepsis; 2010, 155-72.
24. Neal PR, Kleiman MB, Reynolds JK, Allen SD, Lemons JA, Yu PL *et al.* Volume of blood submitted for culture from neonates. ClinMicrobiol. 1986; 24:353-6. [PMCID: PMC268912]
25. Costakos DT, Walden J, Rinzel MT, Dahlen L. Painless blood testing to prevent neonatal sepsis. WMJ. 2009; 108:321-2.
26. Deorari A Investigators of National neonatal perinatal database NNPD Changing pattern of bacteriologic profile in neonatal sepsis among intramural babies. J Neonatol. 2006; 20:1.
27. Sundaram V, Kumar P, Narang A. Bacterial profile of early versus late onset neonatal sepsis in a North Indian tertiary care center: Heading towards a change. J Pediatr Infect Dis. 2009; 4:241-5.
28. Bhat YR, Lewis LE, Vandana KE. Bacterial isolates of early-onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: An audit from a center in India. Ital J Pediatr. 2011; 37:32.
29. Pais M, Devi ES, Pai MV, Lewis L, Gorge A, Mayya S, *et al.* Neonatal sepsis, bacterial isolates and antibiotic susceptibility patterns among neonates. Nurs J India. 2012; 103:18-20.