European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 10, Issue 02, 2023

Original Research Article

To compare the sensitivity of blood cultures on Day 1 (24 Hours) Day 3 (72 Hours) & Day 5 (120 Hours) in neonatal sepsis

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Abstract

Aims and Objectives: To compare the sensitivity of blood cultures on Day 1(24 Hours) Day 3 (72 Hours) & Day 5(120 Hours) in neonatal sepsis.

Materials and Methods: The study was a hospital based observational study conducted in the neonatal ICU, Dept. of Paediatrics and Dept. Of Microbiology, SKIMS, Srinagar, from 1st January 2016 to 1st June 2017. All those neonates with features of sepsis like lethargy, refusal of feeds, irritability, fever, seizure and deranged lab values like CRP, Procalcitonin, and CBC were considered in study. Before drawing blood culture samples, neonates already on antibiotics were excluded from the study. In addition, participants with incomplete details were also excluded from the study.

Results: In our study 1060 cultures were sent from neonates suspected to have sepsis. A total of 85 cultures were found to be positive with an overall culture positivity of 8%. Out of them 64 (75.3%) were positive within 24 hours of incubation (subsequently referred to as culture positive on day 1), 17 (20.0%) were culture positive on day 3 (within 72 hours of incubation, but not within 24 hours) and 4 (4.7%) were culture positive on day 5 (within 120 hours of incubation but not within 72 hours of incubation).

Conclusion: We recommend incubating cultures for 5 days to pick up additional 5% cases of neonatal sepsis.

Keywords: Blood culture, neonatal sepsis, clinical syndrome, bacteraemia

ISSN 2515-8260 Volume 10, Issue 02, 2023

Introduction

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteraemia in the first month of life. It encompasses various systemic infections of the newborn such as septicaemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections. It is estimated that in developing countries up to 20% of neonates develop sepsis [1].

Sepsis related mortality is largely preventable with prevention of sepsis itself, timely recognition, rational antimicrobial therapy and aggressive supportive care. Since treatment should be initiated in a neonate suspected to have sepsis without any delay, only minimal and rapid investigations should be undertaken [2] which usually include the sepsis screen, blood culture and analysis of relevant body fluids. All neonates suspected to have sepsis usually have a sepsis screen to corroborate the diagnosis which commonly includes tests such as total leukocyte count (TLC), absolute neutrophil count (ANC), immature to total (IT) neutrophil ratio, micro-erythrocyte sedimentation rate and C reactive protein (CRP) etc. However, sepsis screen is non-specific and the decision to start antibiotics need not be conditional to the sepsis screen [5, 6] results, if there is a strong clinical suspicion of sepsis.

Blood culture is the gold standard for diagnosis of septicaemia and must be performed in all cases of suspected sepsis prior to starting antibiotics [3]. We therefore studied the time to culture positivity in neonatal sepsis and the need of prolonged incubation (> 3 days) in automated blood culture systems. We also studied for potential clinical or laboratory parameters predicting faster detection of organisms using the automated blood culture systems.

Material and Method

The study was conducted to compare the sensitivity of blood cultures on Day 1(24 Hours) Day 3 (72 Hours) & Day 5(120 Hours) in suspected neonatal sepsis. The study was a hospital based observational study conducted in the neonatal ICU, Dept. of Paediatrics and Dept. Of Microbiology, SKIMS, Srinagar from 1st January 2016 to 1st June 2017.

Inclusion criteria

- All neonates admitted in NICU (including term/preterm/inborn/out born) with clinical features of sepsis like lethargy, refusal of feeds, irritability, fever, seizure and deranged lab values [4] like CRP, Procalcitonin and CBC [5] were enrolled in study.

Exclusion criteria

- Neonates already on antibiotics before drawing blood culture samples were excluded from the study.
- Participants with incomplete details were also excluded from the study.

All neonates who presented to us with suspected sepsis underwent full sepsis work up including blood culture. Veni-puncture site was cleaned with povidone iodine solution (10% w/v) followed by a solution containing 70% v/v alcohol and 2.5% v/v chlorhexidine gluconate solution. This sequence was repeated again before drawing 1 ml of venous blood samples which were collected in BacT/ALERT® PF Plus Aerobic Pediatric Blood Culture Bottles (BioMerieux, Inc. Durham, NC 27712).

All blood culture vials were processed in BACT/ALERT® 3D system in the Department of Microbiology, SKIMS, Soura. The BacT/ALERT Microbial Detection System utilizes a

ISSN 2515-8260 Volume 10, Issue 02, 2023

colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO_2) dissolved in the culture medium. If microorganisms are present in the test sample, CO_2 is produced as the organisms metabolize the substrates in the culture medium. When growth of the microorganisms produces CO_2, the color of the gas-permeable sensor installed in the bottom of each culture bottle changes from blue-green to yellow. The lighter color results in an increase of reflectance units monitored by the system. Bottle reflectance is monitored and recorded by the instrument every 10 minutes.

The various clinical and laboratory parameters of the patients with positive blood cultures were also recorded. The data collected was entered into Microsoft Excel worksheet and SPSS version 20 was used for statistical analysis. Continuous variables with non-normal distribution were expressed as Median (IQR) and non-parametric tests were used for statistical analysis. For normally distributed continuous variables Mean ± SD and parametric tests were used. All tests of significance were two tailed and a p value < 0.05 was considered statistically significant.

Results

A total of 1060 cultures were sent from neonates suspected to have sepsis. A total of 85 cultures were found to be positive with an overall culture positivity of 8%. Out of them 64 (75.3%) were positive within 24 hours of incubation (subsequently referred to as culture positive on day 1), 17 (20.0%) were positive on day 3 (i.e. culture positive within 72 hours of incubation, but not within 24 hours) and 4 (4.7%) were positive on day 5 (i.e. within 120 hours of incubation but not within 72 hours of incubation).

Table 1: Frequency distribution of day of culture positivity

Day	N	%
Day 1	64	75.3
Day 3	17	20.0
Day 5	4	4.7
Total	85	100

Significantly higher numbers of blood cultures were positive on days 1 and 3 as compared to day 5. Approximately 95% cultures were positive within 72 hours of incubation.

Klebsiella pneumonia was the leading organism isolated followed by Pseudomonas Aeruginosa as shown in table 2.

Table 2: Frequency and percentage distribution of the organisms isolated in our series

Organism	Frequency	Percentage
Klebsiella pneumonia	26	30.6
Pseudomonas aeruginosa	14	16.5
Candida krusei	7	8.2
Candida albicans	5	5.9
Staphylococcus aureus	11	12.9
CoNS	11	12.9
Acinetobacter baumanii	5	5.9
E coli	2	2.4
GBS	1	1.2
Candida parapsilosis	1	1.2
Enterococcus fecalis	2	2.4
Total	85	100.0

ISSN 2515-8260 Volume 10, Issue 02, 2023

Overall, Gram negative sepsis (57.6%) was significantly higher as compared to Gram positive (27.1%) and fungal sepsis (15.3%) as shown in table 3.
There was no statistically significant relation between the organism class and the day of culture positivity.

Table 3: Day of culture positivity and organism class cross tabulated. P value = 0.697 (Fisher's Exact Test)

		Organism class			Total
		Gram Positive	Gram Negative	Candida spp.	
Day Culture Positive	1	18	38	8	64
	3	4	9	4	17
	5	1	2	1	4
Total		23	49	13	85

The summary characteristics of the variables of the study classified according to the day of culture positivity has been shown below.

Table 4: The summary characteristics of the variables of the study classified according to the day of culture positivity

Parameter	Day 1 culture Median (IQR) Or %	Day 3 culture Median (IQR) Or %	Day 5 culture Median (IQR) Or %	P value
Age (days)	6.00 (4.00, 9.00)	7.00 (3.00, 9.00)	4.50 (1.75, 8.00)	0.577
ANC ($*10^3$/µL)	1.70 (1.20, 12.00)	1.90 (1.60, 10.00)	1.65 (1.375, 11.675)	0.513
Platelet ($*10^3$/µL)	181.0 (144.25, 287.75)	204.0 (175.00, 291)	305.50 (181.25, 404.25)	0.277
Na	138.5 (133.25, 143.00)	140.0 (134.0, 143.0)	137.00 (133.50, 139.75)	0.778
K	3.60 (2.95, 4.40)	3.70 (3.10, 4.20)	3.60 (3.025, 4.55)	0.968
Ca (mg/dl)	8.65 (7.80, 9.975)	9.20 (8.05, 10.05)	8.95 (7.825, 10.00)	0.821

Discussion

More than one-third of estimated four million neonatal deaths around the world each year are caused by severe infections and quarter-around one million deaths are due to neonatal sepsis/pneumonia alone [6]. Early diagnosis and treatment is the key to improve outcomes. Neonates with suspicion of sepsis are treated empirically with antibiotics before definitive diagnosis is made. Although, no single test or combination of tests is 100% sensitive to rule out sepsis, culture remains the gold standard, with antibiotics often being discontinued after negative blood and body fluid cultures. Additionally, cultures are highly specific and provide drug sensitivity testing for targeted antimicrobial therapy. Cultures have historically been incubated for 5-7 days before being declared negative. Several studies on the time to positivity (TTP) of neonatal blood cultures have altered common practice and suggest discontinuing antibiotic therapy if blood cultures are still negative after 48-72 hours in neonates without clinical or laboratory signs of infection. Other studies utilizing automated culture systems suggest a 24-36 hour observation period to rule out sepsis in asymptomatic neonates.
Most of the studies have been conducted in developed countries and data regarding

developing countries is scarce. Therefore, the aim of our study was to study time to culture positivity among neonates of our population. As early identification of pathogens in the blood can be a crucial step in assuring appropriate therapy, and beginning effective antibiotic therapy as early as possible can have a significant impact on the outcome of the disease [7, 8].
In our study we recruited 85 neonates with culture proven sepsis. A total of 1060 cultures were sent from neonates suspected to have sepsis. Therefore overall culture positivity was 8%.
95.3% of cultures in our study were positive by 72 hours of incubation. The percentage of cultures positive by 72 hours of incubation in different studies has been shown below:

Table 5: The percentage of cultures positive by 72 hours of incubation in different studies

Author name (Year)	Total cultures positive	Cultures positive by 72 hrs
Pauli I Jr *et al*. (1999) [9]	49	100%
Garcia-Prats *et al*. (2000) [10]	445	100%
Y Kumar *et al*. (2001) [11]	404	99.8%
Kumar VCS *et al*. (2005) [12]	877	97.8%
Guerti K *et al*. (2011) [13]	437	97.1%
Our study (2017)	85	95.1%

There was no significant association between organism class and day of culture positivity. Some studies have reported delayed culture positivity for Gram negative organisms whereas others have shown delayed culture positivity for Gram positives whereas others have shown comparable growth.

Conclusion

This was a prospective study conducted in the neonatal ICU, Dept. of Paediatrics and Dept. Of Microbiology, SKIMS.
In our study we recruited 85 neonates with culture proven sepsis. A total of 1060 cultures were sent from neonates suspected to have sepsis. Therefore overall culture positivity was 8%.
75.29% cultures were positive by Day 1, 95.29% by Day 3 and 100% by Day 5.
We recommend incubating cultures for 5 days to pick up additional (~5%) cases of neonatal sepsis.

References

1. Stoll BJ. The global impact of neonatal infection. Clin. Perinatol. 1997;24:1-21.
2. Gerdes JS, Polin R. Early diagnosis and treatment of neonatal sepsis. Indian J Pediatr. 1998;65:63-78.
3. Principles and procedures for Blood Cultures; Approved Guideline, CLSI document M47-A. Clinical and Laboratory Standards Institute (CLSI); Wayne, P.A.; c2007.
4. Ng PC. Diagnostic markers of infection in neonates. Arch Dis Child Fetal Neonatal Ed. 2004;89:F229-235.
5. Christoph P, Hornik MD, Daniel K. Benjamin, use of complete blood cell count in early-onset neonatal sepsis. The Pediatric Infectious Disease Journal; c2012.
6. Neonatal sepsis-a major killer to be tackled in communities. WHO; c2009.
7. Garey KW, Rege M, Manjunath P Pai, Mingo DE, Suda KJ, Turpin RS. *et al*. Time to Initiation of Fluconazole Therapy Impacts Mortality in Patients with Candidemia: A Multi-Institutional Study. Clin Infect Dis. 2006;43(1):25-31.
8. Khatib R, Saeed S, Sharma M, Riederer K, Fakih MG, Johnson LB. Impact of initial

antibiotic choice and delayed appropriate treatment on the outcome of Staphylococcus aureus bacteremia. Eur. J Clin. Microbial Infect Dis. 2006;25(3):181-5.

9. Pauli I, Jr. Shekhawat P, Kehl S, Sasidharan P. Early detection of bacteremia in the neonatal intensive care unit using the new BACTEC system. J Perinatol. 1999;19:127-131.
10. Joseph A Garcia-Prats, Timothy R Cooper, Virginia F Schneider, Stager E, Thomas N Hansen. Rapid Detection of Microorganisms in Blood Cultures of Newborn Infants Utilizing an Automated Blood Culture System. AAP News and Journals, 2000 March, 105(3).
11. Kumar Y, Qunibi M, Neal TJ, Yoxall CW. Time to positivity of neonatal blood cultures. Arch Dis Child Fetal Neonatal. 2001;Ed85:F182-F186.
12. Vinod Kumar CS, Neelagaud YF. Incubation period for culture positivity to detect septicaemia in neonates. Indian J Med Microbiol. 2005;23:270-271.
13. Khadija Guerti, Helena Devos, Margareta M Ieven, Ludo M Mahieu. Time to positivity of neonatal blood cultures Fast and furious? Journal of Medical Microbiology. 2011;60:446-v453.