Original Research Article

Enhancing neonatal sepsis detection: A comparative analysis of interleukin-6 and the BacT/ALERT blood culture system

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ABSTRACT

Background: One of the main causes of newborn mortality is neonatal sepsis, which has to be diagnosed quickly and precisely. Although the Bact/ALERT method for blood culture is commonly used, it is often slow, for early identification of sepsis, interleukin-6 (IL-6) provides a quicker substitute.

Aim and Objective: This study evaluates how well the BacT/ALERT system and IL-6 diagnose newborn sepsis, aiming to determine the most reliable and timely method for improving neonatal outcomes.

Materials and Methods: A clinician at the National Institute of Medical Sciences & Research who admitted neonates to the neonatal intensive care unit (NICU) suspected 120 newborns of having sepsis. The inclusion criteria were satisfied by newborns between the ages of 0 and 28 days who showed clinical signs of sepsis. Exclusion criteria included infants with congenital anomalies or those who had received antibiotics before sampling.

Results: In this study of 120 neonates, IL-6 levels were elevated in 45% of the cases, with a sensitivity of 100%, specificity 86.84%, PPV 81.48% and NPV 100% for. 36.67% of samples had bacterial growth identified by the BacT/ALERT system. In just three hours, IL-6 produced results, which was far quicker than the BacT/ALERT method.

Conclusion: IL-6 is a rapid, sensitive marker for neonatal sepsis, offering faster diagnosis than BacT/ALERT. Combining both methods may improve early detection and treatment outcomes. IL-6 offers rapid, high-sensitivity sepsis detection compared to BacT/ALERT, which, despite being slower, is crucial for pathogen identification. Combining both methods could enhance early diagnosis and treatment.

1. Introduction

During the first 28 days of life, babies are susceptible to neonatal septicaemia, a dangerous blood infection. Because of its high rates of death and morbidity, this illness presents one of the biggest health risks in the area of neonatology. Neonatal septicaemia is one of the main causes of neonatal fatalities, raising urgent public health concerns. Compared to affluent countries, where the average is between 1 and 5 cases per 1,000 live births,

India has an alarmingly high incidence of neonatal sepsis, with 30 to 50 cases per 1,000 live births, according to the National Neonatal-Perinatal Database.[1] The elevated incidence in India can be linked to various factors, including a high rate of preterm births, low birth weight, inadequate infection control measures, and the prevalence of maternal infections during pregnancy.[2] Despite recent advancements in newborn care, there has been little success in reducing mortality from neonatal sepsis, which is a major source of morbidity and death in the paediatric age group.[3] With over a million fatalities worldwide, neonatal sepsis ranks as the ninth most common cause of death for children under five.[2]

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According to WHO estimates, the incidence of neonatal sepsis in Asia ranges from 7 to 38 per 1000 live births.[4] In underdeveloped nations, it continues to rank among the top three causes of newborn mortality.[5] Despite all the advances in medical practices in recent years, septicemia continues to be a serious problem that needs immediate attention and treatment.[6] Blood culture, the gold standard for the diagnosis of septicemia requires up to 48-72 hours before the results are known & almost half of the positive samples do not show growth.[7] When bacterial infection activates monocytes and macrophages, they release the proinflammatory cytokine IL-6. Interleukin-6 is one of the markers of infection which appears earlier and remains elevated in first 24 hours after which it declines.[8] So this study has been designed to comparison between IL-6 detection and blood culture system for early diagnosis of neonatal sepsis.

2. Materials and Methods

It was across-sectional study. This study was carried out in the department of Microbiology and NIMS Hospital during the period of January 2024 to September 2024. One hundred twenty cases of suspected septicemia were enrolled in the study and One hundred twenty healthy newborn were taken for comparison. The samples were randomly collected from neonatology unit, Department of Pediatrics, NIMS hospital. Informed Verbal consent from patient's care taker was obtained for healthy control group.

2.1. Laboratory procedure

Blood cultures were performed by BacT/ALERT system method using BD Blood culture bottles which contains soybean-casein digest broth with anticoagulant (sodium polyanethol sulfonate) with 2-3 ml of blood from each patient and incubate in BacT/ALERT system. When any microbial growth occurs within culture bottle indicates by a beep. Subcultures were done on Blood agar and MaCconkey's agar 24 hours, 48 hours and once before discarding the culture bottle i.e after 5 days. The organisms were identified by their colony morphology, staining character, pigment production, motility and relevant biochemical tests as per standard methods[9]. 2 ml of blood from each patient were collected in a plain vial and centrifuged at 10,000 rpm for 5 minutes. Serum were taken by using micropipette in eppendrop and preserved for estimation of IL-6.IL-6 estimation was carried out by ELISA method using commercial available kit Fine Test.

2.2. ELISA Procedure

Sandwich enzyme-linked immune-sorbent assay technology formed the basis for this kit. 96-well plates were pre-coated with anti-IL-6 antibody. Additionally, as detecting antibodies, the biotin-conjugated anti-IL-6 antibody was employed. Standards, test specimens, and detection of biotin conjugation after that, antibodies were added to the wells, and wash buffer was used to clean them. After adding HRP-Streptavidin, unbound conjugates were eliminated using wash buffer. Utilizing TMB substrates, the HRP enzymatic reaction was observed. HRP catalyzed TMB to yield a blue product, which became yellow with the addition of an acidic stop solution. The amount of IL-6 sample that is caught in the plate is directly correlated with the density of yellow. Calculating the concentration of IL-6 involves reading the O.D. absorbance in a microplate reader at 450 nm.

3. Results

Table 1: Age & gender of neonates studied

Neonates characteristics	No. of neonates	Percentage
Age in days		
0-3 Days	59	49.16
4-28 Days	61	50.84
Gender		
Male	84	70
Female	36	30

In (Table 1) out of 120 neonates with clinical suspicion of septicemia studied 59(49.16%) and 61(50.84%) belonged to EOS (0-3 days) and LOS (4-28 days) respectively. Among suspected cases 70% were males and 30% were females respectively.

Table 2: Spectrum of microorganisms isolated from blood culture

Culture findings	No. of Isolates	Percentage
Blood culture positive	44	36.67
Blood culture negative	76	63.34
Staphylococcus aureus	02	4.54
Enterococcus faecalis	01	2.27
CONS	04	9.09
Micrococcus spp.	02	4.54
Bacillus spp.	01	2.27
Escherichia coli	04	9.09
Klebsiella pneumonia	14	31.81
Acinetobacter baumannii	04	9.09
Pseudomonas aeruginosa	05	11.36
Burkholderia cepecia	01	2.27
Candida spp.	06	13.63

From the above (Table 2) it can be observed that 36.67% were Blood culture positive and rest 63.34% were Blood culture negative.

Table 2 shows out of 44 isolates. *Staphylococcusaureus* was 2(4.54%), CONS 4(9.09%), *Enterococcusfaecalis* 1(2.27%), *Micrococcus* spp. 2(9.09%), *Bacillus* spp. 1(2.27%), *Escherichia coli* 4(9.09%), *Klebsiellapneumonia* 14(31.81%), *Acinetobacterbaumannii* 4(9.09%), *Pseudomonas aeruginosa* 5(11.36%), *Burkholderia cepacia* 1(2.27%) and *Candida* spp. 6(13.63%).

Table 3: Diagnosis of sepsis by IL-6 detection and blood culture

Method	No of cases	Percentage
IL-6 positive	54	45%
IL-6 negative	66	55%
Blood culture positive	44	36.67%
Blood culture negative	76	63.34%

In (Table 3) out of total no of suspected cases we observed the elevation of IL-6 in 45% of cases while 55% cases were normal.

Table 4: Blood culture positive result among IL-6 positive cases (n=54)

Blood culture positive	44	81.48%
Blood culture negative	10	18.52%
Total	54	100%

Among 54 IL-6 positive cases Blood culture method showed positive result in 44 (81.48%) while in 10 (18.52%) cases blood culture method failed to diagnosis sepsis.(Table 4)

Table 5: Comparison of IL-6 with gold standard (Blood culture method)

Method	Blood culture positive	Blood culture negative	Total
IL-6 positive	44 (True positive)	10 (False positive)	54
IL-6 negative	0 (False negative)	66 (True negative)	66
Total	44	76	120
Sensitivity	Specificity	Positive predictive value	Negative predictive value
100%	86.84%	81.48%	100%

In (Table 5) on comparison we observed IL-6 was 100% sensitive and 86.84% specific for diagnosis of sepsis with 81.48% and 100% positive and negative predictive value respectively.

4. Discussion

Neonatal sepsis is a dangerous condition that affects between 0.1-1% of, newborns but among extremely preterm infants incidence rates as high as 30-40% have been reported. Neonatal sepsis causes increased mortality and morbidity,[10–12] with consequences such as poor neurological outcome, broncho pulmonary dysplasia and necrotizing enterocolitis, leading to prolonged hospital stays and increased costs.[13,14]

Diagnosis of neonatal sepsis is based on bacteraemia demonstrated by a positive blood culture, a method with well-known limitations in turnaround time, sensitivity, and specificity. There is no widely accepted definition for neonatal sepsis, but most definitions demand bacteraemia together with clinical signs of sepsis or increased inflammatory parameters.[15] Since blood culture has a poor sensitivity, sepsis treatment is often administered to patients with a clinical picture of sepsis but negative blood cultures; this condition is normally called clinical or suspected sepsis. The standard treatment for neonatal sepsis is intravenous broad spectrum antibiotics together with supportive intensive care. If neonatal sepsis was easier to diagnose, fewer infants would receive antibiotic treatment and the overall antibiotic consumption in neonatal intensive care could diminish.

There were 36.67% of neonates with suspected bacteriologic sepsis confirmed by culture results. A study by Gupta L.K et al.[16] reported 32.75% cases confirmed by blood culture. Worldwide records show that the isolation rates on blood cultures vary from 6.7% to 55.4%.[17] Blood Culture still remains the gold standard for confirming the diagnosis of neonatal sepsis. The success of isolating bacteria from a blood sample depends on the volume of blood culture, timing and frequency of culture, duration and dilution of culture media and the choice of the culture system.[18]

The etiological agents of neonatal sepsis vary between developed and developing countries. *Klebsiella pneumoniae* and other Gram-negative organisms were the common causes of sepsis in the present study as well other studies from.[19,20]

We observed 100% sensitivity, 86.84% specificity, 81.48% PPV and 100% NPP. Sensitivity, specificity, PPV and NPP of IL-6 depends on the cut-off value of test. Tessema B et al,[21] observed 73.1% sensitivity, 80.2% specificity, 37.6% PPV, and 94.8% NPV. In another study, a cut-off level for IL-6 was 21.5 pg/mL, with 75% sensitivity, 82% specificity, 92% PPV, and 52% NPV for neonates with culture confirmed sepsis.[22] Other previous studies have calculated varying cut-off levels and reported inconsistent sensitivity, specificity, PPV, and NPV for IL-6. The cut-off values for IL-6 have been shown to be from 10 to 500 pg/mL, with most falling from 10 to 30 pg/mL.[23] A previous study conducted among 34 neonates with culture confirmed and clinical sepsis reported a cut-off value for IL-6 at 20 pg/mL, with 91% sensitivity, 74% specificity, 78% PPV, and 89% NPV.[24] A study conducted by Ng et al. among 45 neonates with culture confirmed sepsis reported a cut-off level for IL-6 at 31 pg/mL, with 89% sensitivity, 96% specificity, 95% PPV, and 91% NPV.[25]

5. Conclusion

Although blood culture is gold standard method but it is time consuming. IL-6 concentrations were sensitive predictors of neonatal sepsis in neonates we believe that only the measurement of IL-6 concentration may be sufficiently

sensitive for this purpose. We conclude that IL-6 is a useful marker for the early diagnosis of neonatal sepsis, especially with respect to the high negative predictive value.

6. Ethical Approval

This study was conducted after taking approval from the Institute Ethical reviewer Board, with ref. no. NIMSUR/IEC/2022/363.

7. Source of Funding

None.

8. Conflict of Interest

None.

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