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Laboratory Investigations for Neonatal Sepsis

Akshay Tiwari, Manasvee Dewan, Ashok Rattan*

Pathkind Labs, Udyog Vihar, Gurugram,India

***Corresponding Author:** Ashok Rattan, Pathkind Labs, Udyog Vihar, Gurugram, India.

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Abstract

Sepsis is the commonest cause of neonatal mortality and is responsible for 30 to 50 % of total neonatal deaths in developing countries. The mortality due to neonatal sepsis can be prevented with early diagnosis, appropriate antimicrobial therapy and aggressive supportive care.

Due to the subtle and non specific signs and symptoms, prompt and accurate diagnosis of neonatal sepsis is difficult. Blood culture is the gold standard test, but it is slow (positive initial report takes 48 hours or more) and is positive in only a fraction (30%) of total cases. On the other hand, inability to adequately exclude the diagnosis of neonatal sepsis results in prolonged and unnecessary exposure to antibiotics.

Different tests available for diagnosis of early onset sepsis (EOS) as well as late onset sepsis (LOS) together with their strengths

Keywords: Neonatal Sepsis; Blood Culture; CRP; Procalcitonin; Multiplex PCR (Biofire); T2MR

Introduction

Sepsis is the commonest cause of neonatal mortality and is responsible for about 30 to 50 % of the total neonatal deaths in developing countries. Approximately 1% of neonatal deaths have been attributed to sepsis. In developed countries, the incidence of sepsis in term and late preterm infants is low. But as there is potential for serious adverse outcomes, consequently, paediatricians have a low threshold for evaluation and empiric treatment for possible sepsis in neonates [1].

Sepsis is classified according to the infant's age at the onset of symptoms [2].

Early onset sepsis (EOS) is defined as the onset of symptoms before 7 days of age, although some experts limit the definition to infections occurring within the first 72 hours of life. Late onset sepsis (LOS) is defined as the onset of symptoms at > 7 days of age. Similar to EOS, there is variability in the definition, ranging from an onset at > 72 hours of life to > 7 days of age.

EOS is associated with acquisition of microorganisms from the mother. Ascending colonization and infection of the uterine compartment with maternal gastro-intestinal and genitourinary flora during labour with subsequent colonization and invasive infection of the foetus and / or foetal aspiration of infected amniotic fluid. Whether acquired haematogenous across the placenta or via the ascending route, bacterial infection can be a cause of still birth in the third trimester. Listeria monocytogenes, is usually transmitted from the mother to the foetus by the transplacental route. LOS is acquired from the caregiving environment. The organisms implicated in EOS and LOS are:

The infants' skin, respiratory tract, conjunctivae, gastrointestinal tract and umbilicus may become colonized from the environment and such colonization may lead to late onset sepsis from invasive microorganisms. Pneumonia is more common in early onset sepsis, whereas meningitis and bacteraemia are more common in late onset sepsis. When neonatal sepsis is suspected, treatment should be initiated immediately because of relative immunosuppression in neonates. Empiric Antibiotics should be initiated as soon as diagnostic tests are performed.

Bacterial species	Frequency of isolation	
	Early Onset	Late onset
Group B streptococci	+++	++
Escherichia coli	+++	++
Klebsiella pneumoniae	+	++
Enterobacter spp.	+	+
Listeria monocytogenes	++	+
Other enteric gram negatives	+	+
Staphylococcus aureus	+	+++
Citrobacter spp.	-	+
Coagulase Negative Staphylococcus (CONS)	-	++
Pseudomonas spp	-	+
Acinetobacter spp.	-	+

Table 1: +++= commonly associated; ++= frequently associated; +=occasionally associated;- = rarely associated.

When to suspect sepsis [3]

Neonates with signs and symptoms of sepsis require prompt evaluation and initiation of antibiotic therapy. Clinical manifestations range from subtle symptoms to profound septic shock. Signs and symptoms are nonspecific and include temperature instability, irritability, lethargy, respiratory symptoms (e.g. Tachypnea, grunting, hypoxia), poor feeding, tachycardia and hypotension. Laboratory testing is performed in any infant with identifiable risk factors and / or signs and symptoms concerning sepsis.

Diagnostic evaluation of neonates may include

1. Complete Blood count with differential.
2. Micro ESR.
3. I/T ratio.
4. Sepsis screen.
5. Blood culture in all cases.
6. CSF examination.
7. CSF culture in case Blood culture is positive.
8. Urine culture in LOS.
9. Tracheal tube culture (if intubated).
10. C Reactive protein (serial measurement) from blood or saliva.
11. Procalcitonin.
12. Recent advances: Biofire and T2MR.

Complete Blood count with differential and I/T ratios

Total white blood cell counts have poor positive predictive value (PPV) for sepsis. Neutropenia has greater specificity, but the definition of neutropenia is dependent upon gestational age, delivery method and altitude. Low WBC counts (< 5000/mm^3), absolute neutropenia (ANC < 1000 neutrophils/mm^3), relative neutropenia (ANC < 5000 neutrophils/mm^3) and elevated I/T ratio were associated with culture proven sepsis, but none of these are sufficiently sensitive to reliably predict EOS [4,5].

Absolute immature neutrophil counts peak at 12 hours of age, from a maximum value of 1,100 cells/mm3 to 1,500 cells/mm 3 at 12 hours. In contrast, a maximum normal ratio of immature to total white blood cells (I/T ratio) of 0.16 occurs at birth and reaches a nadir of 0.12 with increasing postnatal age. A single value of > 0.3 has a very high negative predictive value (NPV, 99%) but a very poor PPV (25%) for neonatal sepsis. (ref 120) In one study, the I/T squared (I/T divided by the ANC) squared was calculated in order to capture the predictive ability of both ANC and I/T in a single number. Even though it performed better than any of the more traditional tests and was independent of age in hours but had only modest sensitivity and specificity [6].

CBCs obtained 6 to 12 hours after delivery are more predictive of sepsis than those obtained immediately after birth, because WBC and ANC normally increase during the first six hours of life. An elevated I/T ratio (> 0.12) has the best sensitivity for predicting EOS.

In LOS, CBC are frequently relatively more useful in supporting diagnosis of sepsis. WBC counts (< 1000 or >50,000/mm^3), high absolute neutrophil count [ANC] (> 17,670/mm^3), elevated I/T ratio (> 0.2) and low platelet counts (< 50,000/mm^3) are frequently associated with culture positive cases, however, sensitivity is inadequate to reliably predict LOS [7].

Micro ESR

The micro ESR is a popular constituent of the screening tests undertaken in developing countries to detect neonatal sepsis. A normal micro ESR has been considered as "day of life plus 3 mm/hr to maximum 15 mm/hr. The test is normally performed by collecting blood after heal prick into standard 75 mm heparinized microhematocrit tube with internal diameter of 1.1 mm. Both ends of the capillary tube is blocked with plastercin and kept undisturbed vertical, height of plasma column is measured after one hour and reported as micro ESR/hr [8,9].

Sepsis screen [10]

Early diagnosis of neonatal sepsis has been a difficult experience even in developed countries. Due to the subtle and non specific signs and symptoms, prompt and correct diagnosis is difficult. Blood culture is gold standard test but is slow and is positive in only 30 % of cases. Inability to adequately exclude the diagnosis of neonatal sepsis early would result in prolonged and unnecessary exposure to antibiotics. Since each individual test lacks sensitivity and specificity, it has been suggested that combination of tests constitute a Sepsis Screen and if two or more of them are positive, should a tentative diagnosis of Neonatal sepsis be made.

Sepsis screen is considered positive if any two of the following were present:

1. Total Leucocyte count (TLC) pf < 5000/mm^3 or > 20,000/mm^3
2. Absolute Leucocyte count (ANC) of < 1800/mm^3
3. I/T ratio of > 0.2.
4. Micro ESR > 15 mm in 1st hour.
5. Platelet count pf < 150,000/mm^3
6. CRP > 1 mg/dL.

Blood culture in all cases [3]

All neonates suspected of having sepsis should have a blood sample sent for culture. In modern blood culture systems, optimized enriched culture medium with antimicrobial neutralization properties, continues read detection system and specialised paediatric culture bottles are used. Neonates tend to have at least one log higher concentration of bacteria in their blood stream than adults and hence a smaller volume of blood (between 05 mf to 1 ml) may be adequate. Special paediatric bottles are available which have 40 ml of medium which also contains resins to adsorb toxic substances including antibiotics so that pathogenic bacteria can grow in it. The blood is most frequently drawn from a peripheral vein, but samples obtained from an umbilical artery catheter shortly after insertion are also acceptable. Samples from umbilical vein has greater risk of being contaminated unless obtained during delivery from a carefully cleaned segment of a doubly clamped cord.

One bottle is generally all that can be sent. The bottle must be incubated as soon as possible in a continuous monitoring system which looks for growth by monitoring the change in pH or fluorescence every ten minutes round the clock and would alert the technician when there is continuous increase in the signal. These systems are used to reliably detect bacteraemia at a level of 1 to 10 colony forming units per ml if a minimum blood volume of 1 ml is inoculated. No adverse effect of intrapartum antibiotic therapy has been noticed on time to positivity. A medium blood culture time to positivity of < 24 hours is reported among term infants when using contemporary blood culture techniques.

It is expected that the technician would take out the bottle from its incubation chamber and prepare a smear which would be Gram stained and results communicated by telephone to the treating paediatrician. Subculture onto solid media and antibiotic susceptibility testing may take another 48 hours. This time can be shortened by using MALDI TOF. If there is no growth in the bottle, Sterile reports are usually released after 5 days of incubation.

CSF examination

Meningitis was diagnosed clinically in 4% of EOS cases in many surveillance studies in the developed countries. CSF findings in infective neonatal meningitis are:

1. Elevated WBC count (predominantly Neutrophils).
2. Elevated protein level.
3. Decreased glucose concentration.

CSF cultures are present in some neonates who have bacteraemia and CSF culture should be requested in blood culture positive cases to determine if the meninges are infected.

Urine culture

This is of some value in LOS as the bacteria is acquired from the environment. In EOS, since the bacteria is acquired by vertical transmission from the mother, Urine culture may not be of additional value. Sample for culture could be obtained by suprapubic puncture and growth of > 1000 cfu/ml would indicate infection.

If the infant had been intubated, Tracheal swab may be cultured.

Various biomarkers (alone or in combination) are used in the diagnosis of sepsis, including procalcitonin (PCT), C-reactive protein (CRP), interleukin (IL), and soluble form of triggering receptor expressed on myeloid cells-1 (Strem-1). However, the clinical value of these biomarkers is still controversy. Moreover, blood culture is treated as the gold criteria for sepsis diagnosis, but it always takes 48 -72 hours to obtain the outcome when this approach is used.

Blood culture has a low positive rate, which results in diagnosis delay, and the best treatment time is missed. Therefore, finding a reliable biomarker for the early and rapid diagnosis of sepsis is critical.A number of acute phase reactants have been used to identify infected newborns. Many of these tests have a high sensitivity, however, they lack specificity, resulting in poor predictive value. Serial measurements may be of greater assistance than one measurement alone [2,3].

Creactive protein (serial measurement) from blood or saliva

CRP is an acute phase protein associated with tissue injury. CRP levels rise secondary to macrophage, T cell and adipocyte production of interleukin 6 (IL-6). CRP levels begin to rise within 4 to 6 hours of the onset of infection, become abnormal within 24 hours of infection, peak within 2 to 3 days and remain elevated until the infection resolves. However, CRP should not be used as a sole test for making a positive diagnosis of neonatal infection. However, serial examination of blood or saliva for CRP and finding results to be lower than 1 mg/dL may indicate that sepsis is unlikely. CRP levels can be helpful in guiding the duration of antibiotic therapy. Infants with elevated CRP levels that decrease to < 1 mg/dL in 24

to 48 hours after initiation of antibiotic therapy typically are not infected and generally do not require further antibiotic treatment if cultures are negative.

Procalcitonin

It is a peptide precursor of calcitonin produced in monocytes and in the liver. It is released by parenchymal cells in response to bacterial LPS. PCT may be a useful marker to identify neonates who are infected. A physiological increase in procalcitonin occurs within the first 24 hours of birth and elevated levels in serum can occur under non-infectious conditions (e.g. Respiratory distress syndrome, haemodynamic instability and diabetic mothers) and decline rapidly with appropriate therapy. The normal levels for neonates > 72 hours of age is usually < 0.1 ng/ ml. . Its half life is about 24 hours in peripheral blood. Procalcitonin is more sensitive for early detection of sepsis than is CRP. The probability of sepsis is doubled with a PCT of > .5 ng/ml.

Presepsin [11]

It was first described in 2004 that a glycoprotein expressed on monocytes and macrophages, cluster of differentiation 14 (CD14) serves as a receptor of the lipopolysaccharide (LPS)-lipopolysaccharide binding protein complexes and activates a series of signal transduction pathways and inflammatory cascades that finally lead to SIRS. CD14 has two forms, namely, a membrane-bound CD14 (mCD14) and soluble CD14 (sCD14). sCD14 plays an important role in mediating the immune responses to LPS of CD14-negative cells, such as endothelial and epithelial cells. During inflammatory stress, sCD14 is cleaved in plasma, and the N-terminal fragment of 13 kDa has been identified as sCD14 subtype (sCD14-ST; also known as presepsin). Preseps in had some superiority in the management of patients, and may be ahelpful and valuable biomarker in early diagnosis of sepsis. However, preseasons shown a moderate diagnostic accuracy in differentiating sepsis from non-sepsis which prevents it from being recommended as a definitive test for diagnosing sepsis in isolation.

Traditional culture methods are slow while biomarkers are non specific for definitive diagnosis of neonatal sepsis. There is adequate evidence that early initiation of appropriate antibiotic therapy can save lives. In response to these felt needs, several microbiological methods for rapid and specific identification of infectious agents from positive blood culture bottles have been suggested, including pathogen-specific real-time PCR, fluorescence *in situ* hybridization using peptide nucleic acid probes(PNA-FISH), PCR coupled to high-resolution melting curve analysis, and direct matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS).These methods are, however, relatively labor-intensive and in some instances have a narrow diagnostic spectrum. Moreover, one of them has the capacity to evaluate important antimicrobial susceptibility markers, including *mecA, vanA*, and *vanB*. There is aneed for reliable, simple, and direct identification methods with short hands-on time involving limited expertise. Bio Fire Diagnostic's Film Array system (FA; Bio Fire, Salt Lake City, UT) is a PCR based platform developed and tested for the diagnosis of several infectious agents involved in different diseases including sepsis.

Recent advances: Biofire and T2MR

The Film Array platform (FA; Bio Fire, Salt Lake City, UT) is a closed diagnostic system allowing high-order multiplex PCR analysis with automated readout of results directly from positive blood cultures in 1 h. Film Array blood culture identification (BCID) panel, includes 19 bacteria, five yeasts, and three antibiotic resistance genes. In total, 206 blood culture bottles were included in the study. In a clinical trial, The Film Array could identify microorganisms in 153/167 (91.6%) samples with monomicrobial growth. Thirteen of the 167 (7.8%) microorganisms were not covered by the Film Array BCID panel. In 6/167 (3.6%) samples, the Film Array detected an additional microorganism compared to blood culture. When polymicrobial growth was analyzed, the Film Array could detect all target microorganisms in 17/24 (71%) samples. Twelve blood culture bottles that yielded a positive signal but showed no growth were also negative by Film Array. The results of the Film Array were reproducible, as demonstrated by the testing and retesting of five bottles in the same day and a longitudinal follow-up of five other blood cultures up to 4 weeks. The study showed that the Film Array is a rapid identification method with high performance in direct identification of bacteria and yeasts from positive blood culture bottles [12].

T2 Magnetic Resonance (T2MR) is a miniaturized, magnetic resonance based diagnostic approach that measures how water molecules react in the presence of magnetic fields. The method is capable of detecting a variety of targets, including: molecular targets (e.g., DNA); immunodiagnostics (e.g., proteins); and a broad range of hemostasis markers. For molecular and immunodiagnostic targets, T2MR utilizes advances in the field of direct sample detection by deploying superparamagnetic particles that enable T2MR signals to be sensitive to specific targets. When particles coated with target-specific-binding agents are added to a sample containing the target, the particles bind and cluster around the target. This clustering changes the microscopic environment of water in the sample, which in turn alters the T2MR signal, or the T2 relaxation signal, indicating the presence of the target. T2MR is the first technology that can rapidly and accurately detect the presence of molecular targets within a sample without the need for time and labor-intensive purification or extraction of target molecules from the sample. This method differs from traditional PCR, where 90% or more of the target can be lost. T2MR holds a distinct advantage in speed and LOD when compared with PCR. T2MR can detect microbes at a density as low as 1 colony forming unit (CFU) per ml of whole blood, compared with the 100 to 1000 CFU/ml typically required

for conventional PCR-based methods. Run on the fully automated T2Dx Instrument, the T2Candida Panel (T2 Biosystems) is an FDA-cleared rapid diagnostic approach that enables sensitive and specific detection of Candida pathogens directly in whole blood without the need for culture or nucleic acid extraction steps. It is simple to obtain results with the T2Candida Panel; one simply attaches a 4 ml EDTA vacutainer to a T2Candida Cartridge and loads it onto the T2Dx Instrument with minimal hands on time (e.g. typically amplified products directly in concentrated sample matrix; and finally bleach decontamination of all liquids on the T2 Candida Cartridge (T2Candida Panel IFU). The assay workflow on the T2Dx and the 1 CFU/ml LOD is made possible by the attributes of T2MR detection. T2MR detection uses magnetic resonance relaxometry to measure the clustering of superparamagnetic particles when their attached probes bind to amplified nucleic acid directly in the sample matrix. The presence of target nucleic acid sequence leads to clustering of the particles and an increase in the T2MR signal. Because two different capture probes must hybridize to a single nucleic acid target and only ~1 × 10^9 copies of nucleic acid are necessary to elicit a detectable change in T2MR signal, the T2MR detection method enables highly specific detection of minute amounts (femtomoles) of nucleic acid in a highly complex background that can include large amounts of nontarget nucleic acid, protein and other biological debris. These attributes of T2MR enable the utilization of a highly efficient assay workflow on the T2Dx Instrument to select for freely circulating or white-cell encapsulated intact pathogens and avoid detection of freely circulating DNA, also known as DNAemia, which has been reported to confound test performance. Because the target pathogen is not separated from the original sample matrix, the LOD is 1 CFU/ml and detection (Future Microbiol [13].

Summary

Blood culture remains the gold standard test for diagnosis of neonatal sepsis. Unfortunately it is both slow and positive in only a fraction (30%) of cases. Many tests have been used, either alone or in various combinations, for rapid diagnosis, but lack either the sensitivity or specificity. Inability to rapidly rule out neonatal sepsis leads to prolonged unnecessary exposure to antibiotics. The availability of multiplex PCR and its ability to detect presence or absence of 27 bacterial, fungal and viral pathogens as well the presence or absence of drug resistance genes as well as the recent USA FDA approval of T2MR for diagnosis of presence of bacteria in a sample without the need for culture, has the potential to help paediatricians to quickly and accurately diagnose neonatal sepsis, however, cost being the major impediment at present.

Bibliography

1. Sholl BJ. "The global impact of neonatal infection". *Clinics in Perinatology* 24.1 (1997): 1-21.
2. Anderson-Berry AL. "Neonatal sepsis". *Medscape* (2015): 1-22.
3. Simonsen KA., *et al.* "Early onset neonatal sepsis". *Clinical Microbiology Reviews* 27.1 (2014): 21-47.
4. Newman TB., *et al.* "Interpreting complete blood counts soon after birth in newborn at risk for sepsis". *Pediatrics* 126.5 (2010): 903-909.
5. Hornik CP., *et al.* "Use of the complete blood cell count in EOS". *The Pediatric Infectious Disease Journal* 31.8 (2012): 799-802.
6. Newman TB., *et al.* "Combining immature and total neutrophil count to predict EOS in term and late preterm newborn: use of I/T2Ped". *The Journal of Infectious Diseases* 33.8 (2014): 798-802.
7. Puopolo KM., *et al.* "Management of neonates born at >35 0/7 weeks' gestation with suspected or proven EOS". *American Academy of Pediatrics* 142.6 (2018): e2894.
8. Diwakar KK. "Revised look at micro ESR in neonates". *Indian Pediatrics* 36.7 (1999): 703-705.
9. Preet K., *et al.* "Evaluation of micro ESR method with Westergren method for determination of ESR". *Indian Journal of Medical Sciences* 6 (2018): 628-631.
10. Vinay BS., *et al.* "Evaluation of Sepsis screen as a diagnostic tool for neonatal sepsis in a tertiary hospital in Mysore". *Scholars Journal of Applied Medical Sciences* 3 (2015):1005-1010.
11. Wu J., *et al.* "Accuracy of Presepsin in sepsis diagnosis: a systematic review and metaanalysis". *PLOS One* 10.7 (2015):1-15.
12. Altum O., *et al.* "Clinical evaluation of the FilmArray Blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles". *Journal of Clinical Microbiology* 51.12 (2013): 4130-4136.
13. Pfaller M., *et al.* "T2MR and T2 candida. Novel technology for the rapid diagnosis of candidemia and invasive candidiasis". *Future Micro* 11 (2015): 103-117.