

Towards an ideal neonatal sepsis screen panel - A review

Karthikeyan Gengaimuthu¹, Vaishni Karthikeyan²

From ¹Department of Pediatrics, Karuna Medical College, Palakkad, Kerala, ²Department of Microbiology, Karpagam Medical College, Coimbatore, Tamil Nadu, India

Correspondence to: Karthikeyan Gengaimuthu, Department of Pediatrics, Karuna Medical College, Palakkad - 678 103, Kerala, India. Phone: +91-8072895913. E-mail: karthikdubai.neo@gmail.com

Received - 28 July 2017

Initial Review - 21 August 2017

Published Online - 12 November 2017

ABSTRACT

Neonatal sepsis is an important determinant of survival in the neonatal units. Early recognition and institution of antibiotic therapy is the key for successful treatment. Complete blood count and C-reactive protein are the ritualistic commonly used tests that aid the clinician in the initiation and stopping of antibiotic therapy. Procalcitonin at 24 h is useful in diagnosing early-onset neonatal sepsis (EOS). Interleukin 6 and CD 11 β for EOS and CD 64 for late-onset sepsis are promising candidates for future sepsis screen panel as logistical issues like the cost factor rule out their routine use currently. We summarize the current available evidence on the use of these laboratory markers and the clinical sepsis score and suggest a practical guide for antibiotic therapy (initiation and duration of treatment) for the benefit of practicing pediatricians.

Key words: cell surface markers, C-reactive protein, neonate, procalcitonin, sepsis screening

Sepsis is an important determinant of neonatal mortality and morbidity that accounts for a majority of neonatal deaths in the community [1]. Early recognition is very important but is notoriously difficult as the clinical signs of sepsis mimic almost every other neonatal problem. Blood culture which is the gold standard for the confirmation of neonatal sepsis is associated with high false negative rate, it being positive in only up to 50–60% of cases [2]. Hence, it is important to rely upon screening laboratory tests to assist us in the diagnosis of sepsis in both symptomatic babies and also in asymptomatic babies in the presence of risk factors for sepsis such as prematurity, prolonged rupture of membranes (PROM), maternal intrapartum fever >38°C, and asphyxia. Traditionally, neonatal sepsis is sub-classified into early onset and late onset based on the day of onset of symptoms, the cutoff being 72 h (3 days). The logic is that the probable acquisition of organisms is vertical (maternal vaginal flora) in the former, whereas horizontal (from the hospital environment) in the later. We will discuss the usefulness and limitations of available sepsis screen parameters in both these instances. This article is not an exhaustive theoretical structured review of all the mundane markers of neonatal sepsis for which the readers are referred to another recent article [3]. Rather, the focus is on providing practical tips to pediatricians in the all-important antibiotic therapy of neonatal sepsis by intelligent and judicious use of the routinely available sepsis screen markers.

REQUISITES OF AN IDEAL NEONATAL SEPSIS SCREEN

1. It should have 100% sensitivity and 100% negative predictive value (NPV) (i.e.) it should not miss any case of neonatal sepsis, but some overtreatment can be accepted.
2. It should be positive early in the course of the disease so that appropriate interventions that are initiated are effective in preventing morbidity and mortality.
3. It should have a short turn over time.
4. It should give consistent results when repeated in different centers.
5. It should be cost-effective and easier to perform and interpret.

No single test fulfills these criteria, and hence, commonly a battery of tests are grouped together to form a sepsis screen panel.

LABORATORY TESTS USED IN SEPSIS SCREENING

These are generally divided into hematological and non-hematological parameters. The hematological parameters that are used in sepsis screening (Rodwell *et al.* hematological sepsis score) [4] are as follows:

1. Abnormal total leukocyte count (TLC)
2. Abnormal total neutrophil count
3. Abnormal immature neutrophil count (band count)
4. Abnormal immature: Total neutrophil count (I.T ratio >0.2)

5. Abnormal immature: Mature neutrophil count >0.3
6. Decreased platelet count ($<150,000$)
7. Degenerative changes in the polymorphs (toxic granulations etc.).

A score of 3 or more is reported to have a sensitivity of 93% and an NPV of 98% [4]. It is important to bear in mind the physiological changes in the total leukocyte count and absolute neutrophil count (ANC) that occurs in the 1st week of life (Figs. 1 and 2) [5]. There is a physiological surge in the total leukocyte and ANC 6–12 h after delivery and base levels are reached after day 3 of life [5]. Hence, this hematological sepsis score is not generally used alone but almost always combined with acute phase reactants, especially the C-reactive protein (CRP).

ACUTE PHASE REACTANTS IN DIAGNOSIS OF NEONATAL SEPSIS

Acute phase reactants are proteins secreted by the liver in response to an acute stress such as sepsis, trauma, and surgery. CRP along with complete blood count is the widely used ritualistic sepsis screen panel [6]. Among the many other acute phase reactants evaluated as diagnostic markers, only procalcitonin (PCT) has been integrated in clinical practice [3].

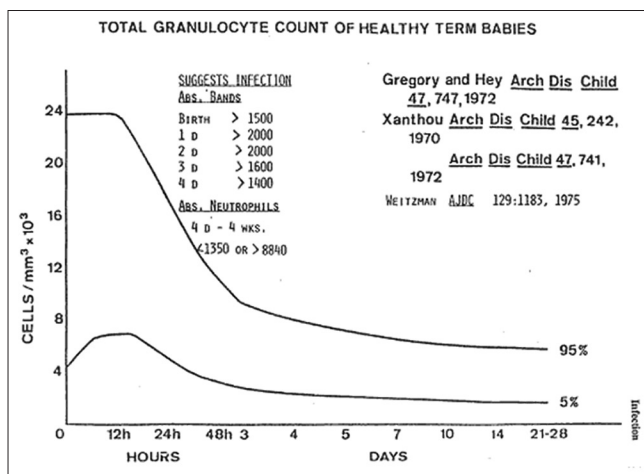


Figure 1: Total leukocyte count in healthy neonates

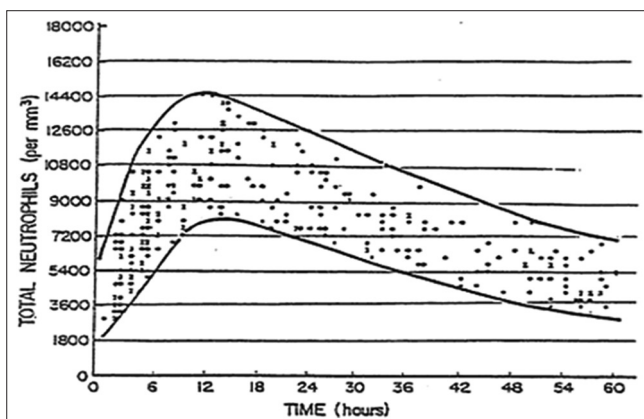


Figure 2: Neutrophil count in healthy neonates over the 1st week of life

CRP IN NEONATAL SEPSIS

CRP is the one universally used in sepsis screen panels. CRP coupled with complete blood count (CBC) is almost the ritualistic work up constituting the laboratory sepsis screen panel. It is an acute phase reactant with a half-life of 24–48 h in neonates and readily available test kits can estimate its levels [7]. A value of >0.6 mg/dl (6 mg/L) is considered as positive. In a recently published meta-analysis, CRP was found to have a sensitivity ranging from 30% to 80% but a higher specificity 83–100% at the onset of symptoms [3]. If done later say 24 h and 48 h after the neonate became symptomatic, there was an increasing trend in sensitivity (after 24 h) and specificity (after 48 h) [3]. It takes 10–12 h for the levels to increase significantly after the onset of sepsis, and hence, it is a specific but relatively late marker of neonatal sepsis [6]. However, it cannot be used as a stand-alone marker as its sensitivity is low and levels can be significantly elevated with non-infectious causes such as trauma, meconium aspiration, and tissue injuries. Moreover, its levels are also influenced by gestational age with preterm infants having a poor CRP response following the onset of infection. Serial values are more useful than a single measurement in deciding the initiation and the duration of antibiotic therapy.

PCT IN NEONATAL SEPSIS

PCT is a glycoprotein consisting of 116 amino acids and upon cleavage in the C (parafollicular) cells of thyroid is converted into the active hormone calcitonin that consists of 32 amino acids [8]. PCT rises early during the illness by 4 h of onset of sepsis and returns to baseline sooner than CRP. PCT levels also show a physiological raise 24 h after delivery returning to baseline by the 3rd day, thereby having less specificity than CRP [6]. PCT response is more rapid than CRP, and hence, a PCT at 24 h of life can be a superior test to diagnose early-onset sepsis (EOS) [9]. However, beyond 24 h, the value of PCT in the diagnosis of EOS decreases due to its postnatal raise pattern. PCT levels are only minimally or not affected by trauma, meconium aspiration, and viral infections, and hence, it may be particularly useful in situations like diagnosing sepsis in a postsurgical neonate [10].

MICRO-ERYTHROCYTE SEDIMENTATION RATE (MICRO-ESR)

Micro-ESR is an easy bedside test that is performed using heparinized capillary tubes (75 mm long and 1.1 mm internal diameter) and is validated in many Indian studies. A fall of ≥ 15 mm in the 1st h is considered as positive, and it is included in the pentad sepsis screen panel recommended by National Neonatology Forum (NNF) [11]. The NNF sepsis screen panel consists of the following five parameters (Positive screen is two or more parameters positive).

1. Leukopenia (TLC <5000)
2. Neutropenia (ANC <1800)
3. Immature: Total neutrophil count (I:T ratio >0.2)

4. Micro-ESR >15 mm in 1st h
5. Positive CRP.

CYTOKINES AND CELL SURFACE MARKERS AS ADJUNCTS TO DIAGNOSIS

Cytokines such as interleukin 6 (IL 6) and tumor necrosis factor have been evaluated in experimental studies [12]. Cytokines rise very early in the course of illness before even the acute phase reactants. IL 6 levels raise earlier than CRP and PCT in the course of neonatal sepsis and cord blood IL 6 levels are consistently elevated with EOS with sensitivity of 87–100% and NPV of 93–100% in studies [13,14]. Its half-life is short and levels normalize rapidly with treatment thus lowering its sensitivity at 24 and 48 h [13]. Hence, it is an early and sensitive marker. IL 6 and PCT might be the better combination to diagnose EOS while CRP ± PCT and CBC may be the choice of screening for late-onset neonatal sepsis (LOS).

With advances in flow cytometry technology, cell surface markers in blood cells can be quantified and can be useful adjuncts in sepsis screen panels. Neutrophil surface markers CD 11β and CD 64A have been found to be useful in diagnosing EOS and LOS, respectively [15]. Levels rise as soon as 5 minutes after the onset of infection [16,17]. However, the usefulness of the cell surface markers in routine clinical practice is limited due to the cost factor and sophisticated technology and the unduly long processing time.

MOLECULAR AND GENOMIC TESTS

Gene-based molecular test to detect signature bacterial marker (16S rRNA) by real-time polymerase chain reaction (PCR) has been used to confirm bacterial sepsis in neonates as well as for genus characterization [18,19]. An Indian study found PCR for 16S rRNA useful in neonatal sepsis with sensitivity of 100% and specificity of 95.6% [19].

CLINICAL SEPSIS SCREENING

For those in the underprivileged countries who cannot afford to have laboratory tests done a clinical sepsis screening has been devised. A WHO multicentric study had devised a clinical sepsis risk score using nine clinical parameters that can be recognized by community health workers to predict a serious bacterial illness in young infants [20]. A study from Chandigarh, India, evaluated the known clinical signs of neonatal sepsis using a set criteria of likelihood ratio of a positive test (LR⁺) >1 and a positive predictive value (PPV) of >30% identified the following seven clinical signs and suggested that a clinical risk score including these seven parameters will be beneficial to the clinicians to guide antibiotic treatment for LOS [21].

1. Abdominal distension
2. Increased pre-feed aspirates
3. Hyperthermia (present on at least two occasions 1 h apart)

4. Tachycardia (present on at least two occasions 1 h apart)
5. Chest retractions
6. Grunting
7. Lethargy.

When this clinical risk score along with a laboratory, sepsis screen panel of four parameters (micro-ESR, CRP, ANC, and immature to mature leucocytes ratio) was evaluated in a validation cohort of 220 LOS episodes in 195 neonates in 2005, clinical sepsis score of one or more positive clinical signs at 0 h (the putative onset of LOS by the defined clinical signs) had a sensitivity of 90% and a NPV of 85.7%, whereas coupled with the laboratory sepsis screen panel the sensitivity increased to 95% and NPV to 90.6% [22]. A cutoff score of 1 had the highest NPV and negative likelihood ratio (85% and 0.44), whereas cutoff score of 2 or more had the best PPV and positive likelihood ratio (52% and 2.65) in diagnosis of LOS. In a subsequent study, to validate this clinical score in a cohort of very low birth weight babies, the authors report that the clinical score when coupled with a laboratory sepsis screen (2 or more positive of abnormal ANC, immature: Total neutrophil count, micro-ESR, and CRP) had a sensitivity of 95% and NPV of 90.6% [22].

In a study, 13% of well and ill-appearing neonate's ≥34 weeks were evaluated for EOS and 11% were treated with antibiotics while only 0.04% of that cohort of 7004 neonates had culture-proven sepsis [23]. Hence, the study evaluated the usefulness of a stratification strategy of term and late preterm neonates at risk of EOS using defined clinical risk factors, namely, the duration of rupture of membranes, highest maternal temperature, gestational age, Group B streptococcus carrier state, and intrapartum antibiotic prophylaxis, and newborn clinical examination parameters such as tachycardia, tachypnea, hyperthermia, need for vasopressors, respiratory distress, and need for breathing support such as oxygen therapy, CPAP, and ventilation [24]. Thus, the following three categories of neonates could be stratified, namely, clinical illness, equivocal presentation, and well-appearing babies with the respective pathways of treatment being empirical antibiotic therapy, evaluate, and antibiotics if indicated by the information gathered from the tests and continued clinical observation [24].

CHOICE OF SEPSIS SCREEN PANELS IN EOS AND LOS

Combination of CBC & CRP is the almost ritualistic and cost-effective panel used routinely in neonatal units. Serial values help in decision-making process than a single measurement. Addition of PCT to this duo for the diagnosis of LOS may help in its earlier recognition and initiation of antibiotic therapy. Whereas PCT added to this duo in setting of EOS is helpful only if PCT is done at 24 h of life as its physiological rise after 24 h attenuates the advantage conferred by this added test. IL 6 (especially umbilical cord IL 6) and CD 11β may be of particular value in early diagnosis of EOS, whereas CD 64 may assist in earlier diagnosis of LOS, but these markers are at present experimental only and have not been adapted for routine clinical practice.

ANTIBIOTIC USE AND LABORATORY SEPSIS SCREEN

The universal rule is that blood culture and cultures of other body fluids like cerebrospinal fluid and urine (as applicable) should be taken before initiation of antibiotic therapy in any newborn and antibiotics should be continued until culture results are known and thereafter, in case of positive cultures, duration of antibiotic therapy is dictated by the isolated pathogen. However, in the remaining culture negative cases ($\approx 50\%$), decision has to be made making the best use of commonly available laboratory sepsis screen markers. The following guidelines are suggested in such situations.

1. In the symptomatic newborn, antibiotics should not be withheld on the face of a negative sepsis screen as none of the available screens has 100% NPV. However, antibiotics can be stopped in such a situation once the cultures (blood and cerebrospinal fluid [CSF]) are negative and a repeat sepsis screen remains negative.
2. In the symptomatic newborn with a positive sepsis screen whose cultures are also negative, antibiotics should be continued for 24–48 h after the repeat screen is negative.
3. In the asymptomatic newborn with a negative sepsis screen in whom antibiotics are started because of the presence of major or multiple perinatal risk factors for sepsis, antibiotics can be safely stopped once cultures are negative and a repeat screen is also negative.
4. In the asymptomatic newborn with a negative sepsis screen in the presence of only a single minor risk factor for sepsis, antibiotics can be withheld with the provision that the neonate should be observed for the next 24 h for symptoms and signs of sepsis.
5. In the asymptomatic newborn with a positive sepsis screen, antibiotics should be administered and continued until the cultures are reported negative or for 24–48 h after a negative repeat screen whichever is later.

CASE STUDIES TO ILLUSTRATE ANTIBIOTIC USE IN NEONATES

1. Term/3.2 kg/male, vaginal delivery, presents at 36 h with poor feeding, lethargy, and grunting. PROM for 28 h.
 - Symptomatic newborn, EOS picture
 - Sepsis screen, blood culture, lumbar puncture, chest X-ray, and start antibiotics till review with culture results.
 - Culture positive cases, antibiotic therapy will be based on culture results irrespective of sepsis screen. Blood culture positive: 10–14 days based on the organism and clinical response. CSF culture positive: 14–21 days
 - Culture-negative and first sepsis screen negative: Antibiotics can be safely stopped if a repeat sepsis screen (at least 24 h apart from the first screen) remains negative
 - Culture-negative and first sepsis screen positive:

Continue antibiotics until 24–48 h after repeat sepsis screens are negative.

2. Term/3 kg/female, vaginal delivery with a history of PROM for 30 h, asymptomatic, and feeding well.
 - Observe the baby for 24–48 h for symptoms of sepsis. No antibiotics
 - If the baby becomes symptomatic anytime then manage as discussed with case 1.
3. 34 weeks/2.1 kg/male, asymptomatic, PROM 3 days
 - Two significant risk factors for EOS.
 - Sepsis screen, blood culture, and antibiotics. Some clinicians will do lumbar puncture in this setting only if screen is positive or blood culture is positive
 - If culture positive then antibiotics will be guided by culture results as discussed in case 1.
 - If culture negative then follows the same approach as discussed in case 1.
4. 30 weeks/1.6 kg/female, ventilated for 4 days for hyaline membrane disease presents on the 7th day with apnea and episodes of duskiness.
 - LOS picture, symptomatic
 - Sepsis screen, blood culture, LP, chest X-ray, and start antibiotics
 - Culture positive = 10–14 days for blood culture, 14–21 days for CSF
 - Culture-negative = antibiotics till 24–48 h after a negative sepsis screen or empirically for 5–7 days whichever is later.
5. Term/2.9 kg/male, no risk factors, on breastfeed presents on the 5th day with tachypnea and vomiting. Blood gas: 7.51/ PO_2 68/ PCO_2 28/ HCO_3 19 (respiratory alkalosis)
 - LOS like picture, symptomatic
 - Sepsis screen, blood culture, LP, chest X-ray, and start antibiotics
 - Further decisions for antibiotic therapy as in case 1
 - In the real clinical scenario, for this baby, cultures were negative, screens repeatedly negative but baby's clinical condition deteriorated despite antibiotics. On further evaluation, this baby was diagnosed with inborn error of metabolism (urea cycle disorder).

REFERENCES

1. Gerdes JS, Polin R. Early diagnosis and treatment of neonatal sepsis. *Indian J Pediatr* 1998;65:63-78.
2. Chiesa C, Panero A, Osborn JF, Simonetti AF, Pacifico L. Diagnosis of neonatal sepsis: A clinical and laboratory challenge. *Clin Chem* 2004;50:279-87.
3. Sharma D, Farahbakhsh N, Shastri S, Sharma P. Biomarkers for diagnosis of neonatal sepsis: A literature review. *J Matern Fetal Neonatal Med* 2017;7:1-14.
4. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a haematological scoring system. *J Pediatr* 1988;112:761-7.
5. Schmutz N, Henry E, Jopling J, Christensen RD. Expected ranges for blood neutrophil concentrations of neonates: The manroe and mouzinho charts revisited. *J Perinatol* 2008;28:275-81.
6. Shah BA, Padbury JF. Neonatal sepsis: An old problem with new insights. *Virulence* 2014;5:170-8.

7. Hofer N, Zacharias E, Müller W, Resch B. An update on the use of C-reactive protein in early-onset neonatal sepsis: Current insights and new tasks. *Neonatology* 2012;102:25-36.
8. Park IH, Lee SH, Yu ST, Oh YK. Serum procalcitonin as a diagnostic marker of neonatal sepsis. *Korean J Pediatr* 2014;57:451-6.
9. Altunhan H, Annagür A, Örs R, Mehmetoglu I. Procalcitonin measurement at 24 hours of age may be helpful in the prompt diagnosis of early-onset neonatal sepsis. *Int J Infect Dis* 2011;15:e854-8.
10. Chiesa C, Pellegrini G, Panero A, Osborn JF, Signore F, Assumma M, *et al.* C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: Influence of illness severity, risk status, antenatal and perinatal complications, and infection. *Clin Chem* 2003;49:60-8.
11. NNF Teaching Aids: Neonatal Sepsis. Available from: <http://www.newbornwho.org/pdf/teaching-aids/neonatalesepsis.pdf>. [Last accessed on 2017 Jul 27].
12. Mehr S, Doyle LW. Cytokines as markers of bacterial sepsis in newborn infants: A review. *Pediatr Infect Dis J* 2000;19:879-87.
13. Cernada M, Badía N, Modesto V, Alonso R, Mejias A, Golombek S, *et al.* Cord blood interleukin-6 as a predictor of early-onset neonatal sepsis. *Acta Paediatr* 2012;101:e203-7.
14. Mishra UK, Jacobs SE, Doyle LW, Garland SM. Newer approaches to the diagnosis of early onset neonatal sepsis. *Arch Dis Child Fetal Neonatal Ed* 2006;91:F208-12.
15. Ng PC. Diagnostic markers of infection in neonates. *Arch Dis Child Fetal Neonatal Ed* 2004;89:F229-35.
16. Weirich E, Rabin RL, Maldonado Y, Benitz W, Modler S, Herzenberg LA, *et al.* Neutrophil CD11b expression as a diagnostic marker for early-onset neonatal infection. *J Pediatr* 1998;132:445-51.
17. Ng PC, Li K, Wong RP, Chui KM, Wong E, Fok TF. Neutrophil CD64 expression: A sensitive diagnostic marker for late-onset nosocomial infection in very low birth weight infants. *Pediatr Res* 2002;51:296-303.
18. Mussap M, Molinari MP, Senno E, Gritti P, Soro B, Mannelli S, *et al.* New diagnostic tools for neonatal sepsis: The role of a real-time polymerase chain reaction for the early detection and identification of bacterial and fungal species in blood samples. *J Chemother* 2007;19 Suppl 2:31-4.
19. Yadav AK, Wilson CG, Prasad PL, Menon PK. Polymerase chain reaction in rapid diagnosis of neonatal sepsis. *Indian Pediatr* 2005;42:681-5.
20. Clinical Prediction of Serious Bacterial Infections in Young Infants in Developing Countries. The WHO young infants study group. *Pediatr Infect Dis J* 1999;18 10 Suppl:S23-31.
21. Singh SA, Dutta S, Narang A. Predictive clinical scores for diagnosis of late onset neonatal septicemia. *J Trop Pediatr* 2003;49:235-9.
22. Kudawla M, Dutta S, Narang A. Validation of a clinical score for the diagnosis of late onset neonatal septicemia in babies weighing 1000-2500 g. *J Trop Pediatr* 2008;54:66-9.
23. Escobar GJ. The neonatal "sepsis work-up": Personal reflections on the development of an evidence-based approach toward newborn infections in a managed care organization. *Pediatrics* 1999;103 1 Suppl E:360-73.
24. Escobar GJ, Puopolo KM, Wi S, Turk BJ, Kuzniewicz MW, Walsh EM, *et al.* Stratification of risk of early-onset sepsis in newborns = 34 weeks' gestation. *Pediatrics* 2014;133:30-6.

Funding: None; Conflict of Interest: None Stated.

How to cite this article: Gengaimuthu K, Karthikeyan V. Towards an ideal neonatal sepsis screen panel - A review. *Indian J Child Health*. 2017; 4(4):614-618.