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Deepak Sharma, Nazanin Farahbakhsh, Sweta Shastri & Pradeep Sharma

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Biomarkers for diagnosis of neonatal sepsis: a literature review

Deepak Sharmaa, Nazanin Farahbakhshb, Sweta Shastric and Pradeep Sharmad

aDepartment of Neonatology, National Institute of Medical Sciences, Jaipur, Rajasthan, India; bDepartment of Pulmonology, Mofid Pediatrics Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran; cDepartment of Pathology, N.K.P. Salve Medical College, Nagpur, Maharashtra, India; dDepartment of Medicine, Mahatma Gandhi Medical College, Jaipur, Rajasthan, India

ABSTRACT
Sepsis is an important cause of mortality and morbidity in neonatal populations. There has been constant search of an ideal sepsis biomarker that have high sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), so that both the diagnosis and exclusion of neonatal sepsis can be made at the earliest possible and appropriate antibiotics can be started to neonate. Ideal sepsis biomarker will help in guiding us when not to start antibiotics in case of suspect sepsis and total duration of antibiotics course in case of proven sepsis. There are numerous sepsis biomarkers that have been evaluated for early detection of neonatal sepsis but till date there is no single ideal biomarker that fulfills all essential criteria’s for being an ideal biomarker. The most commonly used biomarkers are C-reactive protein (CRP) and procalcitonin (PCT), but both have shown varied sensitivity, specificity, PPV and NPV in different studies. We conducted literature search for various neonatal sepsis biomarkers and this review article will cover briefly all the markers with current available evidence.

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KEYWORDS
Biomarker; neonatal sepsis; sensitivity; specificity; positive predictive value; negative predictive value

Introduction
Neonatal sepsis is the most common causes of morbidity and mortality in term and preterm infants [1]. A recent published study reported prematurity and neonatal sepsis as the major cause of infant mortality [2–4]. The reported incidence of neonatal sepsis ranges from one to five cases per 1000 live births [5,6], and incidence in term neonate (including both early onset sepsis (EOS) and late onset sepsis (LOS)) is reported as one to two cases per 1000 live births [7,8]. Numerous interventions have been tried for decreasing incidence and mortality secondary to neonatal sepsis [9–21].

Neonatal sepsis has been defined as the presence of bacteria in sterile body fluids, namely, blood, urine, cerebrospinal, peritoneal, and pleural fluid [1,22]. It has been classified as early onset sepsis (EOS) and late onset sepsis (LOS) on the basis of time of onset after neonatal birth [23]. EOS is defined when the onset of sepsis is within 72 h of postnatal life and the source of infection is vertical transmission of bacteria from mother to newborn. LOS has been defined as onset of sepsis after 72 h of postnatal life and the source of infection is horizontal transfer of bacteria from health care personal [24]. The importance of defining neonatal sepsis as EOS and LOS is to guide for antibiotic pattern and prognostication [25]. The neonatal population especially very low-birth weight (VLBW) and extremely low-birth weight (ELBW) are more prone to develop neonatal sepsis secondary to immature immune system, prolonged invasive mechanical ventilation and respiratory support, prolonged duration of hospitalization, insertion of central line catheters, endotracheal tubes, and other invasive procedures [26].

The clinical manifestations of neonatal sepsis are nonspecific and have varied clinical features. The various manifestation includes decreased acceptance of feed, respiratory distress, pneumonia, apnea, delayed capillary refill time, cold peripheries, mottling, off color, feed intolerance, necrotizing enterocolitis, temperature instability including hypothermia and hyperthermia, hypotonia, seizures, bulging fontanels, disseminated intravascular coagulation (DIC), bleeding manifestation, and prolonged jaundice [27–35]. These varied features can be seen in other neonatal conditions thus making the diagnosis of neonatal sepsis difficult and leading to overtreatment. The clinical signs and symptoms are sometimes late to manifest, leading the neonate shift from compensated phase to refractory phase of sickness thereby increasing the mortality. The gold
standard for diagnosis of neonatal sepsis is isolation of organism in blood culture from any sterile site [36], but it takes 24–48 h for reporting growth of organism and inoculation of small amount of blood (0.5–1.0 ml) leads to less detection of organism. Thus, neonatal sepsis cannot always be excluded even when blood cultures are showing no growth [37].

Biomarkers for diagnosis of neonatal sepsis have been discovered that help in the early diagnosis of neonatal sepsis, before the onset of clinical manifestation so that early treatment of sepsis can be started and neonate can be properly managed [38]. The ideal biomarker for neonatal sepsis should have various properties (Table 1), but presently none of the available markers fulfills all the required criteria’s [39].

The literature search was performed for this review article by searching the electronic data base namely Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, EMBASE, Web of Science, Scopus, Index Copernicus, African Index Medicus (AIM), Thomson Reuters (ESCI), Chemical Abstracts Service (CAS), SCIWIN (Scientific World Index), Google Scholar, Latin American and Caribbean Health Sciences Information System (LILACS), Index Medicus for the Eastern Mediterranean Region (IMEMR), Index Medicus for the South-East Asian Region (IMSEAR), Western Pacific Region Index Medicus (WPRIM), various sites for ongoing trials namely clinical trial registry (www.clinicaltrials.gov, www.controlled-trials.com, Australian and New Zealand Clinical Trials Registry (http://www.anzctr.org.au), Indian Clinical Trials Registry (http://ctri.nic.in/ Clinicaltrials) and the World Health Organization (WHO) International Clinical Trials Registry and Platform (http://www.who.int/ictrp/search/en/) and abstracts of conferences namely proceedings of Pediatric Academic Societies (American Pediatric Society, Society for Pediatric Research, and European Society for Pediatric Research).

The keywords used for search were “infant newborn,” “neonates,” “sepsis,” “infection,” “early onset sepsis”, “late onset sepsis”, “early diagnosis,” “biomarkers” and “biological markers” with the limitation of “humans”. The various biomarkers those have been used for early diagnosis of neonatal sepsis are discussed in this current review with the present evidence (Table 2).

**Complete blood picture**

Numerous studies have been conducted to assess the role of complete blood picture (CBP), white blood cell (WBC) count, absolute neutrophil count (ANC), and immature to total leukocyte ratio (I:T) in the diagnosis

### Table 1. Characteristics of ideal biomarker for diagnosis of neonatal sepsis.

<table>
<thead>
<tr>
<th>Characteristics</th>
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<tbody>
<tr>
<td>Short half-life (rapid increase with onset of sepsis and rapid decreases)</td>
</tr>
<tr>
<td>Should have high sensitivity (≥100%), specificity (≥85%), negative predictive value (NPV) (≥100%) and positive predictive value (PPV) (≥85%)</td>
</tr>
<tr>
<td>Discriminate etiology of sepsis</td>
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<tr>
<td>Level should not increase with other associated co-morbidities</td>
</tr>
<tr>
<td>Provide clinicians time to intervene for treatment</td>
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<tr>
<td>Guide for starting and stopping of antibiotics</td>
</tr>
<tr>
<td>Should have standardized value</td>
</tr>
<tr>
<td>Should be reliable and precise</td>
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<tr>
<td>Accurately predict severity of disease</td>
</tr>
<tr>
<td>Should help in prognostication</td>
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<tr>
<td>Test should be easily doable</td>
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<tr>
<td>Method for measurement should be readily available</td>
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<tr>
<td>Cost effective</td>
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<tr>
<td>Short turnaround time</td>
</tr>
<tr>
<td>Results comparable between different laboratories</td>
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<tr>
<td>Requires very small amount of sample</td>
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</tbody>
</table>

### Table 2. Various biomarkers that have been used in neonatal population for the diagnosis of neonatal sepsis.

| Name of biomarker                                                                                                                                                                                                 | Characteristics                                                                                                                                                                                                 |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Complete blood picture (CBP):                                                                                                                                                                                                                                             |
| C-reactive protein (CRP)                                                                                                                                                                                                                                                   |
| Highly sensitive C-reactive protein (hs-CRP)                                                                                                                                                                                                                                 |
| Procalcitonin (PCT)                                                                                                                                                                                                                                                        |
| Serum amyloid A (SAA)                                                                                                                                                                                                                                                      |
| Lipopolysaccharide-binding protein (LPB)                                                                                                                                                                                                                                     |
| Interleukin 1β (IL1β)                                                                                                                                                                                                                                                      |
| Interleukin 4 (IL-4)                                                                                                                                                                                                                                                       |
| Interleukin 5 (IL-5)                                                                                                                                                                                                                                                       |
| Interleukin 6 (IL-6)                                                                                                                                                                                                                                                       |
| Interleukin 8 (IL-8)                                                                                                                                                                                                                                                       |
| Interleukin 10 (IL-10)                                                                                                                                                                                                                                                      |
| sIL2R                                                                                                                                                                                                                                                                     |
| Tumour necrosis factor (TNF-α)                                                                                                                                                                                                                                             |
| 11sTNFR-p55                                                                                                                                                                                                                                                              |
| 12sTNFR-p75                                                                                                                                                                                                                                                              |
| CD11β                                                                                                                                                                                                                                                                     |
| Soluble CD163                                                                                                                                                                                                                                                              |
| CD 64                                                                                                                                                                                                                                                                     |
| Pentraxin 3 (PTX3)                                                                                                                                                                                                                                                        |
| Angiopoietins (Ang)                                                                                                                                                                                                                                                        |
| Soluble form of the urokinase-type plasminogen activator receptor (suPAR)                                                                                                                                                                                                  |
| Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1)                                                                                                                                                                                                       |
| Inter alpha inhibitor proteins (Iαlpl)                                                                                                                                                                                                                                     |
| Interferon-γ (IFN)                                                                                                                                                                                                                                                        |
| CXCL12                                                                                                                                                                                                                                                                     |
| sCD14-5T or presepsin                                                                                                                                                                                                                                                      |
| Intracellular adhesion molecule-1 (ICAM-1)                                                                                                                                                                                                                                  |
| Vascular cell adhesion molecule-1 (VCAM-1)                                                                                                                                                                                                                                  |
| E-selectin                                                                                                                                                                                                                                                                |
| L-selectin                                                                                                                                                                                                                                                                |
| Fibronectin                                                                                                                                                                                                                                                               |
| Haptoglobin                                                                                                                                                                                                                                                               |
| Neopterin                                                                                                                                                                                                                                                                |
| Orosomucoid                                                                                                                                                                                                                                                               |
| Complement activation products (C3a-desArg, C3bBbp, sC5b-9), Asymmetric dimethylarginine (ADMA)                                                                                                                                                                          |
| L-arginine                                                                                                                                                                                                                                                                |
| Mannose-binding lectin                                                                                                                                                                                                                                                     |
| Melatonin                                                                                                                                                                                                                                                                  |
| α1-acid glycoprotein (α1AG)                                                                                                                                                                                                                                               |
| 16S rDNA                                                                                                                                                                                                                                                                  |
| Lactoferrin                                                                                                                                                                                                                                                               |
| Serum ischemia-modified albumin (IMA)                                                                                                                                                                                                                                     |
of neonatal sepsis. The usefulness of CBP as biomarker of neonatal sepsis has not been proven yet, as it have poor positive predictive value (PPV) and negative predictive value (NPV) but studies have shown that serial normal CBP helps in safely ruling out of neonatal sepsis [40–42]. The other components of CBP that has shown correlation with neonatal sepsis are WBC count, absolute neutrophil counts (ANC), and immature-to-total neutrophil ratio (I:T). WBC count of <5000 to 7500/mm³ have been used for the diagnosis of neonatal sepsis. Leucopenia has shown to have low sensitivity (29%) but high specificity (91%) for diagnosis of neonatal sepsis [29]. In the interpretation of the leucocyte count, the neonatal gestational age has to be taken in consideration as lower limit of ANC decreases with decrease in gestational age [43,44]. ANC and I:T ratio have shown to be having high NPV for excluding neonatal sepsis [45]. The I:T ratio is 0.16 in uninfected newborn in the first 24 h, and gradually decrease in next five days to 0.12 [46]. I:T ratio of >0.2 is considered to be favoring neonatal sepsis. The condition that leads to fallacious changes in the I:T ratio are perinatal asphyxia, maternal hypertension, labor stress, and prolonged induction with oxytocin [45,47]. The study conducted by Hornik et al. showed that low WBC counts, low ANC and high I: T neutrophil ratios were associated with increased odds of infection (highest odds ratios: 5.38, 6.84 and 7.97, respectively) and thus concluded that all these marker have high specificity and NPV and low sensitivity [48]. Murphy et al. showed that two normal I: T ratio and sterile blood culture had 100% NPV in diagnosis of neonatal sepsis [40]. Neutropenia has shown to be more predictive of neonatal sepsis than neutrophilia [49]. In conclusion, WBC, ANC, and I/T ratio have significant limitations in the diagnosis of neonatal sepsis.

C-reactive protein

C-reactive protein (CRP) is the most extensively studied, easily available, and is the most commonly used laboratory tests in the diagnosis of neonatal sepsis [38,50]. It is a pentameric structure protein and is an acute phase reactant protein [51]. The source of CRP are hepatocytes and its synthesis is stimulated by cytokines, with important stimulatory cytokines being interleukin (IL)-6, IL-1, and tumor necrosis factor-α [52,53]. The half-life of CRP is 24 to 48 h and it takes around 10 to 12 h for level to increase, hence making it unreliable for early diagnosis of neonatal sepsis (low sensitivity) [54]. Serial measurement of CRP at 24 to 48 h after the onset of symptoms has shown to increase its sensitivity for diagnosis of neonatal sepsis [54,55], and serial CRP is also used in monitoring the response to treatment in infected neonates, who are on antibiotics course for the same [56,57]. Serial normal value of CRP is strong indicator of absence of neonatal sepsis (99% NPV) and can be used as guide for stopping antibiotics [58]. The studies that evaluated the role of CRP in diagnosis of EOS, reported different sensitivities and specificities ranging from 29 to 100% and from 6 to 100%, respectively. There are numerous conditions where there is spurious increase in level of CRP like meconium aspiration syndrome (MAS), delayed transition after birth, hemolysis, tissue injury, surgery, premature infant exposure to glucocorticoids, maternal fever during labor, prolonged rupture of membranes, stressfull delivery or fetal distress, prolonged labor, perinatal asphyxia or shock, surfactant administration, intraventricular hemorrhage (IVH), and pneumothorax thus making it a nonspecific biomarker for diagnosis of neonatal sepsis [54,59]. In a recently published meta-analysis that included 37 studies showed, CRP having great variability in sensitivity (30–80%) but showed higher specificity (83–100%) at onset of symptom. There was a tendency towards an increasing sensitivity and specificity after 24 and 48 h, respectively [38].

Highly sensitive C-reactive protein (hs-CRP)

The normal accepted cut off for significant level of CRP is >6 mg/l. Highly sensitive CRP (hs-CRP) is more sensitive than the conventional CRP for diagnosis of neonatal sepsis. hs-CRP assay have lower cut off value than conventional CRP assays, with value of hs-CRP <1 mg/l having increased sensitivity for neonatal infection [60]. Edgar et al. reported significant increase in level of hs-CRP in both infected and culture positive newborns when compared to noninfected neonate [61]. This findings were further confirmed by Abdollahi et al. [62] A recently published study from India that compared IL-6, CRP and hs-CRP as early markers of neonatal sepsis failed to show good sensitivity and specificity of hs-CRP when compared with conventional CRP. In this study hs-CRP value <0.5 mg/l indicated no risk of infection, value 0.5–1 mg/l indicated low risk, the value 1–3 mg/l indicated average risk and the value >3 mg/l indicated high risk of infection in neonates [63]. There is need for more studies to evaluate the role of hs-CRP for use as neonatal sepsis marker.
Procalcitonin

Procalcitonin (PCT) is an acute phase reactant protein and is peptide prohormone of calcitonin. PCT is composed of 116 amino acids with molecular weight of 14-kDa and is encoded by the Calc-I gene along with calcitonin and katalcalcin [64]. The levels of PCT are not affected by calcitonin levels and the source of PCT is macrophages and hepatocytes [65]. PCT has shown to be associated with immunomodulation and vascular response associated with systemic inflammatory response syndrome (SIRS), especially caused by systemic bacterial infection. There is rapid rise in the level of PCT within 2–4 h of bacterial endotoxin exposure and peak levels are reached at 6–8 h and it remain elevated for the next 24 h [66]. The half-life of PCT is 24–30 h. The rapid rise of PCT with the onset of bacterial sepsis makes it a good marker for early diagnosis of neonatal sepsis when compared to CRP. Other added advantage is the increase in PCT level in bacterial sepsis is not affected by gestational age of the neonate, but recently published study showed that reference PCT levels of infants with GA \( \leq 32 \) weeks were affected by post-natal age, thus needing cautious interpretation in these gestational age neonates [67]. Serum PCT levels increase significantly during systemic bacterial infection (EOS and LOS) and necrotizing enterocolitis (NEC) [64,68]. Chiesa et al. showed that in bacterial infection (EOS and LOS) and necrotizing enterocolitis (NEC), PCT had sensitivity of 92%, specificity of 97%, PPV of 94%, and NPV of 96% [68]. The other added advantage of PCT is that its serum concentrations remain high when compared to other sepsis biomarkers like tumor necrosis factor alpha (TNF-α) and IL-6, thus making PCT more useful in predicting the severity of infection and response to treatment [64,68]. False increase of PCT is seen in many conditions like nonspecific elevation in healthy newborn, premature newborn, intracranial hemorrhage, birth asphyxia, neonatal hypoxemia, neonate requiring resuscitation, infants born to mothers with chorioamnionitis in the absence of neonatal infection [69], maternal GBS colonization and prolonged rupture of membranes \( \geq 18 \) h [70], prenatal disuse of antibiotics, surfactant administration, postnatal use of antibiotics, and VLBW thus emphasizing the proper assessment of increase in PCT in neonatal period [71]. Kordek et al. compared clinical usefulness of blood PCT concentrations in the diagnosis and therapeutic monitoring of nosocomial neonatal sepsis and showed that PCT had better sensitivity, specificity, PPV and NPV when compared with CRP and WBC count [72]. Similarly, Luzzani et al. in their study showed PCT as a better marker of sepsis than CRP [73], but Hahn et al. showed that CRP was better in detection of sepsis from healthy controls compared with PCT [74]. A recently published meta-analysis concluded that PCT is a helpful biomarker for early diagnosis of sepsis in critically ill patients [75]. As there are numerous conditions for false increase in PCT, therefore, PCT needs to be studied further in larger groups of infants so as to improve its diagnostic accuracy.

Serum amyloid A

Serum amyloid A (SAA) is an early acute phase reactant apolipoprotein and source of SAA is liver. The other sources of SAA are endothelial cells, monocytes, and smooth muscle cells. The synthesis of SAA is regulated by IL1, IL6, and TNFα [76], and SAA is released in response to infection and injury. The level of SAA changes with age, therefore, the interpretation of the result should be considered after taking care of the age of the patient. The lowest levels are seen in umbilical cord blood with highest levels seen in old age patients [77]. SAA have role in inflammation and it stimulates the secretion of IL-8 from neutrophils. Arnon et al. enrolled 42 preterm infants with fulminant sepsis and non-fulminant sepsis, and measured CRP, SAA, IL-6 levels and WBC counts at the first suspicion of LOS and after 8, 24 and 48 h. The authors reported that SAA was the earliest prognostic marker and in addition of SAA, CRP and WBC counts can also be used as prognostic markers in LOS in preterm infants [78]. In another study Arnon et al. showed that SAA increased quickly at onset of neonatal sepsis, followed by a gradual decline thereafter, whereas CRP levels increased only after 24 h of neonatal sepsis onset. The study reported that SAA at 10 mug/ml concentration showed highest sensitivity (95%, 100% and 97%, respectively) and NPV (97%, 100% and 98%, respectively) at 0, 8 and 24 h after onset of sepsis [79]. Arnon et al. in other study showed that SAA levels in the septic infants were significantly higher when compared with non-septic infants at 0, 24 and 48 h (\( p < .01 \) for all time points). The rise of SAA was faster and sharper when compared to CRP and similarly the levels of SAA returned to normal rapidly when compared to CRP. At 0 h, post-sepsis evaluation, serum SAA had good diagnostic accuracy for predicting EOS when compared to CRP (sensitivity (96% vs. 30%), specificity (95% vs. 98%), PPV (85% vs. 78%), NPV (99% vs. 83%), positive likelihood ratio (19 vs. 12), and negative likelihood ratio (0.05 vs. 0.71) [80]. Cetinkaya et al. conducted study to compare SAA concentrations with CRP and PCT in diagnosis and follow-up of neonatal sepsis in premature infants. The result showed that SAA had better sensitivity and area under curve when
compared with CRP and PCT, although the difference was not statistically significant [81]. SAA is a good marker for the detection of neonatal sepsis, with quick and reliable SAA detection kits, it can make SAA as an useful marker for the detection of neonatal sepsis.

**Lipopolysaccharide-binding protein**

Lipopolysaccharide-binding protein (LPB) is a soluble pattern-recognition molecule that plays an important role in interaction with endotoxin released during Gram-negative bacterial infections. LPB recognizes microbial-associated molecular patterns of bacteria to transport endotoxin to CD14 immune effector cells in response to infection [82,83]. LPB binds with lipopolysaccharide component of the Gram-negative bacteria, thus forming a complex which initiates acute infection response by leukocytes [84]. The sources of LPB are hepatocytes, epithelial and muscle cells, and rapid rise of LPB levels are seen within 6–8 h, after onset of an acute infection. Thus, making LPB as a good marker with high sensitivity and NPV for diagnosis of EOS. The other added advantages of LPB are that it has stable level with less physiological fluctuations in the first two days of post-natal life, and is less affected by other obstetrical events [85,86]. Behrendt et al. studied serum concentrations of LPB in preterm infants with neonatal bacterial infection (NBI). The study enrolled 57 preterm and 17 term infants and result showed that maximum LPB concentrations in infants with NBI were greatly increased compared with infants without NBI (13.0–46.0 μg/ml (median 20.0 μg/ml) vs. 0.6–17.4 μg/ml (median 4.2 μg/ml)) [82]. Pavcnik-Arnol et al. compared LPB with PCT, IL-6 and CRP in critically ill neonates and children with suspected infection. The study enrolled 29 neonates with <48 h of post-natal life and reported that in critically ill neonates aged <48 h, LPB on the first day of suspected infection was a better marker of sepsis than IL-6 and PCT, and was similar to CRP. In critically ill neonates aged >48 h and children, LPB was a better marker than IL-6 and CRP, and was comparable to PCT [84]. Berner et al. showed that in septic neonates, LPB levels (median, 36.6 vs. 7.8 microg/ml; \( p < .001 \)) were significantly increased when compared to healthy neonates. The LPB levels in septic neonates analyzed between 24 and 48 h of life were further increased when compared to samples obtained at or shortly after delivery (median, 36.6 vs. 60 microg/ml; \( p = .038 \)). Therefore, the author concluded that the levels of LPB in plasma of neonates with EOS are significantly elevated and this increase in plasma LPB levels persist for more than 24 h, providing clinician with a prolonged time period to identify the newborn with bacterial sepsis [87].

**Interleukin 6**

During the acute phase of an infection, IL-6 is produced by B and T lymphocytes and the other source of IL-6 are monocytes, endothelial cells and fibroblasts. This IL-6, induces hepatic cells to produce acute phase reactants such as CRP [88,89]. The structure of IL-6 consists of 184 amino acids with two N-glycosylation sites and four cysteine residues [90]. The advantage of using IL-6 as sepsis marker is that there is rapid rise in its concentration after the onset of bacteremia, even before the rise of CRP level. The disadvantage of IL-6 is that it has very short half-life and its level normalize within 24 h of starting antimicrobials, therefore IL-6 have narrow window of opportunity [91,92]. The umbilical cord IL-6 levels are increased in newborns having EOS [93,94]. The cord blood IL-6 levels used in predicting neonatal sepsis has shown sensitivity of 87–100%, and NPV of 93–100% [94–96]. Smulian et al. compared clinical chorioamnionitis with IL-6 (plasma level \( \geq 25 \) pg/ml) for diagnosis of EOS and showed IL 6 having better sensitivity (42.9% vs. 92.9%), specificity (71.4% vs. 92.9%), PPV (60% vs. 92.9%) and NPV (55.6% vs. 92.9%) for predicting early neonatal sepsis [93]. The studies have shown that when IL-6 is used as an early phase biomarker, it has better sensitivity and NPV when compared with CRP [97]. The combination of IL-6 with other sepsis marker like TNF-α and CRP leads to better sensitivity and NPV for diagnosis of EOS [98]. Raynor et al. conducted study in neonates >3 days of postnatal age and measured cytokine levels for the diagnosis of neonatal sepsis and postulated cytokine scores. They reported that cytokine score using thresholds for granulocyte colony-stimulating factor (G-CSF), IL-6, IL-8, and tumor necrosis factor (TNF)-α had 100% sensitivity and 69% PPV for diagnosis of gram negative bacilli infection. Isolated IL-6 <130 pg/ml showed 100% sensitivity and 52% PPV for ruling out neonatal sepsis [91]. Hou et al. in recently published meta-analysis showed pooled sensitivity of IL-6 for the diagnosis of sepsis was 80% (95% CI, 77–83%) and specificity was 85% (95% CI, 81–88%). In neonate subgroup, IL-6 had a pooled sensitivity of 77.0% (95% CI, 73.0–81.0%) and specificity of 91.0% (95% CI, 86.0–94.0%) for sepsis diagnosis [99].

**Interleukin 8**

IL-8 is pro-inflammatory cytokine and the sources of IL-8 are monocytes, macrophages, fibroblast and...
endothelial cells. IL-8 plays role in migration and activation of neutrophils during infection [100,101]. It serves as marker of infection and also correlates well with the severity of the illness. There is rapid rise of IL-8 levels after initiation of infection with concentrations of IL-8 rising within 2–4 h, followed by rapid decline by 4 h, thus making it useful as an early marker of infection, similar to IL-6 [102]. IL-8 has sensitivity and specificity ranging from 80 to 91% and 76 to 100%, respectively, in diagnosis of neonatal sepsis [97,103]. Ng et al. showed that combination of IL-8 with CRP as sepsis biomarker leads to better diagnosis (improved sensitivity and specificity) and reduction in use of antibiotics in neonates admitted for suspected sepsis [97]. The advantages of using chemokines as marker of neonatal sepsis is that they have rapid rise in level (within 2–4 h of infection), much prior than rise in level of CRP but the major disadvantages are their rapid fall in level with initiation of treatment (with 24 h), need for sophisticated instruments for detection and lack of measurement facility at majority of places, thus limiting its widespread use [95]. Boskabadi et al. enrolled 80 neonates having postnatal age >72 h and showed that serum concentrations of IL-8 were 3.3 times higher in neonates with mortality when compared to discharged neonates. With cut off value of >60 pg/ml, IL-8 showed sensitivity, specificity, PPV and NPV value of 95%, 10%, 97% and 10%, respectively [104]. Boskabadi et al. in other cohort of neonates showed that with cutoff value of 60.05 pg/ml, IL-8 was valuable for diagnosing definitive infection [105]. Kocabas et al. showed that pretreatment mean serum IL-8 levels were significantly higher in the septic neonates when compared with healthy ones [106]. A recently published meta-analysis showed pooled sensitivity and specificity of IL-8 as 78% and 84%, respectively, thus showing moderate accuracy in the diagnosis of neonatal sepsis [107]. Although the results are promising to seek the role of IL-8 in the diagnosis of neonatal sepsis, but still there is need for further studies to further strengthen its use.

**Tumor necrosis factor (TNF-α)**

It is a proinflammatory chemokines/cytokines and source being activated phagocytes and is produced during systemic infection and inflammation. The characteristics and pharmacokinetics of TNF-α are almost similar to IL-6 [103], thus having rapid rise within 2–4 h of infection onset. This proinflammatory response of increase in TNF-α level is not affected by gestational or postnatal age of neonate [97]. The concentrations of TNF-α is significantly higher in septic newborn when compared to normal healthy newborn [108,109]. Silveira et al. studied cohort of 117 newborn infants to see role of IL-6 and TNF-α for diagnosis of neonatal sepsis. The median TNF-α levels were significantly higher in group of patients with a diagnosis of clinical sepsis when compared with control and the optimal cutoff point was 12 pg/ml. The combination of IL-6 and TNF-α showed a sensitivity of 98.5% [98]. de Bont et al. showed that when TNF-α and IL-6 levels were combined for the diagnosis of neonatal sepsis, then sensitivity and specificity increased to 60% and 100%, respectively [110]. The meta-analysis that included 23 trials showed that TNF-α had moderate accuracy in the diagnosis of neonatal sepsis both in EOS (sensitivity = 66%, specificity = 76%) and LOS (sensitivity = 68%, specificity = 89%) [111].

**CD11β**

CD11β (Mac-1, CR3) is the α-subunit of the β2-integrin adhesion molecule and is expressed in low concentration in inactivated neutrophils. This is involved in various functions of neutrophil during inflammatory reaction like adhesion, diapedesis, and phagocytosis. Bacterial infection leads to rise of level within five minutes, thereby making it good biomarker for early detection of neonatal sepsis [97]. Weirich et al. sought neutrophil CD11β expression as a diagnostic marker for EOS and reported that neutrophilic CD11β was detectable in all infants diagnosed to have confirmed infection and was not detectable in neonates without sepsis. The NPV, PPV, sensitivity, and specificity of CD11β for diagnosis of neonatal infection at initial evaluation were 100%, 99%, 96%, and 100%, respectively [112]. Nupponen et al. in another study showed that CD11β expression was increased maximum in sepsis when compared to possible infection and healthy neonates and CD11β had 100% sensitivity and specificity for the detection of neonatal sepsis [113]. Adib et al. conducted study in 65 neonates with gestational age of 27 to 38 weeks with suspected sepsis within 28 days of life for evaluation of CD11β expression on peripheral blood neutrophils for early detection of neonatal sepsis. They reported that CD11β had sensitivity of 75%, specificity of 100%, PPV of 100% and NPV of 86% for detection of neonatal sepsis. The combination of CD11β and CRP made sensitivity and NPV to reach 100% [114]. These studies have shown that CD11β has very good diagnostic accuracy as biomarker for neonatal sepsis but lack of detection facility and cost effectiveness may be important hindering factors.
sCD 163
Soluble CD163 is a macrophage cell surface glycoprotein receptor and belongs to the scavenger receptor cysteine rich (SRCR) domain family. It decreases the oxidative damage caused by hemolysis through clearing the circulating free hemoglobin with the help of haptoglobin [115]. sCD163 binds to Gram-negative and Gram-positive bacteria and promote synthesis of proinflammatory cytokines like TNF-α, IL-1β, IL-6 and IL-10 [116]. Prashant et al. showed that for sCD163 at >896.78 ng/ml, there was sensitivity of 100%, specificity of 88% for the diagnosis of infection before antibiotics. In this study they compared CRP, IL-6, IL-8, TNF-α and sCD163 and concluded that sCD163 was the most powerful predictor to differentiate between the non-infected and infected neonates before antibiotics [117].

CD 64
CD64/Fc gamma-receptor I is a high-affinity Fc receptor for immunoglobulin G1 and G3 and is expressed in low level on the surface of inactivated neutrophil, approximately 1000 molecules per cell [111]. Its level increase up to ten times in activated neutrophil after onset of bacterial infection within next four to six hours. The expression levels return to normal in next few days after the infection stimulus is removed by the immune system [112,113]. CD64 expression plays an important role in innate immunity by promoting neutrophilic phagocytosis [114,115]. Bhandari et al. conducted a study to evaluate neutrophil CD64 as a diagnostic marker. They enrolled 163 infants with 293 episodes of sepsis and neutrophil CD64 indices correlated with the diagnosis of confirmed and suspected sepsis. The results of the study showed that there was higher neutrophil CD64 indices (5.61 ± 0.85 vs. 2.63 ± 0.20) in sepsis episode. The area under curve in ROC analysis for cases of all sepsis episodes for CD64 was 0.74 with a cut off value of 2.30. When CD64 index was combined with the ANC, it reached NPV of 93% for ruling out sepsis and 95% sensitivity for diagnosing sepsis [116]. The other recent studies have shown similar results with CD64 having good sensitivity in diagnosing neonatal sepsis [117,118]. Yang et al. showed that neutrophil CD64 combined with PCT, CRP, and WBC improves the sensitivity for the early diagnosis of neonatal sepsis [119]. In a recently published meta-analysis that included 17 studies with 3478 participants showed overall pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR) of 77% (95% CI: 74–79%), 74%(95% CI: 72–75%), 3.58 (95% CI: 2.85–4.49), 0.29 (95% CI: 0.22–0.37) and 15.18 (95% CI: 9.75–23.62), respectively. The area under the summary (SROC) curve was 0.8666, and no threshold effect was found based on the Spearman correlation analysis (p = .616) [120]. Thus, neutrophil CD64 can combine with other specific hematologic criteria as an additional marker for the diagnosis of neonatal sepsis because it has many features required for being ideal neonatal sepsis biomarker [114].

Pentraxin 3
Pentraxin (PTX) is an acute phase glycoprotein and the sources of PTX3 are endothelial cells and mononuclear phagocytes. It is an important component of innate immune system and it plays major roles as pattern recognition receptors in defense against microbes and clearance of apoptotic or necrotic cells [118]. The production of PTX3 is induced by microbial endotoxin and cytokines. PTX3 interacts with growth factors, extracellular matrix components, and selected pathogens, and play role in complement activation, facilitating pathogen recognition, and act as a predecessor of antibodies [119]. Structurally PTX3 shares almost 98% homology with TNFα. High levels of PTX3 has shown to act as prognostic indicator in pediatric meningococcal shock. Currently, there are no studies that have evaluated the role of PTX3 in diagnosis of neonatal sepsis [120].

Angiopoietins
Angiopoietins (Ang)-1 and Ang-2 are antagonist to endothelial cell growth factors and play an important role in vascular permeability. They both have been studied extensively in proliferative diseases such as retinopathy [121,122]. Ang-2 leads to more vascular permeability especially against host responses to TNFα and IL-6 [123]. Mankhambo et al. conducted study to see role of angiogenic factors in predicting clinical outcome in severe bacterial infection in 293 children. The authors reported that in children with sepsis mean Ang-1 was significantly increased, and Ang-2 was significantly decreased in survivors compared with non-survivors (6000 vs. 3900 pg/ml, p = .03; and 7700 vs. 11,900 pg/ml, p = .02, respectively). Thus, lower Ang-1 and higher Ang-2 predicted poor prognostic outcome, and low levels of Ang-1 predicted high mortality [124]. Therefore, both of them can be a useful biomarker to see response to therapy in neonatal sepsis. Presently there are no studies to see the role of
Ang-1 and Ang-2 as sepsis marker in neonatal population.

**Soluble form of the urokinase-type plasminogen activator receptor (suPAR)**

suPAR is expressed on immune and endothelial cells and play role in immune regulation. It is multifunctional glycoprotein released during inflammation. suPAR is present in various body fluids, such as plasma, pleural, bronchoalveolar lavage, urine and cerebrospinal fluid [125,126]. Siahanidou et al. conducted study to see clinical value of plasma suPAR levels in term neonates with infection or sepsis. The study enrolled 47 term neonates with infection (19 bacterial and 28 viral) and 18 healthy control neonates and results showed that plasma suPAR levels were significantly increased in infected neonates upon admission, and the levels were highest in septic neonates, in comparison with controls (p < .001) and correlated positively with serum CRP levels (r = .001). At infection subsidence, suPAR concentrations decreased significantly in comparison with baseline (p < .001) but still remained higher than controls (p = .01). The authors concluded that suPAR is a diagnostic biomarker of infection or sepsis in term neonates; but, it cannot discriminate bacterial from viral infections and also its utility for monitoring the response to treatment was less reliable [127]. Similarly, Okulu et al. conducted study to see serum levels of suPAR in infants with LOS. The results of the study showed that there were significantly higher levels in patient group (18.8 ng/mL (range 6.8–30.1 ng/mL)) when compared to control [6.0 ng/mL (range 3.7–10.8 ng/mL)] (p < .001). There was significant decrease in suPAR level from the inclusion to the third day and end of the treatment (p < .001). At a cutoff value of 11.3 ng/mL, suPAR had specificity of 100% and sensitivity of 82.5%, respectively, and there was a positive correlation between laboratory values of CRP and suPAR (r: 0.359, p = .003) [128].

**Soluble triggering receptor expressed on myeloid cells-1**

Soluble triggering receptor is included in the immunoglobulin family and is expressed by phagocytes (sTREM-1). sTREM-1 plays an important role in innate inflammatory response and sepsis [129]. Adly et al. conducted study to see role of sTREM-1 as diagnostic and prognostic marker in neonatal sepsis and showed that baseline sTREM-1 levels were significantly elevated in culture-proven (1461.1 ± 523 pg/mL) and culture-negative sepsis (1194 ± 485 pg/mL) compared to controls (162.2 ± 61 pg/mL) with no significant difference between both septic groups. At concentration limits of 310 pg/mL, sensitivity and specificity reached 100%, while the cutoff value 1100 pg/mL was predictive of survival with 100% sensitivity and 97% specificity [130]. Similarly, Saldir et al. showed that sTREM-1 levels were significantly higher in septic neonates in comparison with non-septic neonates [131]. Recently published systematic review that included four neonatal studies concluded that there are insufficient data to support the role for sTREM in the diagnosis and follow-up for pediatric sepsis [132].

**Inter alpha inhibitor proteins**

Inter alpha inhibitor proteins (IαIP) is a group of serine protease inhibitors, playing an important role in multiple biological actions like tumor invasion, extracellular matrix stabilization, inflammation, and wound healing. IαIP is postulated to play an important anti-inflammatory and regulatory role during infection [133,134]. Its concentration is independent of gestational age, postnatal age, and is similar to adult levels. Baek et al. studied 135 newborn infants (24–42 weeks’ gestational age) and reported that circulating IαIP concentrations were independent of gestational age. Its concentration was significantly reduced in septic neonates when compared with controls (169 ± 126 mg/L vs. 613 ± 286 mg/L, p < .0001). The investigators suggested that IαIP may be useful in assisting the decision to start antibiotics in infants admitted with suspected sepsis and also can act as a prognostic indicator for monitoring the progress of treatment as levels of IαIP are increased with treatment [135]. Chaaban et al. evaluated IαIP as a diagnostic marker in neonatal sepsis and reported that IαIP levels were significantly lower in the septic group (121 ± 71 mg/L) than in the non-septic group (322 ± 91 mg/L). The optimal cutoff value with the ROC curve was ≤177 mg/L (sensitivity= 89.5%; specificity= 99%; PPV= 95%; NPV= 98%) with area under the curve of 0.94. The authors thus concluded that IαIP is a more reliable diagnostic marker for neonatal sepsis than other available tests [136]. But still there is need for further studies to evaluate the role of IαIP as neonatal sepsis biomarker [137].

There are many other biomarkers that have been studied for the diagnosis of neonatal sepsis, namely, IL1b, sIL2R, IL4, IL5, IL10, interferon-γ, CXCL12 [138], sCD14-ST or presepsin [139,140], 11sTNFR-p55, 12sTNFR-p75, intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, L-selectin, fibronectin, haptoglobin,
neopterin, orosomucoid, complement activation products (C3a-desArg, C3bBbP, sC5b-9), asymmetric dimethylarginine, l-arginine [141], mannose-binding lectin [142,143], melatonin [144], α1-acid glycoprotein (α1AG) [145], 16S rDNA [146], serum ischemia-modified albumin (IMA) [147], and lactoferrin [148], but none of these could gain popularity in the usage, either they did not fulfill the criteria of being ideal biomarkers, or are not cost effective, or lack appropriate technology for detection or delay in getting the results [97].

Recently there have been advances in “Omics” technology, namely, genomics, proteomics, and metabolomics (new clinical biochemistry). Genomics include identification of the genes that show altered regulation during the pathogenesis of neonatal sepsis [149]. Petarakou et al. conducted study in 42 premature neonates to see IL-8 and monocyte chemotactic protein-1 mRNA expression in perinatally infected (PI) and asphyxiated preterm neonates. They reported that IL-8 mRNA levels were significantly increased in whole blood both during perinatal asphyxia (PA) and PI. In vitro activated lymphocytes expressed significantly increased IL-8 mRNA levels during PI, whereas no increase was observed during PA [150]. Proteomic and metabolomics study about the functional expression of proteins or metabolites in the sample and can be used for the diagnosis of neonatal bacterial and fungal sepsis [151]. These technologies for the detection of neonatal sepsis are in initial phase and still long way to go before being used easily in all the neonatal intensive care units [152,153].

Conclusions
Presently there are many markers for the early diagnosis of neonatal sepsis but CRP, PCT are the most commonly used and holds good promise for the use as sepsis markers. These two markers have been studied in numerous neonates. Presently there is no single biomarker that fulfills all the criteria’s for becoming an ideal biomarker. There is still research going on for finding the biomarker that has high sensitivity, specificity, PPV and NPV for the detection of neonatal sepsis.

Disclosure statement
No potential conflict of interest was reported by the authors.

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