Early Diagnosis of Neonatal Sepsis: A Review of the Current Methods in Clinical Practice

B. O. Kayode-Adeleji

1Neonatal Unit, Princess Royal Maternity Hospital, Glasgow, UK.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

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ABSTRACT

Introduction: The burden of neonatal sepsis continues to be significant, more so in the preterm population. It is quite challenging to make early diagnosis because the early signs of sepsis may be subtle, and resemble those of other non-infectious processes; furthermore, cultures take time and the yield is quite low with wide inter-laboratory variation. Alternative diagnostic tests are simply not accurate enough. The resulting implications of these challenges include prolonged empirical antibiotic therapy for at-risk neonates. An early diagnostic test that is highly accurate will be immensely beneficial in guiding clinicians in neonatal units on commencement of antibiotics and when to stop. This metanalysis assesses the usefulness, reliability, limitations and challenges in clinical practice of some of the tests for the early diagnosis of neonatal sepsis.

Methods: A metanalysis of articles on the different methods of diagnosis of neonatal sepsis in clinical practice as well as scientific papers comparing various methods was carried out. Sources include but not limited to Pubmed, HINARI and EMBASE database.

Conclusions: Extensive work is being performed to find the ideal test for early diagnosis of neonatal sepsis. Despite numerous studies on acute phase reactants, their use in clinical setting is...
limited to CRP and PCT to some extent. There is still need for further research work to find an ideal test for early diagnosis of neonatal sepsis. However, the methodologies and study designs are to be harmonized in order to obtain ideal cut-off acceptable values.

Keywords: Sepsis; diagnosis; early; practices.

1. INTRODUCTION

Neonatal sepsis remains a major problem associated with high morbidity and mortality in newborns, particularly amongst preterm infants [1-4]. Mortality rate is between 11-68 per 1000 live birth in developing countries and 5 per 1000 live birth in developed countries [5,6]. Neonatal Sepsis is implicated in 25-40% of neonatal mortality in Nigeria [7,8]. It is quite challenging to make early diagnosis because the early signs of sepsis may be subtle, and resemble those of various non-infectious processes; furthermore, cultures take time and the yield is quite low with wide inter-laboratory variation [9,10]. The clinical course of neonatal sepsis can be fulminant and fatal if treatment is not commenced promptly [11]. In clinical practice, patients with suspected sepsis are empirically treated with antibiotics [12-14]. When such empirical antibiotics are given, the duration of treatment is another issue. The practice of empirical treatment of newborns with suspected sepsis can contribute to drug resistance in addition to unnecessary venous cannulation with its attendant problems.

The accuracy of the tests available are not satisfactory; there are cases of false negatives and more commonly false positives. The relevance of an early diagnostic test is mostly in the evaluation of at-risk neonates and those with subtle, non-specific signs, where delay in instituting appropriate antimicrobial therapy can lead to significant morbidity and possibly fatality [15].

A number of acute-phase reactants (APR) have been evaluated with reasonably high specificity and sensitivity, however none has been proven to be accurately diagnostic [16,17]. This review draws attention to the limitations of some of the tests for the early diagnosis of neonatal sepsis.

2. METHODS

A metanalysis of articles on the different methods of diagnosis of neonatal sepsis as well as scientific papers comparing various methods was carried out. Sources include but not limited to Pubmed, HINARI and EMBASE database.

In order to draw attention to the challenges of the current methods of diagnosing neonatal sepsis, it would be important to highlight the features of the ideal test.

The Ideal Test should have the following features: well defined cut-off values, very high diagnostic utilities (sensitivity, specificity, positive and negative predictive values), early detection of infection, ability to monitor treatment, easy to measure, quick turnover time, comparable inter-laboratory results and cheap [10].

2.1 Diagnostic Tests

2.1.1 Haematologic scoring system (HSS)

Total leukocyte count (TLC) is of little clinical use in the diagnosis of neonatal infection because of wide variation in values. As no single individual hematological parameter is superior in comparison to another in predicting neonatal sepsis, a combination of these parameters in the form of HSS has been found to be quite useful [18].

The HSS has practical advantages; it is applicable to all infants, including those who have received antibiotic therapy prior to evaluation and simplifies the interpretation of hematologic profile [18].

In a Bangladeshi study by Khalada et al. involving 100 neonates, a score of 3 was highly significant (P<0.05), with sensitivity of 100%, specificity of 21%, PPV 15%, NPV 100%. These results were consistent with other studies. In the study, sensitivity of score >4 was 100%, specificity 60%, PPV 26%, NPV 100%. In comparison with score >3, score >4 was more sensitive (P<.001) and specificity and PPV were
significantly higher as well, 60% and 26% respectively. But considering the high specificity and positive predictive value, the study implied that score > 4 was more reliable as a screening tool for sepsis than any of the individual hematological parameter.

In the study, I/T ratio >0.2 had a sensitivity, specificity, PPV and NPV of 100%, 04%, 13% and 100% respectively. Specificity and positive predictive value was low because of large number of false positive results. While an I/T ratio >0.2 suggested by Rodwell, Lesilie, and Tudehope had a sensitivity of 96% and NPV of 99%. So this result for an elevated I/T ratio was consistent with other reports. The sensitivity of I/M ratio (>0.3) was 100%, specificity 07%, PPV 11% and NPV 100%. Rodwell et al. used I/M ratio of 0.3 as a predictor of infection and reported sensitivity, specificity, PPV and NPV of 93%, 81%, 32% and 99% respectively [11,18,19,21-23].

Ghosh et al. [22] evaluated the haematologic parameters of 105 Indian neonates, a score of three was taken as the cut of point as it provided acceptable levels of sensitivity, specificity and positive predictive values. A score of 3 identified 28 of the 30 babies with sepsis and in addition five infants without sepsis. Each of the seven criteria of the HSS was evaluated. An abnormal 1:T ratio had the highest sensitivity (93%) and identified more than 90% of the infants with sepsis of probable infection. An abnormal 1:M ratio was also found to be highly sensitive (92%) in identifying infants with sepsis. These two criteria along with thrombocytopenia (<150,000/cm³) had a high negative predictive value over 94%. Hence the likelihood of sepsis being present in the absence of these findings is low. The study also found that the higher the score the greater the certainty that sepsis was present. The five false positive cases all had a borderline score of 3.

The HSS is potentially a very useful tool in resource poor settings and its right application should significantly minimize the problems associated with prolonged empirical treatment of at-risk babies. One of the drawbacks of the HSS is the observer variation in the assessment of blood film for white cell properties.

2.1.2 Biological biomarkers

Biological biomarkers are human blood components that increase in response to inflammation. The most commonly used acute phase reactant (ARP) is the C-reactive protein (CRP). However, the CRP takes 12–24 hours to increase to measurable levels; its half life is very long and it takes 5–7 days to normalize after eradication of the infectious agent. Cytokines such as IL6, IL8, TNF-α, and procalcitonin have also been studied [24,25]. Cytokines rise quickly after infection even in neonates, and are more sensitive to low concentrations of pathogens than CRP.

2.1.2.1 The C-reactive protein

The C-reactive protein is the most analyzed parameter for the detection of bacterial infections for years [26,27]. It is an annular pentameric protein of hepatic origin that increases following interleukin-6 secretion from macrophages and T cells. It was discovered by Tillett and Francis in 1930 [28].

<table>
<thead>
<tr>
<th>Table 1. Hematological scoring system</th>
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<tr>
<td>Criteria</td>
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<tr>
<td>Total WBC count</td>
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<tr>
<td>Total PMN count</td>
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<tr>
<td>count</td>
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<tr>
<td>Immature PMN count</td>
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<td>I:T PMN ratio</td>
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<td>I:M PMN ratio</td>
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<td>PMN changes</td>
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<td>Platelet count</td>
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WBC- White blood cell, PMN- Polymorphonuclear, I:T- Immature to total ratio, I:M- Immature to mature ratio.


Interpretation: < 2 Sepsis is very unlikely, 3 or 4 Sepsis is suspected, > 5 Sepsis or infection is very likely

C-reactive protein is a good discriminatory marker of bacterial sepsis in newborns, its half-life of 48 hours is constant, and therefore its level is determined by the rate of production. The predictive value of CRP improves with time and is most predictive between 24 and 48 hours after presentation with suspected infection [29,30].
Table 2. Summary of specificity and sensitivity of CRP in various studies

<table>
<thead>
<tr>
<th>Authors and sample size</th>
<th>Testing method</th>
<th>Cutoff value</th>
<th>Sampling interval</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Russel et al. n= 58</td>
<td>Quantitative Nephelometry</td>
<td>&gt; 8 mg/L</td>
<td>Daily</td>
<td>71%</td>
<td>72%</td>
</tr>
<tr>
<td>Poucyrous et al. n= 187</td>
<td>Immune-nephelometry</td>
<td>&gt; 9 mg/dl</td>
<td>12 hourly</td>
<td>91%</td>
<td></td>
</tr>
<tr>
<td>Kamawura et al. n= 348</td>
<td>Laser-Nephelometry</td>
<td>&gt; 10 mg/dl</td>
<td>3 levels</td>
<td>75%</td>
<td>98%</td>
</tr>
<tr>
<td>Franz et al. n=223</td>
<td>Nephelometry</td>
<td>&gt; 10 mg/dl</td>
<td>4 levels</td>
<td>93%</td>
<td>100%</td>
</tr>
<tr>
<td>Benitz et al. n= 1002</td>
<td>Quantitative Nephelometry</td>
<td>&gt; 10 mg/dl</td>
<td>5 levels</td>
<td>89%</td>
<td>97%</td>
</tr>
</tbody>
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Several hours are needed for CRP levels to increase in serum (including activation of neutrophils, elaboration of interleukin-6, and induction of hepatic synthesis of CRP) therefore limiting the sensitivity of this test in diagnosing neonatal sepsis (NNS). Its levels are consistently elevated 24 to 48 hours after the onset of infection; therefore serial normal levels done at 18 to 24 hour intervals may be useful for identification of infants who do not have bacterial infection [31,32]. It is not a highly specific marker, as false positive elevation of CRP is found in viral infections, meconium aspiration syndrome, cephalhaematoma, bruising and surgery [33].

The wide variation in sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) reported in various studies are partly due to different cut-off values ranging from 5 to 20 mg/l, as well as different testing methods. Han et al. reported the sensitivity, specificity, PPV and NPV of serial CRP as 82%, 78%, 15% and 98.9% respectively. Ayazi et al. reported 55%, 80%, 24% and 90% respectively, while Borma reported 79%, 85%, 36% and 97% respectively. Nuntnarumit et al. reported a sensitivity of 100%, specificity of 94%, PPV and NPV of 91.6% and 100% respectively of CRP for detecting proven sepsis and localized infection at cut off point ≥5 mg/l. Himayun et al. found 70%, 72.3%,28% and 94% respectively at a cut-off of 8 mg/l [2,31,34,35].

The NPV of CRP is consistently high and it is most useful in indicating an absence of infection. A negative CRP value is more important than a positive CRP value in that it excludes infection with a high certainty and can guide the decision to discontinue antibiotics.

The challenge with interpretation and application of the studies is defining the reliability, in this case NPV and PPV, generally low threshold values for diagnosis gives a higher PPV and lower NPV, the converse applies to high cut-off values.

In an Australian study, CRP values greater than 15 mg/l was said to confer a 7% risk of death and up to 21% when levels are greater than 30 mg/l. With regards to the utility of CRP as a prognostic marker, the majority of studies failed to demonstrate any correlation between CRP concentration and mortality [36-38].

Serial measurements of CRP is required to make decisions in clinical practice, this can be expensive in resource limited settings where health insurance is grossly suboptimal.

2.1.2.2 Procalcitonin (PCT)

Procalcitonin (PCT) has been reported as a measurable laboratory marker in the inflammatory response to infection in some studies.

Procalcitonin is a 116-amino acid protein, a precursor of calcitonin which is produced by the thyroid. In sepsis, macrophages and the monocytic cells of the liver are involved in the synthesis of PCT. A number of studies have reported on the usefulness of the quantitative measurement of PCT for an early diagnosis of sepsis in newborns [39].

In healthy persons, PCT levels are undetectably low, however in severe bacterial, fungal, parasitic infections with systemic manifestations, a significant rise is seen. In this condition the production site is the extra thyroid tissues [40].

In healthy individuals, production of PCT and subsequently calcitonin is restricted to the thyroid
C-cells. Bacterial infections selectively induce an increase in the concentration of PCT; because both endotoxins released from the bacterial cell wall as well as the host responses to infection activate the production of PCT mainly in parenchymal tissues [41,42].

In 1993, PCT was first described as a marker of the extent and course of systemic inflammatory response to bacterial and fungal infections. The advantage of PCT as compared to C-reactive protein is that the increase of the former in bacterial infection and its restoration to normal is more rapid. It can therefore help in deciding the duration of antibiotics administration [43,44]. Furthermore, the finding that PCT is released into the circulation within 3 h after endotoxin injection, plateaus at 6 h, and remains elevated for 24 h, makes PCT a promising new agent for early and sensitive identification of infected patients [45].

The results of recent studies on the usefulness of PCT for early diagnosis of neonatal sepsis have been inconclusive [27,46-53]. Some authors have reported markedly increased concentrations during the first 48 h of life in newborn infants without bacterial infection [53-55]. In these studies, PCT sensitivity in the early diagnosis of neonatal sepsis was found to be 83-100% while the specificity was 70-100% [54,56,57].

The sensitivity of PCT in initial determinations for the diagnosis of early-onset neonatal sepsis has been reported to vary from 61% to 85%, increasing to 72–100% within the subsequent 24 h [51,53,54].

Sastre et al. [12] evaluated 317 newborns in a multicentre Spanish study (confirmed vertical sepsis: 31, vertical clinical sepsis: 38, non-infectious diseases: 79). In asymptomatic neonates, PCT values at 12–24 h were significantly higher than at birth and at 36–48 h of life. Neonates with confirmed vertical sepsis showed significantly higher PCT values than those with clinical sepsis. PCT thresholds for the diagnosis of sepsis were 0.55 ng/mL at birth (sensitivity 75.4%, specificity 72.3%); 4.7 ng/mL within 12–24 h of life (sensitivity 73.8%, specificity 80.8%); and 1.7 ng/mL within 36–48 h of life (sensitivity 77.6%, specificity 79.2%). Active resuscitation at birth was independently associated with high PCT values.

Ali et al. in a study of 70 Neonates admitted in NICU, 31.4%, 42.9% and 25.7% were categorized as proven sepsis, suspected sepsis and clinical sepsis respectively. Procalcitonin was positive in 100% compared to the CRP positivity in 63.6% of the proven sepsis cases. Chiesa et al. determined reference ranges for PCT across the range of postnatal hours from 0 to 48 h and, in a further study, these authors showed that PCT sensitivity and specificity were greater than those of CRP or interleukin 6 if different cutoff points at birth and at 24 h and 48 h of life were used [29,36].

In these studies, it was reported that serum levels had also increased in non-infected neonates with maternal chorioamnionitis, GBS colonization, perinatal asphyxia, intracranial hemorrhage, pneumothorax, or after resuscitation, and these conditions had negatively affected the specificity of PCT. Janota et al. reported a significant increase in serum PCT concentrations within 72 h of age in preterm infected and uninfected newborns born to mothers with chorioamnionitis. Gendrel and Bohuon suggested that hypoxemia may be responsible for increased PCT values in neonates. They also found that neonates born to mothers with preeclampsia had higher PCT concentrations at both 24 and 48 h of life [58,59].

Although PCT may cross the placental barrier, the findings of higher PCT concentrations in cord sera compared with maternal samples, with even larger differences at 24 and 48 h of age, cannot be explained on the grounds of maternal transfer alone; therefore, the postnatal surge of PCT may represent endogenous synthesis [27]. This phenomenon might be attributed to direct stress on the baby during the perinatal period or to the adaptation to the extrauterine environment [60,61].

Higher concentrations of PCT have been observed in uninfected infants with respiratory disorders (mostly respiratory distress syndrome (RDS) compared with asymptomatic infants. Because no significant effect related to the severity of RDS could be detected, Monneret et al. suggested that hypoxemia (which is transient during delivery) could be responsible for these increased PCT values, providing further support for the hypothesis of pulmonary PCT synthesis [53,62].

The wide disparity in cut-off points used in these studies (0.5–5 ng/mL) may account for the differences in results. The inability of PCT to differentiate bacterial infections from other
aetiologies limits its potential benefit of preventing unnecessary antibiotic therapy.

It has been speculated that in addition to diagnosis of sepsis, PCT may be helpful as a tool for prognosis. Ali et al. and Brunkhorst et al. reported that the risks of septic shock and mortality are significantly increased in proven sepsis if PCT levels are greater than 10 ng/ml. Jensen et al. in a study in Denmark reported that PCT rise of more than 1 ng/ml per day was associated with increased mortality [63,64].

In spite of the huge potentials of PCT however, it is not yet used in routine clinical practice; it still largely remains in the realms of research.

3. CONCLUSION

The Haematologic scoring system is widely used but does not fulfill the criteria of an ideal test. The negative predictive value of CRP has been quite useful in stopping antibiotics, however an initially normal result does not exclude sepsis. Procalcitonin has been shown to have a huge potential, but the methodologies and study design are to be harmonized in order to obtain ideal cut-off acceptable values. There is still need for further research work to find an ideal test for early diagnosis of neonatal sepsis.

The requirement for early diagnosis may appear to be less relevant for low and middle income countries where care seeking behaviour is suboptimal and late presentation is common. However, early diagnosis is important in low resource developing countries to guide the management of at-risk neonates and those with suspected sepsis, particularly in the face of limited bed space for neonatal care and the high cost of hospital care.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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