Early Detection of Neonatal Sepsis, Dilemma of Diagnosis

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ABSTRACT

Background and objectives: Accurate identification of neonatal sepsis is an increasing problem facing neonatologists due to non specific clinical signs with no existing single reliable marker of infection. Molecular assays for the detection of bacterial DNA in the blood represent possible diagnostic tools for early identification of bacterial causes. Procalcitonin (PCT) is a promising marker distinguishing between infection and inflammation which cannot be differentiated by acute phase proteins as C-reactive protein (CRP). The aim of the study was to compare results of blood cultures with eubacterial PCR, PCT and CRP as early markers of neonatal sepsis.

Methods: In this study, neonates with clinically suspected sepsis admitted to neonatal intensive care unit (NICU) in Mansoura University Children Hospital were included. Based on blood culture positive results, they were divided into 2 groups: proven sepsis and clinical sepsis. Comparing the 2 groups, sensitivity and specificity for, PCR, PCT and CRP were evaluated. Using receiver operating characteristic (ROC) curves, threshold value for both PCT and CRP were estimated.

Results: Out of 141 neonates with clinically suspected sepsis, 56 (39.7%) were confirmed as proven sepsis. Compared to blood culture, the diagnosis of bacterial proven sepsis by PCR revealed 100% sensitivity and 93% specificity. This study revealed that PCT >6.5 ng/ml had 83.9% sensitivity and 98.8% specificity, whereas CRP >3.5 mg/dl had 83.9%, sensitivity and 81.2% specificity for diagnosing sepsis.

Conclusion: This study confirms the value of PCR and PCT as rapid diagnostic tools for early detection of neonatal sepsis.

INTRODUCTION

Neonatal sepsis is the most serious problem in neonatal intensive care. It is associated with significant morbidity and mortality because the patients handled are often very immature, and consequently, immunocompromised. The lack of specific criteria for sepsis definition particularly early in the course of disease has resulted in mistaken or late diagnosis. Moreover, the serious outcome of missing a case of neonatal infection has led to overuse of antibiotics and the emergence of resistant organisms in the neonatal intensive care environment.

Several studies have investigated different tests for early diagnosis of neonatal sepsis. The gold standard for diagnosing sepsis is the positive organism-specific blood culture, yet its lack of sensitivity, being a time-consuming method and contamination by skin microorganisms can be problematic, which necessitated more-rapid and more-sensitive techniques. Broad-range bacterial PCR detect rRNA gene component present in all bacteria, is one such technique.

Although PCR cannot discriminate viable and nonviable bacteria, its value may be improved if it is coupled with a host response biomarker indicative of infection and systemic inflammation. Procalcitonin (PCT) represents an early marker used in diagnosis of neonatal sepsis. It is produced by monocytes and hepatocytes while C-reactive protein (CRP) is an acute phase reactant that is released by hepatocytes. In the setting of severe bacterial infections and sepsis, PCT levels may increase by hundreds to thousands fold ranging from 1 to higher than 700 ng/ml. Increases in PCT occur more rapidly than in CRP. Besides, it normalizes more rapidly than CRP.

The aim of the present study was to compare the results of eubacterial PCR, PCT and CRP with those of the conventional blood culture method highlighting their value as markers for rapid diagnosis of neonatal sepsis.

SUBJECTS AND METHODS

The current study enrolled 141 neonates who had clinical manifestations of sepsis as diagnosed by the treating physician. It was carried out in the NICU in Mansoura University Children Hospital starting from July 2009 until the end of December 2011. All neonates were subjected to clinical monitoring and laboratory tests for septic work up including complete blood count, blood culture, erythrocyte...
sedimentation rate, CRP, urine analysis, urine culture, chest X ray, cerebrospinal fluid (CSF) analysis and culture. Data were obtained from patient medical records, direct observation as well as laboratory and radiology reports.

Based on clinical suspicion and results of blood culture, the study population were divided into 2 groups: Group I consists of 56 clinically septic neonates with positive blood cultures (proven sepsis) and group II comprised 85 clinically septic neonates with negative blood cultures (clinical sepsis). Exclusion criteria were antibiotic administration therapy prior to admission, birth asphyxia, aspiration syndromes, laboratory finding suggestive of inborn error of metabolism and congenital anomalies. Such conditions increase serum PCT concentrations.

**Eubacterial PCR.**

Bacterial DNA was extracted from 300µl of patient blood specimens using a QIAamp DNA Mini Kit (blood protocol). A 241-bp fragment of the bacterial 16S DNA sequence was amplified using forward primer UNI-1 (5′-GAGGAAGGTGGGGTGAGC-3′) and reverse primer UNI-2 (5′-TGGTGACGCGGCGGAC-3′).

A mastercycler instrument Peltier-Effect cycling MJ Research. Inc was used with the following reaction conditions: 95°C for 15 min, followed by 35 cycles of 95°C for 45 s and 60°C for 30 s and a final extension at 72°C for 2 min (with or without the addition of the target DNA).

**Post-PCR analysis.**

Following PCR amplification, aliquots of amplified (10-µl) samples were electrophoresed through 1.5 high-resolution agarose (Sigma Aldrich) gels, visualized by UV fluorescence after ethidium bromide staining, and compared with 1kb DNA ladder. (Wide range DNA ladder – Biomiga)

**C-reactive protein Testing**

C-reactive protein was analyzed with a turbidimetric assay (CRP Flex reagent cartridge; Dimensionl Dade Behring, Inc., Marburg, Germany) (15)

**Procalcitonin measurement**

Serum samples for PCT measurement were collected then stored at -20°C until analyzed. PCT levels were measured by ELISA assay following the manufacturer’s instructions (Ray Biotech, Inc., USA). Color intensity of the reaction was measured at 450 nm in microplate reader (Bio-Tek Instruments, Winooski,VT, USA). The assay had a detection limit of 0.05 ng/ml and a coefficient variation of <10% intra-assay and <12 % inter-assay.

**Statistical analysis:**

To investigate the discriminative power with which the above mentioned parameters could be distinguished between patients of proven sepsis and those with clinical sepsis, the Roc curves were constructed. The AUCs, which represent the combined measures of sensitivity and specificity of a given parameter, were determined.

Data were analyzed with the aid of computer's SPSS (statistical package for social science) program version 17. And EPI info program version 3.5.1. Mean, median standard deviation (SD), range and percentage were used as descriptive statistics.

**RESULTS**

The data of the 141 included neonates [56 (39.7%) proven sepsis and 85 (60.3%) clinical sepsis] were analyzed. Of these 74 (52.5%) were males and 67 (47.5%) females. The mean age was 11 ± 4.8 days. Mortality was 7.8% (n = 11), which included 8 (14.3%) of proven sepsis and 3 (3.5%) of clinical sepsis group. Among the proven sepsis group, 23 (41%) Gram positive (13 Staphylococcus aureus, 8 CoNS and 2 enterococcus) and 33 (59%) Gram negative bacteria (17 Klebsiella, 4 Acinetobacter, 2 Enterbacter, 2 E.coli, 2 Citrobacter and 2 proteus) were isolated from blood cultures. No organisms detected in CSF or urine cultures.

Eubacterial PCR revealed, (100%) positivity among the 56 proven clinical sepsis by positive blood culture. PCR further detected 6 (7%) cases which were blood culture negative. The sensitivity and specificity were 100% and 93% respectively. (Data shown in table 1 – Figure 1)

Serum median levels of both CRP and PCT in the study groups are presented in figure (2, 3). Within the proven sepsis group, median serum PCT concentration in the group of Gram negative bacteremia was 37 ng/dl (SD 3.5) compared to those of Gram positive bacteremia patients which was 7 ng/dl (SD 2.7) with (P value = 0.04).While, it was 1.3 ng/dl (SD 2.3) and 48 ng/dl (SD 4.1) in survivors and in non survivors respectively (P value =0.017).

The ROC plots for PCT and CRP are shown in figure (4). From ROC curves optimum cutoff values, sensitivity and specificity for PCT and CRP are listed in table (2).
Table (1): Comparison of PCR and culture in diagnosis neonatal sepsis

<table>
<thead>
<tr>
<th>PCR</th>
<th>Blood culture</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
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<tr>
<td>Positive</td>
<td>56</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
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Figure (1): Agarose gel electrophoresis (1.5% agarose with ethidium bromide) showing the results of PCR applied on blood specimens using eubacterial primers targeting 16SrDNA. Lane 1: 1kbp DNA ladder, Lane 2 and 3: blood sample positive results.

Fig. (2) Serum PCT level are presented as box plots (median value and interquartile range). Significant differences in PCT between proven sepsis and clinical sepsis $p < 0.0001$. Line within box: median; edges of box: quartiles (Q1, Q3); whiskers: range of values; open circles: outliers value.
Fig. (3): CRP level are presented as box plots (median value and interquartile range). No significant differences in CRP between proven sepsis and clinical sepsis \( p < 0.0001 \). Line within box: median; edges of box: quartiles (Q1, Q3); whiskers: range of values; open circles: outliers value.

Figure (4): ROC-curves representing the sensitivity and specificity of PCT (solid line, blue) and CRP (dashed line, green) for a diagnosis neonatal septicemia.

Table (2): Diagnostic efficiency parameters of CRP and PCT tests.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CRP assay</th>
<th>PCT assay</th>
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<tbody>
<tr>
<td>Cutoff value</td>
<td>3.5 mg/dl</td>
<td>6.5 ng/ml</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>83.9%</td>
<td>83.9%</td>
</tr>
<tr>
<td>Specificity</td>
<td>81.2%</td>
<td>98.8%</td>
</tr>
<tr>
<td>AUC</td>
<td>0.944 (95% CI: 0.911-0.977)</td>
<td>0.997(95% CI:0.990-1.003)</td>
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</tbody>
</table>

CI: confidence interval
DISCUSSION

The enhanced sensitivity of PCR cause it to be a likely helpful method for diagnosing septicemia. The use of biomarkers as a diagnostic tool for the early discontinuation of empirical antibiotic treatment for infants with suspected sepsis shows a valuable potential, but necessitates additional studies.

In this study, PCR with sensitivity and specificity of 100% and 93% respectively met some of the criteria of an ideal diagnostic test for neonatal sepsis: Furthermore, its short time will allow the clinician to avoid the unnecessary use of antimicrobials. However, it does not provide information about antibiotic drug resistance, the assays are not yet standardized and it is costly. Similar results have been reported by Yadav, et al., and Shang et al. with sensitivity of 100% and specificity of 95.6% and 97.8% respectively.

A major concern about PCR is false positive results due to dead bacterial DNA, so its significance can be enhanced by using it in combination with a biomarker which signifies infection and systemic inflammation.

Finding a single cutoff value of biomarkers of infection will remain a problem since a zone of uncertainty will always found causing lack of clinical relevance of the results.

Determination of a high level of PCT (>6.5 ng/ml) in blood found to be compatible with the diagnosis of neonatal sepsis with sensitivity of 83.4% and specificity of 100%. Analogous to our results, Carrol et al mentioned that PCT yielded a sensitivity of 92.6% and specificity of 100%. A great discrepancy in sensitivity (50 -100%) and specificity (50 – 99%) was detected in numerous studies of PCT testing in neonatal sepsis.

Median PCT concentration was significantly higher in proven sepsis than clinical sepsis group (P= 0.0001). Likewise, several studies had shown a significant increase in neonates with proven sepsis. While, Zahedpasha et al., did not find a significant difference between the studied groups which might be due to small sample size in the carried study.

Procalcitonin levels were significantly higher in Gram-negative sepsis (p=0.04) than in Gram-positive, which is in accordance with a study held by Kyung-Eun Kim and Jin-Yeong Han.

Regarding mortality, a statistical significance was revealed in PCT levels (P= 0.04), suggesting that high levels may affect the survival of neonates with sepsis. However, other authors showed no difference in PCT level between survivors and non survivors.

The median CRP concentration was found to be significantly higher in proven sepsis than clinical sepsis group (P= 0.001). However many findings from different studies ranged widely. Some authors observed much lower sensitivity (60%) and higher specificity (96%) in proven sepsis compared to clinical regarding CRP level. Lower specificity in this study might be due to single measurement of CRP. Sequential testing may improve the diagnostic value of the test.

In the current study, PCT compared with CRP had equivalent sensitivity but better specificity in differentiation of proven and clinical sepsis groups of neonates. This finding gives the advantage of avoiding over diagnosis of neonatal sepsis.

The contemporary data however are not in line with others who found that PCT was not a better marker of infection than CRP and not recommend it for routine use in diagnosis of bacterial sepsis in neonates.

The contradictory study results are not surprising and in part related to differences in the postnatal ages of the neonates studied, differing cutoff points for PCT and CRP and wide variations in study groups (a homogeneous group of neonates were studied with clinically suspected sepsis). In contrast, some authors used a control group formed by asymptomatic infants that may overestimate the reliability of a diagnostic test.

In conclusion, if CRP was speculated useful, the availability of PCR has direct clinical application for rapid, early, and accurate diagnosis of sepsis. Also, PCT determination could be included in clinical prediction rules serves as a point-of-care test to diagnose sepsis, especially in critical settings like the NICU.

To summarize, both PCR and PCT determination may be useful as rapid tests for detecting septicemia holding hope as methods included in the diagnostic guidelines for sepsis and guiding empirical antibiotic therapy for this vulnerable population.

REFERENCES


