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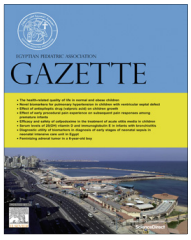

Diagnostic utility of biomarkers in diagnosis of early stages of neonatal sepsis in neonatal intensive care unit in Egypt

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KEYWORDS

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Abstract *Background:* Neonatal sepsis is considered one of the major causes of morbidity and mortality in NICUs. To avoid unnecessary treatment of non-infected neonates, emergence of multidrug resistance organisms, prolonged hospitalization and a considerable economic burden, particularly in developing countries with poorly-equipped NICUs, an early, sensitive and specific laboratory test would be helpful to guide clinicians in neonatal units to decide whether or not to start antibiotics.

Objective: C-reactive protein (CRP), tumor necrosis factor-α (TNF), interleukin-6 (IL-6) and interleukin-1 (IL-1) were measured in an attempt to identify a set of tests which can confirm or refute the diagnosis of neonatal sepsis at an early stage before administration of antibiotics.

Methods: Assessment of serum levels of CRP, TNF-α, IL-6 and IL-1 was done using quantitative enzyme immunoassay sandwich technique in 116 neonates (36 newborns with clinically suspected sepsis, 48 newborns with culture-proven sepsis and 32 infection-free neonates).

Results: The cutoff levels for CRP at >12 mg/l had a sensitivity of 91% and specificity of 100%, for TNF-α at >113.2 ng/ml had a sensitivity of 83% and specificity of 100%, for IL-6 at >16.8 pg/ml had a sensitivity of 100% and specificity of 47%, and for IL-1 at >15 pg/ml had a sensitivity of 100% and specificity of 47% for the diagnosis of infection before antibiotics.

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Conclusion: The area under ROC curve (AUC) of TNF and CRP in the diagnosis of sepsis was superior to determinations of IL-1 and IL-6. From our data analysis and based on our financial backgrounds, we can conclude that abnormal of CRP levels together with immature-to-total neutrophil ratio above 0.2 with or without elevated IL-1, IL-6 or TNF can be used as early markers of sepsis in neonates.

Introduction

Neonatal sepsis is considered one of the major causes of morbidity and mortality in neonatal intensive care units (NICUs), despite major advances in the management of newborn infants.[1] Blood culture has been considered the gold standard diagnostic test but its analysis takes too long time and lacks sensitivity at early stages.[2] It is also thought that total leukocyte count (TLC), total neutrophil count, immature-to-total neutrophil ratio (I/T), and platelet count also failed to reach the appropriate sensitivity and specificity in this disease.[3]

However, rapid diagnosis is still a major challenge in the management of neonatal sepsis especially at early stages due to non-specific clinical signs that may be minimal and resemble those caused by various non-infective conditions and the fact that infection markers showed difficulty to be interpreted during the early neonatal sepsis.[4–6] Hence it is becoming increasingly important to find an early sensitive and specific biochemical test to differentiate sick newborns with or without infection, especially to minimize the empirical use of antibiotics, emergence of multidrug resistance organisms, prolonged hospitalization and a considerable economic burden, particularly in developing countries with poorly-equipped NICUs like Egypt.[7,8]

Finding a reliable laboratory test as a marker for immediate detection of infection with acceptable sensitivity and specificity has always been controversial among investigators. Recently various biochemical markers, for example C-reactive protein (CRP), tumor necrosis factor (TNF-α) and interleukins have been evaluated as potential indicators for early identification of septic infants.[7]

The aim of this study was to evaluate CRP, TNF-α, interleukin-6 (IL-6) and interleukin-1 (IL-1) as potential early diagnostic markers of neonatal infection. We also aimed to determine the specificity and sensitivity of interleukins in early detection of neonatal infection, and suggest cutoff values for studied interleukins in order to detect infections.

Subjects and methods

Subjects

All neonates admitted to the NICU of Cairo University during the period from June 2014 to December 2014, were enrolled in this study. Of 181 eligible infants, 116 were enrolled in the study; 65 infants were excluded because insufficient blood sampling, incomplete documentation, history of perinatal asphyxia, inter-current illnesses, known congenital anomalies, chromosomal abnormalities or inborn errors of metabolism, confirmed intrauterine viral infection and who were already receiving parenteral antibiotic at the time of study.

The study protocol was approved by the Ethical Committee of Cairo University & the Ethical Committee of National Research Center, Cairo, Egypt. A written consent was obtained from parents of neonates included in the study.

Full medical history was obtained from the parents then all neonates are subjected to full clinical examination, especially for the clinical signs of infections (poor peripheral perfusion, capillary refilling time >3 s, hypotension, hypothermia or hyperthermia, poor neonatal reflexes, hypotonia, abdominal distension, tachypnea, increased or decreased heart rate). Routine complete blood count, differential TLC, blood cultures and other relevant cultures were done for all patients at the time of enrollment.

According to results of previous parameters, neonates enrolled in the study were divided into three groups:

1- Culture-proven sepsis group where sepsis was confirmed by a positive blood culture or other relevant cultures accompanied by compatible signs and symptoms.
2- Clinically suspected sepsis group that was defined as clinical symptoms and/or signs suggestive of sepsis and necessitated the start of antibiotic therapy but not confirmed by laboratory tests (negative culture).
3- Control group that includes all infection-free neonates, without clinical findings or maternal risk factors for infection, admitted for minor problems or nursed in the neonatal ward at the same period.

Methods

Adequate venous blood samples were taken from each infant for analysis in the first 6 h of admission and before administration of antibiotics. Blood samples were collected into plain evacuated blood tubes and were allowed to clot for 60 min then centrifuged at 4000 rpm for 10 min. Hemolyzed samples were excluded from analysis. After separation, routine analysis and assessment of CRP were done and aliquots of serum were frozen at -80 °C for TNF-α, IL-6 and IL-1 analysis.

CRP, TNF-α, IL-6 and IL-1 were assessed using Quantikine ELISA kit, R&D, Bio-Techne, Minneapolis, USA.[3]

Statistical analysis

Statistical calculations were done using Statistical Package for the Social Science (SPSS Inc., Chicago, IL, USA) version 16 for Microsoft Windows. Data were statistically presented in terms of mean, standard deviation (SD), and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using one way analysis of variance (ANOVA) test. For comparing categorical data, Chi square test was performed. Exact test was used instead when the expected

frequency is less than 5. Receiver operating characteristics (ROC) curve was made and area under the curve (AUC) was calculated. The optimal cutoff was determined for the variables required. *P* value was considered to be significant if less than 0.05.

Results

- The demographic and clinical characteristics of each group are shown in Table 1.
- The laboratory data showed that septic neonates and neonates with suspected sepsis had significantly elevated levels of CRP, TNF-α, IL-6, IL-1 and when compared to the control group (Table 2).
- On comparing the laboratory data of septic neonates and neonates with suspected sepsis, we found that TLC, I/T ratio, and IL-6 were comparable between both groups ($P > 0.05$). CRP and TNF were significantly higher and PLT and IL-1 were lower in septic group when compared to neonates with suspected sepsis (Table 3).
- The sensitivity of IL-1 and IL-6 was 100% with specificity below 50% and their AUC was significant in diagnosing sepsis. The specificity of TNF and CRP in the diagnosis of sepsis and their AUC were superior to determinations of IL-1 and IL-6 (Table 4 and Fig. 1).

Discussion

Neonatal sepsis is considered one of the major causes of morbidity and mortality in NICUs. To avoid unnecessary treatment of non-infected neonates, emergence of multidrug resistance organisms, prolonged hospitalization and a considerable economic burden, particularly in developing countries with poorly-equipped NICUs, an early, sensitive and specific laboratory test would be helpful to guide clinicians in neonatal units to decide whether or not to start antibiotics.

This study was done for a period of six months in NICU in Cairo University which considered the biggest unit in Egypt. We have assessed hematological data and routine laboratory

Table 1 Neonatal characteristics of the studied groups.

Variables	Culture-proven sepsis group ($n = 48$)	Suspicion group ($n = 36$)	Control group ($n = 32$)
Sex (n, %):			
Males	44 (91.7%)	36 (100%)	12 (37.5%)
Females	4 (8.3%)	0	20 (62.5%)
Gestational age (weeks):			
Median (range)	38 (33–40)	34 (32–37)	35 (31–38)
Birth weight (kg):			
Median (range)	2.9 (1.3–3.6)	1.9 (1.45–2.56)	1.9 (1.3–3.5)
Postnatal age:			
Median (range)	11.5 (2–28)	11 (10–25)	6.5 (4–25)

Table 2 Laboratory characteristics of the studied groups.

Variables	Culture-proven sepsis group ($n = 48$)		Sepsis suspected group ($n = 36$)		Control group ($n = 32$)		*P*-value
	Median	Range	Median	Range	Median	Range	
CRP (mg/l)	48	12–192	12	12–12	3	3.0–4.0	0.001
TNF-α (ng/ml)	153.3	105–120	107.9	97–113	12.6	11–21	0.001
IL-6 (pg/ml)	36.0	27.1–45.9	37.9	35.2–43.3	8.5	3.6–16.8	0.001
IL-1 (pg/ml)	26.0	21.4–41.7	30	26.4–42.7	12.0	11.3–15.0	0.001

Table 3 Laboratory characteristics of septic neonates and suspicious neonates.

Variables	Culture-proven sepsis group ($n = 48$)		Suspicion group ($n = 36$)		*P* value
	Median	Range	Median	Range	
TLC ($\times 10^3$/ccm)	9.6	1.7–28.9	8.5	5.4–11.2	0.192
I/T ratio	0.33	0.25–0.77	0.35	0.25–0.45	0.069
Platelet ($\times 10^3$/ccm)	95.0	10–200	165	130–336	0.001
CRP (ng/ml)	48	12–192	12	12–12	0.001
IL-1 (pg/ml)	26.0	21.4–41.7	30	26.4–42.7	0.001
IL-6 (pg/ml)	36.0	27.1–45.9	37.9	35.2–43.3	0.141
TNF-α (ng/ml)	153.3	105–120	107.9	97–113	0.001

Table 4 Comparison of the AUC of CRP, IL-1, IL-6 and TNF in diagnosing sepsis with the sensitivity and specificity at their optimum cutoff level.

Variable	Cutoff	Sensitivity	Specificity	AUC	SE	95% CI
CRP (mg/l)	> 12	91.7	100	0.978	0.0110	0.932–0.996
IL-6 (pg/ml)	> 16.8	100	47.06	0.686	0.0494	0.593–0.769
IL-1 (pg/ml)	> 15.04	100	47.06	0.642	0.0526	0.547–0.728
TNF-α (ng/ml)	> 113.28	83.33	100	0.967	0.0145	0.916–0.991

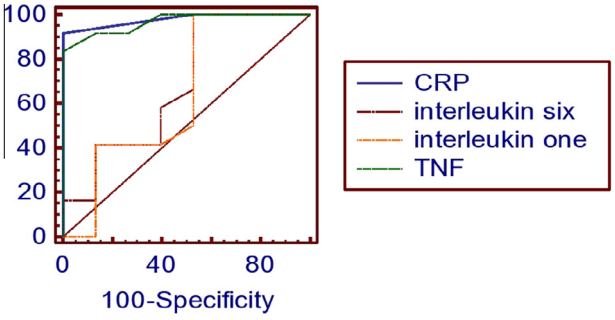

Figure 1 ROC curves of the AUC of CRP, IL-1, IL-6 and TNF in diagnosing sepsis.

tests. We have also estimated TNF-α, CRP, IL-6 and IL-1 in the serum of the infected, clinically suspected sepsis and control neonates within 6 h of admission and before administration of antibiotics.

We demonstrated that septic neonates and neonates with clinically suspected sepsis had significantly elevated levels of CRP. This was in line with the previous studies,[3,11] and this means that this parameter can differentiate healthy infants from those with proven or suspected sepsis.

In addition, CRP was significantly higher in septic group when compared to neonates with suspected sepsis. This means that this parameter was affected in suspected sepsis group and differentiates them from healthy neonates and progressively increases through the course of illness reaching significantly higher levels when sepsis is proven. Our data confirmed the previous reports on the advantage of repeated CRP measurement as a diagnostic tool in neonatal sepsis.[5,12] One study looked at whether the time from onset of fever at which CRP was measured affected its sensitivity or specificity. They found no significant difference in sensitivity or specificity between CRP values collected before or after 12 h from the onset of fever.[13] However, some studies reported that the optimum sensitivity and specificity for CRP were obtained during the window of 24–48 h after the onset of symptoms.[14] Some studies also suggested that serial measurements of CRP over a period of 2–3 days after onset clinical symptom, using varying cutoff values, improved the diagnostic performance of CRP.[15,16] However, another study indicated that measuring CRP levels is not efficient for the early diagnosis of infections in infants.[17] There are several studies which have used different cutoff values for CRP ranging from 4.8 to 70 mg/l and have reported that the sensitivity of CRP for identifying neonatal infection ranges from 63% to 95%, and specificity ranges from 40% to 97%.[18,19] In our study, CRP $\geqslant$12 mg/l was found to be the most appropriate cutoff value by using ROC curves. However, low test sensitivity and low negative predictive values for CRP in many studies have led them to think that this test alone will not be sufficient in the early diagnosis of neonatal sepsis.[20] From the available study, there seems to be appropriate recommendation for the use of CRP in sepsis patients. High CRP does suggest the presence of neonatal sepsis but must be used together with other markers to inform clinical decision-making on a case-by-case basis.

In our study, we reported increased levels of TNF-α in both culture proven sepsis group and clinically suspected sepsis group compared to control group. Levels of TNF-α were also higher in septic group when compared to neonates with suspected sepsis. This also means that this parameter progressively increases through the course of illness reaching significantly higher levels when sepsis is proven. However, publishing data regarding TNF levels in neonatal sepsis is also divergent. Some studies reported significant increase of this inflammatory mediator[3,7] while others demonstrated comparable or even lower levels in infected newborns compared to healthy newborns.[21,22] Prashant et al. reported increase in TNF levels in infected and clinically suspected sepsis neonates compared to healthy subjects although levels in infected and clinically suspected sepsis neonates were comparable.[7] We reported high sensitivity (83.33%) and high specificity (100%) of TNF in diagnosis of early stages of neonatal sepsis at concentration > 113.28 ng/ml. However, publishing studies reported that the sensitivity of TNF ranged from 20.8% to 100% and specificity ranged from 43.1% to 100%.[23]

IL-6 is an important cytokine of the early response to infection. Previous studies have shown IL-6 to be a useful marker of early infection in the newborn.[3,24] In our study, the serum IL-6 levels were significantly increased in newborns with sepsis compared with control and were comparable between septic group and suspected group. Kantar et al. showed that septic preterm newborns had significantly elevated IL-6 levels at the onset of sepsis as compared to the recovery period and the controls.[24] This supports findings reported by Magudumana et al. who mentioned that there is no benefit in serial determination of IL-6 in the diagnosis of neonatal sepsis.[25] Our results showed that IL-6 shows 100% sensitivity and low specificity at 16.8 pg/ml cutoff value. A value of 10 mg/l is the most commonly used cutoff in most published studies.[20] The cutoff values obtained by other investigators who measured IL-6 in neonatal sepsis ranged between 3.6 and 500 pg/ml with mean cutoff value = 76.49 pg/ml and median 30 pg/ml. The mean sensitivity was 77.87% and specificity was 78.61%, at 76.49 pg/ml cutoff value.[14] Although IL-6 is considered one of early markers in neonatal sepsis, some factors do affect its sensitivity and specificity to be widely used in neonatal diagnosis.[26] Its concentration increases sharply after exposure to bacterial infection and it even precedes the increase in CRP but it has a very short half-life, and its concentration falls

dramatically with treatment and becomes undetectable within 24 h. In addition, it was reported that level of IL-6 shows natural fluctuations immediately after the postnatal period and its serum level is affected by gestational age and perinatal complications other than infection.[26,27]

In the other side, IL-1 levels were higher in proven infected neonates and suspected sepsis group compared to control subjects and its levels also showed significant increase in suspected sepsis group compared to proven infected neonates. In the current study, IL-1 shows 100% sensitivity and low specificity at 15 pg/ml cutoff value. Results of different published studies regarding this cytokine are contradictory. Santa et al. reported that IL-1 showed increase in neonatal sepsis and its detection sensitivity and specificity were 60% and 87% respectively.[28] Kurt et al. reported that IL-1 levels in culture positive neonatal sepsis at time of diagnosis were significantly higher compared to healthy subjects and after seven days of treatment.[3] Ucar et al. reported no increase in IL-1 in septic neonates compared with healthy neonates. They explained this finding that the monocytes of newborn infants may be unable to secrete adequate IL-1.[29] However, the previous finding is supported by finding reported in a study done by Atici et al. who reported that IL-1 levels were found to be lower in neonates with sepsis than in healthy controls.[30]

However, reports in the literature on the use of CRP, TNF, IL-6 and IL-1 as early markers of neonatal sepsis are contradictory. Variations in study design, definition of neonatal sepsis, sample size, postnatal age, gestational age, risk factors, inclusion criteria of patients, cutoff points of the markers, test methodology, data analysis and reporting of results lead to difficulties in comparing studies.[14,28,30,31] Thus, it is often difficult to formulate a definitive opinion on the clinical usefulness of infection markers from the published reports.

Our study confirmed previous findings that neonates with bacterial sepsis have reduced platelet count and high I/T ratio.[9,10,29] TLC was comparable between studied groups and this is in agreement with Ucar et al.[29] In our study, I/T ratio, was comparable between septic neonates and neonates with clinically suspected sepsis, which means that abnormal I/T ratio may occur earlier in cases with suspected sepsis and reach levels comparable to those with proven sepsis. This confirmed the previous report of I/T ratio >0.2 as a useful marker of infection.[32] However, hematological parameters have shown significant heterogeneity among many studies. Da Silva et al. reported that the possible sources of heterogeneity were gestational age of subjects, methodological quality, different reference values, different cutoff values and analysis of test results by different laboratory observers.[33]

While evaluating the findings obtained from this study, methodological limitations such as the small sample size and the absence of follow-up for septic and clinically suspected septic neonates must be taken into account. However, findings that were obtained from this study were strengthened by the following factors: the presence of positive blood cultures in all of the septic neonates; taking newborns at risk of neonatal sepsis development and taking healthy newborns as control group; the exclusion of neonates who were either delivered by a mother that was using antibiotics or had used antibiotics before.

At the end, our data analysis demonstrated that AUC of TNF and CRP in the diagnosis of sepsis were superior to determinations of IL-1 and IL-6. From our data analysis and based on our financial backgrounds, we can conclude that abnormal of CRP levels together with I/T ratio above 0.2 with or without elevated IL-1, IL-6 or TNF can be used as early markers of sepsis in neonates who are clinically suspicious and could be used as an indication of starting antibiotic therapy, in spite of negative or unavailable cultures at our NICU.

Conflict of interest

There is no conflict of interest.

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