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RESEARCH ARTICLE

Role of procalcitonin and TNF α as a marker for early detection of neonatal sepsis in Taif, Saudi Arabia

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Abstract

Objectives: A rapid test with the best degree of diagnostic efficiency is required for the early diagnosis and treatment of neonatal sepsis. So this study aimed to evaluate serum procalcitonin (PCT) and TNFα as a diagnostic marker of neonatal sepsis, and comparetheir diagnostic efficiency with C-reactive protein (CRP).

Design and methods: 240 neonates were included and classified into three groups. The patients groups were: Proven septic, suspected septic and control healthy neonates, classified depending on Tollner score.CRP,TNFα and PCT values were analyzed, compared, and their effectiveness as diagnostic markers was determined by using receiver operating characteristic (ROC) curve analysis. Sensitivity, specificity, positive, and negative predictive values of the markerswere also calculated.

Results: significant elevation was detected in serum levels of CRP, PCT and TNFα in both suspected and proved groups when compared with the control group. Also, higher specificity and sensitivity values were detected for PCT and TNFα (PCT: 90%,67& TNFα: 90%, 63 respectively) in comparison with CRP sensitivity of 70% and specificity of 60%. The positive and negative predictive values were higher for PCT followed by TNFα then that for CRP.

Conclusion: PCT and TNFα are the best markers in the diagnosis of neonatal sepsis, and these markers are also could be valuable in following the effectiveness of treatment and determining the prognosis of the disease. The experiments results need to be further studied and the clinical validation is also needed in different region.

INTRODUCTION

Neonatal sepsis (NS)is a common cause of morbidity and mortality in neonates. Rapid diagnosis and treatment of systemic bacterial infection is essential in neonate and infants, since a delay in treatment of severe bacterial infection may lead to improper outcome [1]**.**The clinical signs of NS are nonspecific and indistinguishable from those of noninfectious disorder. Due to non-specific signs and symptoms of sepsis, the diagnosis of NS is quite difficult and

can be misleading because critically ill neonates often manifest systemicinflammatory response syndrome without infection [2]**.**

An early detection and specific clinical intervention has been shown to be crucial for the improved outcome of patients with sepsis [3, 4]**.**During the first hours of life, reliable infection markers are absent in NS.Therefore, neonatologists often begin early antibiotic treatment in newborn infants with risk factors for infection, exposing many neonates to unnecessary treatments. Due to the limitation of the treatment strategy in early diagnosis of sepsis, the isolation of causative organisms from microbiological cultures takes up to 72 h, which cannot be used to identify most infected infants [5]**.**

C- reactive protein (CRP) is a good marker for diagnosis of NS. Elevated CRP levels are seen in infection, in autoimmune disease, in surgery, meconium aspiration and recent vaccination. Also, the CRP values do not rise significantly until almost 14-48 hr after the onset of infection [6].

Indeed, bacteriologic results need time and may be negative in newborns. Besides, it is impractical to obtain blood sample for serial blood culture from infants [6]**.** So, new laboratory methods for early diagnosis of the diseases, evaluation of prognosis and treatment efficiency are needed.

Recently, many studies have found that some new markers also play important roles in the diagnosis of neonatal sepsis. However, the systematic research and comparison of these biomarkers for diagnosing NS are limited [7]**.**So, this study aimed to investigate the value of PCT and TNFα, in comparison to CRP in establishing the early diagnosis of neonatal sepsis.

Subjects and methods

In a case control study, 240neonates who admitted to the Neonatal Intensive Care Units (NICU) atKing Abdul Aziz Hospital, Taif; KSA (from October 2014 to August 2015) were included. The study protocolwas approved by the ethical committee of Faculty of Medicine, Taif University.

We targeted >4-day-old and <7-days-old patients with suspected bacterial infection.The diagnosis of NS was made according to the presence of the following parameters on clinical, laboratory, or culture screen: (1) clinical signs consistent with infection based on the Tollner score[8]**;** (2) CRP values of ≥1.0 mg/dl; (3) positive culture (blood, urine, and cerebrospinal fluid) or pneumonia (chest X-ray findings). The Tollner scoring system uses a range of clinical (change in skin color, peripheral circulation impairment, hypotonia, bradycardia, apnea, respiratory distress, hepatomegaly, abdominal distension) and laboratory (leukocyte count, left shift, thrombocytopenia, metabolic acidosis) parameters. Points are given for each parameter: 0, 1, 2, or 3; a higher number of points reflect a greater severity.

According to this scoring system, a score of ≥10 indicates clinical sepsis, a score of ≤5 indicates no sepsis, and a score of 5–10 indicates possible sepsis. Gestational age, birth weight, history of intrapartum maternal antibiotic administration, maternal fever (≥ 38^{o}C), prolonged rupture of membranes> 18 h, and clinical chorioamnionitis were recorded. The study was approved by the local ethics committee. Both verbal and written informed consent was given by the parents.

Three groups of neonates were investigated;Group(1)included 80 neonates diagnosed as proved sepsis, based on clinical and laboratory findings (a Tollner score of ≥10).Group (2) included 100 neonates diagnosed as suspected sepsis based on a Tollner score of (5-10). Every effort will be done to identify patients with suspected infection as early as possible after admission.Group(3): control group included 60 healthy neonates, with no clinical or biological data of infection, no clinical signs of sepsis during the first week of life, and who were seen to be healthy based on a Tollner score of ≤ 5, will be recruited for this study.

Exclusion criteria were history of giving antibiotics to the mother or the newborn, congenital malformations, congenital infections associated with the TORCH complex, and refusal of parental consent. Where there was a history of maternal antibiotic administration, the neonate was excluded from the study.

2. Procedures:

Written informed consent was obtained from their relatives before enrollment.Clinical assessment was the first step in diagnosing infection. Data collected for each patient included; age, sex and clinical diagnosis. Temperature, heart rate, blood pressure, central venous pressure and respiratory parameters will be recorded.Laboratory tests including; leukocytic count, C-reactive protein,platelet count and the immature to total neutrophil ratio, arterial blood-gas analysis. Routine blood cultures and culture of other specimens from suspected sites, as clinically indicated, will be performed.

Before initiation of antibiotic therapy in infants suspected with sepsis, blood samples for blood culture (1-2 ml), also (1-2 ml) were obtained by peripheral venous puncture for measurements of PCT (procalcitonin) , TNFα (tumor necrosis factor- alpha) and CRP (C- reactive protein)

Blood samples were centrifuged within 30 min ofcollection, and the serum was stored at -20˚C until analysis. PCTwas measured by a specific immunoluminometric (Lumitest PCT, BrahmDiagnostica GmbH, Berlin, Germany). We used latex agglutination test for CRP and Elisa technique for TNF-α.

Data analysis: Data will be analyzed with SPSS version 15.0 (statistical package for the Social Science, Chicago, IL).The results of continuous variables will be expressed as means ± SD. To compare means of the variables, one-way ANOVA test was done by SPSS followed by Tukey post-hoc test for multiple comparisons.

Diagnostic efficiency was defined as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). To determine the diagnostic usefulness of PCT, TNFα and CRP levels, we analyzed the receiver operating characteristic (ROC) curve and calculate the sensitivities, specificities, and positive and negative predictive values. A P-value< 0.05 was considered as significant.

Results:

In this study, 80 neonates with positive blood cultures and clinical sepsis (group 1 or proved group), 100 suspected sepsis neonates (group 2) and 60 healthy neonates (group 3) were enrolled.The Characteristics of the proven, suspected sepsis and control groups are shown in (Table 1).Means of patients BW of all groups were 2339±401, 2358±520, 2715±237g respectively and GAs were 31.55±2.29, 33.9±2.27 and 35.38±4.69 respectively. BW and GA were not significantly different between the groups (table 1). A statistical significant difference was observed between the mean of Apgar score in proved and suspected groups in comparison to control group.

Blood cultures were positive for all patients in group (1). The identified bacteria (table 2) included Staphylococcus aureus (n= 18), coagulase negative Staphylococcus (n= 15), Streptococcus viridans (n= 12), Entrobacter (n= 5), Escherichia coli (n= 3) and Pseudomonas aeruginosa (n= 2).

The mean of CRP, PCT and TNFα in studied groups are shown in (Table 3). There wassignificant difference between the mean of CRP, TNFαand PCT levels in proved septic and suspected groups when comparedwith healthy controls (*P*< 0.05). Also, a significant difference was observed between septic and suspected newborns (*P*< 0.0001).

ROC curve analysis was performed to determine the diagnostic usefulness of PCT and TNFα compared with CRP for earlydetection of neonatal sepsis. In the ROC curve, the area under the curve (AUC) was highest for TNFα (0.83) with a significant p value of 0.002, followed by PCT (0.72) with a significant p value of 0.034.The lowest AUC was detected for CRP (0.43) with non significant P value of 0.512.

CRP is less useful in diagnosis of neonatal sepsis compared to PCT which has the highest sensitivity and specificity (90%, 67% respectively) followed by TNFα with sensitivity and specificity (90%, 63% respectively). The lowest sensitivity and specificity values were detected for CRP (70%, 60% respectively). Moreover, the most sensitive diagnostic cutoff values were 9 mg/L for CRP and 28.4 pg/ml for TNFα and 5.6 ng/ml for PCT. The positive and negative predictive rates were the lowest in CRP (28% and 80% respectively), whereas the positive and negative predictive rate showed high result in case of TNFα and PCT (TNFα positive predictive and negative predictive values were 45% and 95% respectively, while PCT positive predictive and negative predictive values were 53% and 95% respectively).

Table (1): Demographic and clinical characteristics of the study neonates:

	Proved sepsis (n= 80)	Suspected sepsis (n=100)	Control (n= 60)
Sexn(boys: girls)	54:26	55:45	35:25
Mode of delivery n(VD: CS)	30:50	55:45	30:30
Gestational age (weeks)	31.55±2.29	33.9±2.27	35.38±4.69
Birth weight (g) mean ±SD	2339±401	2358±520	2715±237
Apgar score 1***** mean ±SD	6.34±2.3[a]	6.91±0.8[a]	8.3±1.3
Apgar score 5***** mean ±SD	7.70±0.95[a]	8.37±0.92[a]	9.2±0.78
Tollnerscorerange	≥10	5-10	1-4

*****The Apgar score is used as an indicator of the infant's condition in the first and fifth minutes after birth that include: appearance, heart rate, muscle tone, respiratory effort.

Data are presented as means±SEM; n= number of rats in each group. Statistical analysis was done using one-way ANOVA followed by Tukey post-hoc test for multiple comparisons.

[a]significant with control p< 0.05. VD: vaginal delivery, CS: caesarian section

Table (2): Laboratory findings of the studied groups:

	Proved sepsis (n= 80)	Suspected sepsis (n= 100)	Control (n= 60)
Platelet count/mm3 mean ±SD	483124 ± 110205[a]	410560±140000	375890±99800
Leukocyte count/ mm3 (mean ±SD)	18994.4 ± 9289[a]	111384±2350 [a]	8910±2.56
Blood culture			
Coagulase-negative staphylococci	15		
Staphylococcus aureus	18		
Klebsiella pneumonia	10		
Acinetobacter SPP	4		
Pseudomonas aeruginosa	2		
Enterobacter cloaca	5		
Candida tropicalis	1		
Streptococcus viridans	12		
E-Coli	3		
Hemophilis influenza	9		

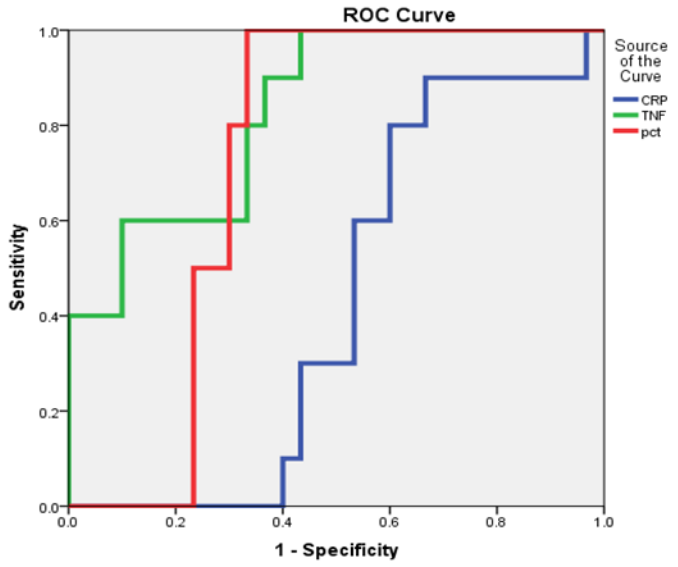
Statistical analysis was done using one-way ANOVA followed by Tukey post-hoc test for multiple comparisons. [a]significant with control p< 0.05**.**

Table (3): comparison between mean± SD of serum levels of CRP and PCT and TNFαin studied groups

Variable	Proved (n=80)	Suspected (n=100)	Control (n= 60)	F test	P value
	Mean ± SD	Mean ± SD	Mean ± SD		
CRP (mg/ml)	40.33± 17.85	`10.63± 4.22	2.76± 1.18	47.4	<0.0001
TNF(pg/ml)	31.97±5.01	28.22±7.75	22.82±3.50	3.2	<0.001
PCT (ng/ml)	10.50±2.71	5.71±2.17	0.84±0.34	54.9	<0.001

Data are presented as means ± SD; n= number of rats in each group. Statistical analysis was done using one-way ANOVA followed by Tukey post-hoc test for multiple comparisons.

Table (4) and figure: Comparison of the receiver operating characteristic (ROC) curves of C-reactive protein (CRP), procalcitonin (PCT) and tumour necrosis factor (TNFα) in suspected neonates (group 2). The area under the curve (AUC) was highest for TNFα (0.83) with a significant p value of 0.002 followed by PCT (0.72) with a significant p value of 0.034.



Screening tests	AUC (95%CI)	P value
CRP	0.43 (0.25 – 0.60)	0.512
TNFα	0.83 (0.70 – 0.97)	0.002
PCT	0.72 (0.57 – 0.88)	0.034

Table (5): Sensitivity, specificity, positive predictive value, and negative predictive value of CRP, TNFα and PCT in diagnosis of neonatal sepsis in suspected neonates (group2).

Accuracy measures	**CRP**	**TNFα**	**PCT**
sensitivity	70%	90%	90%
specificity	60%	63%	67%
cut off point	9	28.4	5.6
+ve predictive value	28%	45%	53%
-ve predictive value	80%	95%	95%

Discussion

Early diagnosis of neonatal sepsis and appropriate antibiotic treatment can be lifesaving and cost effective. At early onset, the clinical symptoms are non-specific and difficult to differentiate from transient problems with extra uterine adaptation [9]**.**Therefore, a rapid test with the best degree of sensitivity, reliability, and predictability is required for the early diagnosis and treatment of neonatal sepsis [10].

The ideal early diagnostic test for infectionwould have 100% sensitivity (all patients withthe disease are always detected by the test) and100% specificity (all patients without the diseasehave a negative test result). However, such anideal test is unlikely ever to be discovered, asmost tests that measured on a continuous scale have an overlap between infected andnon-infected infants. Even if the test is not 100%specific, as long as the specificity is not too low, itwill allow some reduction in antibiotic prescriptions [11]**.**

Multiple studies have examined total leucocytic count, immature to total neutrophil ratio, platelet count, and CRP, and shown that these routine investigations either have low sensitivity and specificity or varying delayed responses early in the course of infection [12].

In the present study we assessed the value of PCT and TNFα in comparison to CRP for the diagnosis of neonatal sepsis at early stage. In consistence with others [13,14], we reported significant increase in the serum levels of CRP in proved and suspected groups. CRP at a level 9 mg/l was found to be the most appropriate cut-off value by using (ROC) curves, and at this cut-off value, test sensitivity was 70%, specificity was 60%, PPV was 28% and NPV was 80%.In other study[14]with cut-off value of 12 mg/l for CRP, the range of reported statistical outcomes is as follows:

sensitivity 68.18–85.28%; specificity 67.23–87.76%; positive predictive accuracy about 80%; and negative predictive accuracy about 81%**.**

CRP is most commonly used in all hospitals during follow-up and diagnosis. Although CRP levels can be obtained easily and rapidly through an automatic method, and has a high sensitivity, its specificity is low, making it difficult to diagnose sepsis [15].The increase in the serum concentration of CRP is rather slow during the first 24-48 hrsof infection and this may negatively affect the sensitivity of the test. In addition, increase in CRP concentration in non-infected clinical conditions such as meconium aspiration, prolong rupture of membranes are thought to affect the specificity of the test [16]**.**CRP estimation does have a role in the diagnosis of neonatal sepsis but the test is not specific enough to be relied upon as the only indicator[17]**.**

Our results suggest that the best cutoff value for diagnosing neonatal sepsis is 5.6ng/ml for PCT and 28.4pg/ml for TNFα. PCT had a sensitivity of 90%, specificity of 67%, PPV of 53% and NPV of 95% while TNFα had a sensitivity of 90%, specificity of 63%, PPV of 45% and NPV of 95% in comparison to CRP which had 70% sensitivity and 60%specificity.

PCT is an important acute phase reactantproduced by monocytes and hepatocytes which begin to risefour hours after exposure to bacterial endotoxin, peaking atsix to eight hours, and remain for at least 24 hourswith a half-life of 25–30 hours. Several studies have shownthat serum PCT concentrations increase appreciablyin systemic bacterial infection, necrotizing enterocolitis, andduring both early and late onset neonatal sepsis [18].It may besuperior to other acute phase proteins, with sensitivity andspecificity ranging from 87% to 100%. It may be useful inassessing the severity of infection, following the progress oftreatment, and predicting outcomes [19]**.**

Previous studies have reported the sensitivity and specificity of PCT as follows: 66.7%, 94.4% [20]**;** 75% and 59% [**21]**;66.7% and 50%; [22]**;** 88.9% and 65.2% [**23]**respectively. These results suggested that PCT is a better clinical marker than CRP—although it is associated with a more expensive cost**.**

In the research of chin et al. [24]**,** sensitivity of 69.5% and specificity of 64.5% for PCT were obtained, compared to 67.25% of sensitivity and 93.9% of specificity for CRP. **Chiesa et al [**25] reported that increment of serum PCT due to prenatal events other than infection was more less than the PCT response against infection.

Adib et al[26] found 75% sensitivity, 80% specificity 80%, positive predictive value and 75% negative predictive value for PCT as a marker for the early diagnosis of neonatal sepsis. Also, the results of study by **Khoshdel et al [**27] in Iran showed that sensitivity, specificity, and positive and negative predictive values of PCT level for neonatal sepsis were 87.5%, 87.4%, 30.4%, and 99.1% respectively. The study of **Zahedpasha et al [**28] in Iran showed that PCT levels were remarkably high in neonates with proven sepsis and the levels dropped dramatically after treatment with antibiotics.

Although CRP level is widely used as an indicator of acute infection, increased CRP levels after the beginning of inflammation is slower than that in PCT levels. PCT is more specific than other markers and is detected faster than CRP. Comparatively, this difference can be attributed to that with the beginning of inflammation;CRP starting to increase 4–6 hours later than PCT, and reaching its peak about 36 hours later [29]**.** Further, although PCT decreases within 24–48 hours later after antibiotics are initiated and its level returns to normal 5 days after, CRP levels remains high for at least 24–48 hours, and then decline 5 days later despite treatment with antibiotics [**30].** Therefore, PCT is considered as a more useful indicator for diagnosing early-onset sepsis in newborns because it increases earlier within 12 hours of life than either CRP or IL-6 levels, and it may also be useful for follow-up examination.However, PCT is not areadily available diagnostic assay in most institutions.Although, some investigators [31,32]reported some difficulties ininterpreting PCT values in the newborn period,**Çelik et al., [**33]confirmed that PCT is still a valuable tool in the diagnosis of early onset sepsis.

TNF-α is a cytokine involved in systemic inflammation, which belongs to a member of a group of cytokines that stimulate the acute phase reaction [34].

In our study, TNFα has sensitivity of 90% and specificity of 60% at cut off value 28.4pg/ml. In consistence with others [9,35], we reported significant elevation in serum level of TNFα in proved septic and suspected neonates when compared with control. In this context, other investigators [36] reportedthat, TNFα were produced rapidly in the early course of neonatal sepsis and peaked on day zero, but their half-life were short and could fall back to its baseline value within 24 hours. This specific property of TNFα rendered it useful as a very early alarm hormone, but could not use alone for the diagnosis of infection, because in most circumstances it was uncertain at which stage of infection blood sample was taken for TNFα determination.**Bokun et al. [**37]recordedTNFα sensitivity ranged from 20.8% to 100%, specificity ranged from 43.1% to 100%**.** In particular, TNFα shows a higher accuracy for the diagnosis of NS and also shows regional variation with higher recorded sensitivity and specificity in northern hemisphere.

Considering the high mortality and potential morbidity associated with neonatal sepsis, diagnostic tests with high sensitivity and negative predictive value are most desirable because all septic infants have to be identified [38]. So, TNFα is good marker in the diagnosis of early neonatal sepsis[37]better than CRP as illustrated by **Zaharie et al[**39]**.**

In summary, our results detected higher sensitivity, specificity and positive and negative predictive values for PCT and TNFα more than that for CRP which shows the moderate accuracy inthe diagnosis of NS.We conclude that PCT and TNFα are the best markers in the diagnosis of neonatal sepsis, and these markers are also could be valuable in following the effectiveness of treatment and determining the prognosis of the disease.The experiments results need to be further studied and the clinical validations also needed in different region.

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