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Procalcitonin as a diagnostic marker for neonatal sepsis

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Abstract

Background: Sepsis is the most common cause of neonatal mortality. Isolation of bacteria from blood is the most specific and standard method used to diagnose neonatal sepsis. The drawback of culture-based diagnosis is the 24–48 hour assay time. Procalcitonin (PCT) has high specificity and positive predictive values. Thus, the current study was undertaken to investigate the released serum-procalcitonin as a biomarker for predicting neonatal sepsis among newly born infants. **Subjects and Methods:** A case-control study design was set to perform the underlying work. Forty-five neonates were included in this study. Blood samples were collected and processed for laboratory investigations and culture. Laboratory investigations included complete blood picture, blood culture and PCT. **Results:** Positive blood cultures were detected in all cases of the confirmed sepsis group (100%) and 66.66% of cases of the suspected sepsis group. The ROC curve analysis, used to evaluate the diagnostic value of serum procalcitonin depending on the area under the curves (AUC), showed a very high AUC (91.7%) corresponds to a better diagnostic test. The optimum cut off value of PCT (<0.05 ng/ml) was with a corresponding sensitivity of 100% and specificity of 83.33%, a positive predictive value (100%), and a negative predictive value (74.99%). **Conclusion:** The current findings showed high sensitivity and specificity of the PCT test for diagnosis of neonatal sepsis. The results so far are very encouraging and may suggest that shortly the PCT may be used routinely for diagnosis of neonatal sepsis and can provide a life-saving approach for newborns.

Keywords: Sepsis, biomarker, Procalcitonin (PCT), neonatal sepsis

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Introduction

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection and is accompanied by bacteremia in the first month of life.⁽¹⁾ Sepsis is the most common cause of neonatal mortality and is responsible for 30-50% of total neonatal deaths, each year in developing countries.⁽²⁻⁴⁾ The term neonatal sepsis refers to the systemic infection of neonates including septicemia, pneumonia, meningitis, arthritis, osteomyelitis, and urinary tract infection (Indian Council of Medical Research, 2003; Sankar et al., 2008)^(4,5).

National Neonatal Forum of India⁽⁴⁾ has defined neonatal sepsis as culture-negative or probable (clinical) sepsis when an infant having a clinical picture suggestive of septicemia and culture-positive sepsis when an infant has a clinical picture suggestive of septicemia, pneumonia or meningitis. Neonatal sepsis has been classified into early-onset (EOS) and late-onset (LOS) sepsis. EOS has onset <72 hours. LOS usually appears after 72 hr of age.⁽⁶⁾ The manifestation of neonatal sepsis is vague and ill-defined. The Apgar score provides a convenient shorthand for reporting the status of neonate infant and the response to resuscitation. The Apgar score, the very first test given to a newborn, is performed in the delivery or birthing room right after the baby's birth. The test was designed to quickly evaluate a newborn's physical condition and to see if there's an immediate need for extra medical or emergency care. It refers to as an acronym for **A**ppearance, **P**ulse, **G**rimace, **A**ctivity, and **R**espiration. The Apgar test is usually given to a baby twice: once at 1 minute after birth, and again at 5 minutes after birth. Sometimes, if there are concerns about the baby's condition or the score at 5 minutes is low, the

test may be scored for a third time at 10 minutes after birth.⁽⁷⁾

Isolation of bacteria from blood is the most specific and standard method used to diagnose neonatal sepsis. The drawback of culture-based diagnosis is the 24–48 hour assay time. Newer diagnostic tests are grouped into acute phase reactants; cell surface markers; granulocyte colony-stimulating factor; cytokines; molecular genetics; and molecular cell proteomics.⁽⁸⁾ Acute phase reactants are groups of endogenous peptides produced by the liver as part of an immediate response to the infection or tissue injury. These reactants are; fibronectin, haptoglobin, lactoferrin, neopterin oromucosoid, C-reactive protein and procalcitonin.⁽⁹⁾

Procalcitonin (PCT) is produced by monocytes and hepatocytes. It begins to rise four hours after exposure to bacterial endotoxin, reaches its peak after six to eight hours, and remains raised for at least 24 hours, with a half-life of 25–30 hours. Procalcitonin (2.3 ng/ml) has high specificity and positive predictive values (97% and 96%, respectively), though with low sensitivity (48%) for sepsis diagnosis. It was reported that procalcitonin >2.3 ng/ml indicates a high likelihood for neonatal sepsis, and antibiotic therapy should be continued even in the presence of sterile cultures. However, it is not a readily available diagnostic assay in most institutions.⁽¹⁰⁾ The study aimed to monitor the released serum-procalcitonin as a biomarker for predicting neonatal sepsis among newly born infants examined at the hospital of Medical Research Institute, University of Alexandria, Egypt, delivered from June to December 2017.

Subjects and Methods

1. Subjects. Forty-five neonates were included in this study and were divided into three groups as follows: **Group I:** (suspected sepsis) involved 15 neonates with symptoms and signs suggestive of sepsis or at high risk of infection; **Group II:** (proven sepsis) included 15 neonates who are septic with a clinical picture of sepsis and laboratory data showing sepsis; and **Group III:** (Controls) included 15 healthy neonates with no evidence of sepsis. A convenient sample of neonates was taken from El-Shatby hospital of maternity, Alexandria University, Egypt. Neonates in groups I and II were of matched age as the control group and informed consent was taken from parents of all contributors in this study.

Ethical considerations. This work was conducted according to the guidelines of the local Ethical Committee for sampling from a human. Oral consents were taken from parents to participate in the study.

Study sample exclusion criteria. Neonates with severe congenital anomalies, chromosomal abnormalities, intrauterine growth retardation, preterm, perinatal asphyxia and infant of a diabetic mother were excluded from the study.

2. Methods

Study design. A case-control study design was set to perform the underlying work.

Study setting. Three studied groups of neonates were involved in this study; Group I: suspected sepsis included 15 neonates; Group II: proven sepsis included 15 neonates; and Group III: healthy controls included 15 neonates. The study was set to collect blood samples from the target three groups previously described. Blood samples were then

processed for laboratory investigations and culture. Venous blood was used for assaying CBC, blood culture and procalcitonin. Laboratory investigations included: complete blood picture (CBC); blood cultures; and serum procalcitonin.

Data collection. To all neonates, the following data were collected: full history recording: (to detect risk factors for sepsis) including obstetric, prenatal, natal, postnatal and present history. Data were obtained from records of data sheets of neonates; and thorough clinical examination including general examination, cardiovascular examination, chest examination, neurological examination, abdominal examination and skin examination.

Blood Sampling. 2.5 millilitres of venous blood were withdrawn from suspected, proven sepsis neonates and healthy control group. For venous blood: The first part (1.5 ml) was pipetted into a tube containing EDTA to be used for assaying CBC and blood culture. The second part (1 ml) was allowed to clot thoroughly for 30 minutes then centrifuged at 1000xg for 15 minutes for separating serum. Serum was used for assaying procalcitonin.

Laboratory Investigations

Complete Blood Picture (CBC). For the determination of CBC, 0.5 millilitres of venous blood was collected in vacutainers containing ethylenediaminetetra-acetic acid (EDTA) and analyzed by auto hematology analyzer Mindray BC-3000 plus (Mindray Bio-Medical Electronics Co., Ltd, United States of America). Calibration of the machine was done every morning before processing. The normal ranges were; 5000 and 30,000/ml for WBC, 4700 and 9400 for neutrophils, 5000 and 5000/ml for lymphocytes and 150,000 and

450,000/ml for platelets. The extreme value on either side was suggestive of ongoing neonatal sepsis.

Blood Culture. Blood culture aseptic techniques were performed as follow; approximately one milliliter of blood was inoculated into the pediatric blood culture bottles (ready to use Hexa-Biotec blood culture Co., Ltd, Egypt). Blood culture bottles were incubated at 37°C for 24 hours after which the aliquots were sub-cultured on solid agar plates; MacConkey, blood and chocolate agars (OXOID Ltd, Basingstoke, Hampshire, England) for up to 96 hours before being regarded as no growth.

Serum Procalcitonin. Serum procalcitonin, PCT, was detected semi-quantitatively by a rapid immunochromatographic technique using a commercially available test kit (PCT-Q, BRAHMS Diagnostica GmbH, Berlin, Germany) in 30 minutes. 200 microliters of serum were applied onto the test strip. PCT in the sample is bound by mouse anti-calcitonin antibodies conjugated with colloidal gold to form a complex. This complex moves using capillarity through an area containing fixed anticalcitonin antibodies to form a sandwich complex that can be seen as a reddish band. The color intensity of the band was directly proportional to the PCT concentration of the sample. Normal serum and plasma levels of PCT are less than 0.5ng/ml. Levels above this value were considered as pathological. The result was read and interpreted as per the manufacturer's recommendations: PCT > 10ng/ml, severe bacterial sepsis or septic shock; PCT 2 to 10ng/ml, severe systemic inflammatory response, most likely due to sepsis unless other causes are known; PCT 0.5 to 2ng/ml, a systemic infection cannot be excluded; and PCT < 0.5ng/ml, local bacterial infection possible; sepsis unlikely.

Statistical Analysis of the Data. Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using mean and standard deviation. For normally distributed data, a comparison between two independent populations was done using an independent t-test while more than two populations were analyzed using F-test (ANOVA) to be used and Post Hoc test (LSD). The significance of the obtained results was judged at the 5% level.

Results

Results revealed that sepsis was more in neonates aged >3 days age (late-onset sepsis) than newborns of <3 days age (early-onset sepsis), as indicated by ANOVA ($F=32.696$, $p=.001$). Incidence of sepsis was more seen in preterm with a gestational age of <37 weeks in comparison to term newborns. This difference was statistically insignificant, using ANOVA ($F=0.253$, $p=0.778$). Incidence of sepsis was more in newborns delivered by caesarean section than normal delivery. This difference was statistically insignificant using ANOVA ($F=0.262$, $p=0.770$). Incidence of sepsis was more among low birth weight newborns than normal birth weight newborn. This difference was statistically insignificant using ANOVA ($F=0.653$, $p=0.526$). The incidence of sepsis was more in male than female newborns. This difference was statistically insignificant using ANOVA ($F=0.263$, $p=0.770$).

Table 1 shows the APGAR score distribution among study subjects. The Apgar score provides a convenient shorthand for reporting the status of the newborn infant and the response to resuscitation. It refers to as an acronym for **A**ppearance, **P**ulse,

Grimace, Activity, and Respiration. The Apgar test is usually given to a baby twice: once at 1 minute after birth, and again at 5 minutes after birth.

APGAR (1 min) and APGAR (5 min) were in the lowest values among group II neonates followed by a group I neonates while the highest APGAR

score was among group III neonates (control). These differences were statistically significant using ANOVA ($F=48.731$, $p=0.002$ and $F=25.345$, $p=0.007$, respectively). Clinical sepsis score was higher among group II newborns more than group I newborns. This difference was statistically significant using a t-test ($T=4.146$, $p=0.016$)

Table 1. APGAR score distribution among study subjects.

	Studied Groups			F	P-value
	Group I N = 15	Group II N = 15	Group III N = 15		
APGAR (Mean \pm SD)					
(1 min)	6.5 \pm 0.13	5.7 \pm 0.15	8.5 \pm 0.17	48.731	0.002
(5 min)	8.5 \pm 0.18	7.8 \pm 0.16	9.9 \pm 0.19	25.345	0.007

Table 2 shows organisms revealed from blood cultures and antibiotic sensitivity. *Klebseilla* and *Staph spp.* showed the highest percentages being (20%) in blood cultures of Group I followed by *E. coli* (13.3%), and lastly *Pseudomonas* and *Enterobacter* (6.6%). In Group II, *Klebseilla* showed the highest percentage being (33.3%) followed by *Staph spp.* (26.6%) followed by *E.coli* (20%) followed by *Pseudomonas* (13.3%), and

lastly *Enterobacter* (6.6%). The organisms in Group I was sensitive to Meropenem (40%) followed by Amikacin, Ciftazidim (13.3%), and then Ceftriaxon, Ciprofloxacin, Cefepim, Gentamycin, Vancomycin (6.6%). Also, the organisms in Group II were sensitive to Meropenem (33.3%) followed by Amikacin (20%) then Vancomycin (13.3%) and Ciprofloxacin, Ceftriaxon, Gentamycin, Ciftazidim (6.6%).

Table 2. Organisms in blood cultures and antibiotic sensitivity.

	Studied Groups		T	P-value
	Group I N = 15	Group II N = 15		
No growth	5 (33.33%)	0 (0.00%)	49.0	0.001
Growth (Organisms)	10 (66.66%)	15 (100%)		
Gram positive	3 (20.00%)	4 (26.66%)	33.28	0.001
Gram negative	7 (46.66%)	11 (73.33%)		
Klebseilla. spp	3 (20.00%)	5 (33.33%)		
Staph. spp	3 (20.00%)	4 (26.66%)		
Escherichia coli	2 (13.33%)	3 (20.00%)		
Pseudomonas	1 (6.66%)	2 (13.33%)		
Enterobacter	1 (6.66%)	1 (6.66%)		

Table 3 shows the serum PCT level among study subjects. Levels of PCT were above normal in most of the newborns with suspected and proven sepsis (Group I & II) compared to healthy control. The differences were statistically significant using ANOVA at $p=0.001$.

Table 3. Serum PCT levels among the study subjects.

	Studied Groups			F	P-value
	Group I N = 15	Group II N = 15	Group III N = 15		
Serum PCT (ng/ml)					
< 0.5 ng/ml	0 (0.00%)	0 (0.00%)	15 (100%)	164.745	0.001
≥ 0.5 ng/ml	7 (46.66%)	0 (0.00%)	0 (0.00%)		
≥ 2 ng/ml	8 (53.33%)	6 (40.00%)	0 (0.00%)		
≥ 10 ng/ml	0 (0.00%)	9 (60.00%)	0 (0.00%)		

Table 4 and Figure 1 show agreement (sensitivity, specificity and accuracy for PCT. The ROC curve analysis was used to compare the diagnostic values of PCT and culture result depending on the area under the curves (AUC). The higher AUC corresponds to a better diagnostic test. PCT showed significant AUC (91.7%, $p<0.001$) with a sensitivity of 83.33% and specificity of 100%.

Table 4. Sensitivity, specificity and accuracy for PCT.

	AUC	P	Sensitivity	Specificity	PPV	NPV	95 % Confidence Interval
Serum PCT	0.917	< .0001	83.33	100.0	100.0	74.99	0.795 - 0.978

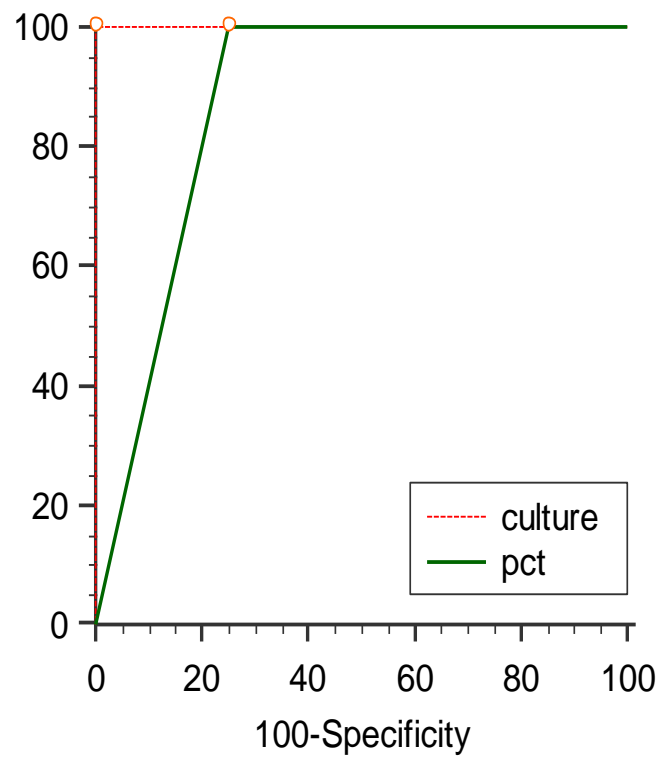


Figure 1. ROC curve for PCT.

Discussion

In the present study, late-onset septicemia (LOS) was more common than early-onset septicemia (EOS). This is probably because EOS reflects transplacental or, more frequently, ascending infections from the maternal genital tract, whereas LOS is associated with the postnatal nosocomial or community environment, with the peak incidence reported to be between the 10th and 22nd day of life.⁽¹¹⁻¹⁵⁾ Since the early 1980s, epidemiological studies have observed a general reduction in EOS, probably due to advances in obstetric care and the use of prophylactic intrapartum antibiotics to prevent infections caused by Group B *Streptococcus*. Meanwhile, the incidence of LOS has increased in parallel with the improved survival of premature infants, especially in those with very low birth weight, indicating the role of hospitalization and life-sustaining medical devices in the pathogenesis of neonatal LOS.^(16,17)

Results revealed that sepsis was more common in preterm neonates with gestational age <37 weeks than in term newborns with gestational age ≥37. This is probably due to complications of labor and delivery and the immaturity of innate and adaptive immunity.^(18,19) Also, one of the important risk factors was a premature rupture of the membrane (PROM) which was more prevalent in 30% of cases. The fact lying here is that PROM poses a risk of ascending infection to the fetus.^(19,20)

Sepsis was shown to be more common in caesarean section (63%) than normal delivery in (37%). This is probably due to an increased number of high-risk pregnancies admitted in this hospital resulting in increased caesarean section.⁽²⁰⁾ Also, it was shown that infection was more common in the low birth weight baby compared to babies of normal

birth weight in agreement with results reported in other studies.^(19,20)

In this study, sepsis was more common in males (60%) than females (40%), which is consistent with the findings of Berger *et al.*⁽²¹⁾ This might be attributed to the attitude of parents to seek medical services more often for their male babies rather for female babies in this region.

It was also found that there were statistically significant differences among the study groups regarding APGAR (1min) and APGAR (5 min) at a p-value equal to 0.007. This agrees with the study of Yousef *et al.*⁽²²⁾ who observed that, a 5-minute Apgar score <7 carries a significantly higher risk of sepsis than infants with higher scores and that Apgar scores less than 5 at one minute may be due to sepsis, especially with the presence of risk factors for infection. Furthermore, low Apgar scores usually necessitate more prolonged and aggressive resuscitation which is a known risk factor for sepsis.⁽²³⁾

In the current study, it was found that positive blood cultures were found in all of the confirmed sepsis group (100%) and 66.67% of the suspected sepsis group. *Klebsiella* and *Staph* spp. showed the highest percentages (20%) in blood cultures of Group I followed by *E. coli* (13.3%), and lastly *Pseudomonas* and *Enterobacter* (6.6%). In Group II, *Klebsiella* showed the highest per cent of incidence (33.3%) followed sequentially by *Staph* spp. (26.6%), *E. coli* (20%), *Pseudomonas* (13.3%), and lastly *Enterobacter* (6.6%). These findings are in agreement with Abdel-Hady and Zaki (2003)⁽²⁴⁾ who reported that *klebsiella* was found in 41.3 % of patients. Also, Badrawi *et al.* (2005)⁽²⁵⁾ reported that *klebsiella* was found in 63.6%. Boseila *et al.* (2011)⁽²⁶⁾ found that *Klebsiella* dominated the

organisms isolated from the blood culture (35%), followed by *Pseudomonas* (20%), Coagulase Negative Staphylococci (10%), Group B Streptococci (10%), *Staph. Aureus* (10%) and *Enterobacter* (15%). These results were consistent with Aletayeb *et al.* (2011)⁽²⁷⁾ and Shah *et al.* (2012)⁽²⁸⁾ who reported that the percentage of Gram-negative isolates from sepsis cases were 92.8% and 52%, respectively. On the other hand, Kaseb *et al.* (2004)⁽²⁹⁾ found that gram-positive bacteria as staphylococcus were isolated in 50% of the cases, while *Klebsiella* was found in 26.7%. Also, Marchant *et al.* (2013)⁽³⁰⁾ found that gram-positive organisms accounted for the majority of neonatal sepsis cases (up to 70%) while sepsis due to gram-negative organisms accounted for (15 to 20%). Ballot *et al.* (2012)⁽³¹⁾ found that gram-positive infections predominated in EOS, with *Streptococcus Agalactiae* being the most common pathogen. Muhammad *et al.* (2010)⁽³²⁾ found that *Klebsiella* and *Moraxella* were the most common isolates of LOS. On the contrary, Li *et al.* (2013)⁽³³⁾ also reported that coagulase-negative *Staphylococcus* (CoNS) was the major gram-positive bacteria in LOS (54.4%).

This variability in the etiology between EOS and LOS can be explained by the fact that EOS is conventionally regarded as maternally-acquired, with causative organisms, such as gram-negative organisms which are usually found in the maternal genital tract, whereas LOS is considered environmental in origin, either hospital or community-acquired.⁽³⁴⁾ Intervention procedures may affect the distribution of microorganisms. As regard EOS, after the introduction of intrapartum GBS prophylaxis, the rate of this infection increases and allows gram-negative predominance to occur. While the predominance of gram-positive

organisms in LOS is attributed to the poor infection control attitude in hospitals especially in developing countries.⁽³⁵⁾

Procalcitonin (PCT) has been proposed as a marker of bacterial sepsis in critically ill patients. PCT is a precursor of calcitonin and is a 116 amino acids protein.^(34,36) In contrast to calcitonin that has a short half-life of 10 min, PCT has a much longer half-life of 25-30 hr. In healthy persons, PCT levels are barely detectable.⁽³⁶⁾ Although the exact sites of production of PCT in sepsis have not been identified, monocytes and hepatic cells are believed to be potential sources.^(34,37) Bacterial lipopolysaccharide (LPS) is a potent inducer of PCT release into the systemic circulation.⁽³⁸⁾ PCT concentration starts to rise from 3-4 hrs after an endotoxin challenge with a peak after about 6 hrs, and remain increased for over 24 hr.^(34,39) Many authors found that PCT is a promising marker for the diagnosis of neonatal sepsis.⁽³⁴⁻⁴⁰⁾ In these studies, PCT sensitivity in the early diagnosis of neonatal sepsis was found to be 83-100% while the specificity was 70-100%.⁽³⁶⁻⁴⁰⁾ But some investigators questioned the diagnostic accuracy of PCT in detecting neonatal sepsis. In these studies, it was reported that serum levels had also increased in non-infected neonates with perinatal asphyxia, intracranial hemorrhage, pneumothorax, or after resuscitation, and these conditions had negatively affected the specificity of PCT.^(40,41)

In the present study, PCT showed a sensitivity of 83.33%, specificity of 100%, and positive predictive value of 100% and negative predictive value of 74.99%. To evaluate the PCT test performance, ROC curve using sensitivity and specificity of PCT was made for cut off value of <0.05 ng/ml. Area under the curve was found to be

equal (0.917). These findings are in agreement with other studies that reported that the PCT test appeared to be useful for the diagnosis of neonatal sepsis.⁽³⁴⁻⁴⁶⁾

The current findings showed high sensitivity and specificity of the PCT test for diagnosis of neonatal sepsis. The results so far are very encouraging and may suggest that shortly the PCT may be used routinely for diagnosis of neonatal sepsis and can provide a life-saving approach for newborns.

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