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The role of procalcitonin and neutrophilCD64 in non culture- based diagnosis of neonatal and infantile septicemia

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ABSTRACT

Sepsis is the body's extensive reaction to an infection. It is a severe medical emergency. Without prompt treatment, sepsis can quickly lead to tissue damage, organ failure, and death. Each year in the worldwide, more than 75,000 neonates and infants develop severe sepsis. Almost 7,000 of these infants die. Neonatal sepsis is defined as a syndrome of clinical features of spread infection and presence of bacteremia increase access to the blood stream, neonatal sepsis is distinct as a syndrome of bacteremia with systemic signs and symptoms of infection in the first 28 days of life.

Aim: The aim of this study is to evaluate a number of non-culture method, procalcitonin and neutrophilCD64 in the diagnosis of sepsis in neonates and infants.

Methods: This study is a case-control study, it includes80 patients neonates and infants(sepsis group) (44 males and 36 females) attended the children at Kerbala pediatric teaching hospital and Kerbala primary health care centers during the period between (May 2019 to September 2019) Their ages ranged between (1 day to 1 year),The control groups(non-sepsis group), included 20 healthy control (12males and 8females) The clinical data were collected from their parents through a questionnaire.

Results: The mean age for neonates of sepsis was (7.40±13) days while the mean age for neonates of control was (5.58±6.75)days, The mean age for infants of sepsis was (4.1±7.971) months while the mean age for infants of control was (4.01±5.66) months **.**Male to female ratio of sepsis group=44/36=1.22.

Conclusions: in this study, PCT, CRP and CD64 showed to be the best indicators of early- and late-onset neonatal and infantile sepsis.

Keywords: NIL

INTRODUCTION

Sepsis refers to the dispersed inflammatory response caused by microbial infections, where fever, tachycardia, and tachypnea are typically developed by the patient. Severe sepsis is linked to at least one organ's dysfunction. The disease is called septic shock when extreme sepsis is supplemented by multiple organ system failures. Extreme sepsis is the sepsis that causes poor functioning of one organ (extreme sepsis and septic shock recorded in the 1991 and 2001 consensus papers(LevyMM and Marshall, 2001)**.**Sepsis is a life-threatening malfunction of the organ due to a deregulated host reaction to

infection(Singer et al., 2016, Sankar et al., 2008)**.**It is one of the very important Causes of fatality for infants and neonatal. Gram-negative sepsis, if left organic, is commonly correlated with rather greater death than gram-positive death(Karunakaran et al., 2007)(Moradi et al**.,** 2015)**.**One of the major causes of neonatal disease and death is bacterial sepsis with a prevalence of 1-5Live births in 1000, and free exposure to infection can occur during delivery and its clinical presence can appear at birth or during the first days of life (Sharma et al.,2020).Neonatal sepsis (NS) is a systemic infection that occurs at ≤28 days of life in neonates and is a significant cause of neonate disease and death (Sankar et al., 2008).Neonatal sepsis is one of the major causes of disease and death among neonates in the developing world (Edmond and Zaidi, 2010)**.**Early-onset neonatal sepsis (EOS) is characterized as occurring in the first 3 days of life and is caused by vertically transmitted bacterial pathogens from the mother to neonates before or during delivery. Late-onset sepsis (LOS) is sepsis occurring after 72 h in the neonatal intensive care unit (NICU)(Bizzarro et al., 2005)**.**Gram-negative organismshave been the most common causes of neonatal sepsis, especially *Escherichia coli (E. coli)* (Stoll et al., 2002a). The primary etiologies of early-onset neonatal sepsis (EOS) stay group B *streptococcus* (GBS) infection(Camacho-Gonzalez et al., 2013)**,**and *Escherichia coli* (*E. coli*), as the major pathogen of neonatal sepsis in early-onset sepsis(Stoll et al., 2011)**,**Infantile septicemia (IS) is most commonly caused by bacteria. But it can be triggered by other microorganisms too. Infants can be exposed in the hospital or at home to infections. The best ways to avoid sepsis are by early diagnosis and treatment. Antibiotic medicine begins as early as possible. Sepsis is the most common cause of death in infants worldwide(Wiens et al., 2012).The identified rate of infantile septicemia Per 1000 live births, it ranges from one to five cases(Van Den Hoogen et al., 2010)(Puopolo and Eichenwald, 2010).clinical diagnosis is difficult to diagnose sepsis in infants and neonates because clinical symptoms are subtle and unspecific, mainly early in the course of the disease, and laboratory tests are not always accurate, including blood culture, the 'gold standard'(Gerdes, 2004)**,**and non - blood culture Clinicians have long sought dependable markers to detect sepsis in infants and neonates early in its course and to reject diseases of noninfectious original(Ng and Lam, 2006)(Mishra et al., 2006).Alternatively, researchers began looking earlier at potential fast-competent diagnostic markers of neonatal and infantile sepsis to allow a strong distinction between neonates and infants with clinical signs due to extreme bacterial sepsis such as Gram-negative infections. Several markers, such as procalcitonin (PCT), C-reactive protein (CRP), and CD64, are examples of these markers investigated with versatile sensitivities and specificities**(**(Døllner et al., 2001).Neutrophil CD64 (nCD64),The receptor for high-affinity Fc is expressed by neutrophils. nCD64 up-regulation of neutrophils is expected to be a very early step in the immune response of the host to bacterial infection. The expression of nCD64 can therefore be used as a symbol at a very early stage to differentiate infected from non-infected neonates and infants. Serum procalcitonin (PCT) is an increasingly interesting biological marker for the identification of serious bacterial infections(Alami Hakami, 2018).Is a precursor peptide of the hormone calcitonin. It is levels increase significantly in the presence of inflammatory stimuli. Serum PCT rises within 4h after onset and attains maximum serum concentrations at 18–24hours(Mussap et al., 2007)**.**The serum procalcitonin (PCT) level increases 3-4 hours after bacterial infection exposure and contributes to a corresponding rise in CRP(Zhydkov et al., 2015).

Material and Methods

Patients

This study involved a total of 100 subjects (patients and controls) of one day to one year of age during the period from May 2019 to September 2019 of neonatesand infants cared for in Kerbala pediatric teaching hospital and Kerbala primary health care centers. A total of 100childrentwo categories have been included and classified. The first group was neonates and infants (a group of sepsis), which consisted of 80 patients (44 males and 36 females) who attend Kerbala teaching hospital.The second group was a healthy control group, which consisted of 20 healthy control (12 males and 8 females) who attend Kerbala primary health care centers. They are of age and sex matching with patients who were diagnosed based on the clinical features and laboratory findings.

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Sample Collection

Approximately 3cc of venous blood was obtained from each child (patient and control) preceded by sterilization of the area with 60% ethanol.2cc of blood was dispensed into EDTA tube (for Complete Blood Count (CBC) and neutrophil CD64), 1 cc of blood (for CRP and PCT), Dispensed into a plain tube, the serumwas separated by centrifugation and allowed to coagulate 4,000 round per minutes (RPM) for 10 minutes. Then the serum was transferred to a new plain tube for determination of PCT and CRP.

Serum inflammatory biomarker detection

Venous blood sample for biomarkers was evaluated before exposure to antibiotics. The samples are placed in a plain tube and were separated by centrifugation at 4,000 rounds per minutes (RPM) for 10 minutes, PCT and CRP are detected by enzymatic immunoassay (EIA).

Statistical analysis

Using the statistical SPSS v 22.0. Statistical analyses were performed To determine the optimal laboratory diagnostic parameters (PCT, CRP, CD64) for predicting neonates and infants with sepsis, the receiver operating characteristic (ROC) curve was developed to calculate the diagnostic importance of blood biomarkers for predicting neonates and infants with sepsis. A ROC curve showed the false-positive rate on the x-axis (specificity) and the true-positive rate on the y-axis (sensitivity) for variable test cut-off values.By determining the point located geometrically close to an ideal test with 100 % specificity and sensitivity, the ideal cut-off criteria for the laboratory results were selected.Efficiency in diagnosis was described as (sensitivity, specificity). Number (n), percent (%), mean, standard deviation (SD), median, and minimum-maximum (min-max) results were presented. This is called a meaningful value of $p < 0.05$. The specifics of laboratory tests were expressed as mean ± standard deviation (M±SD). Statistical Studies Data were analyzed for the classification of sepsis and then further stratified by EOS and LOS.Using the expected likelihood of sepsis from generalized linear mixed modeling, receiver operating characteristic (ROC) curves were created. The ROC curve is a sensitivity specificity design for all observed values of (PCT, CRP, and CD64). To count their predictive value, the region under the ROC curve (AUC) was estimated(Liu and Wu, 2003).

Results

The clinicalpatterns of the two groups

Involved in this analysis100 neonates and infants, of which 80 neonates and infants belonged to sepsis and 20 to the non-sepsis control group. The clinical of patterns the two groups are summarized as in Table (3-1).

Table 3.1: Clinical patterns (sepsis and control group) of the study groups

Clinical patterns	Sepsis group (n=80)		Control group(n=20)	
	(neonates)	(infants)	(neonates)	(infants)
Age(days)	13±7.40	239.13±124.70	6.75±5.58	170±120.4
Temperature(°C)	39.3±0.78	39.004±0.75	37.04±0.518	37±0.1
Gestational age(months).	9.4±0.91	8.94±0.65	9±0.40	8.75±0.66
Birth weight(kg).	3.35±0.84	8.25±2.56	3.01±0.39	6.42±2.94
Male.	7(0.7)	37(0.52)	8(0.6)	4(0.5)

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Female.	3(0.3)	33(0.47)	4(0.3)	4(0.5)
nCD64 (mean±SD)	53.90±18.49	66.33±15.63	14.79±19.65	11.85±16.51
PCT,ng/mL(mean±SD)	2.76±0.52	3.26±1.82	0.72±0.48	0.85±0.61

Gender Distribution

The study result exposed that a higher prevalence of sepsis in males, in whom the percentage of the male was 55%, whereas the percentage was 45% in females. Male to female ratio is 44/36=1.22 .as in Table (3-2).

Table 3.2:-Difference in gender between patients and healthy control.

Sex	Sepsis patients	Healthy children
Male	44 (55%)	12(60%)
Female	36 (45%)	8(40%)
Total	80	20

Maturity of children

The percentage of the maturity of neonatal and infantile of the total sample patientsand healthy control was 21% preterm and 79% term. as inFigure (3-1).

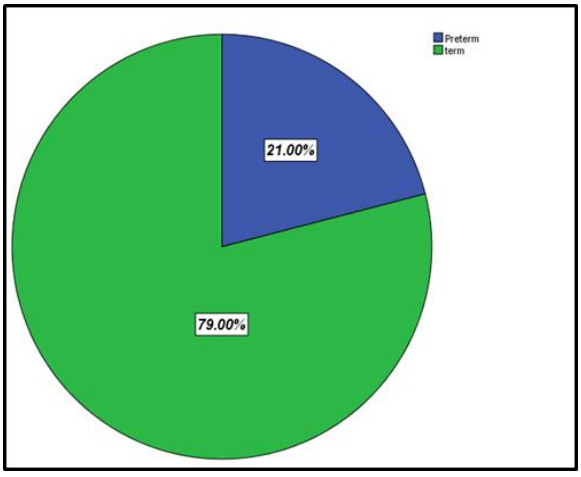

Figure 3.1: The distribution maturity of among the total sample

Use of Optimum inflammatory biomarker cut-off values for sepsis diagnosis

ROC analysis has been used to recognize the sensitivity, specificity, positive predictive value

(PPV), negative predictive value (NPV), and AUC of the tests for the optimal cut-off values select .as in Figure(3-2).

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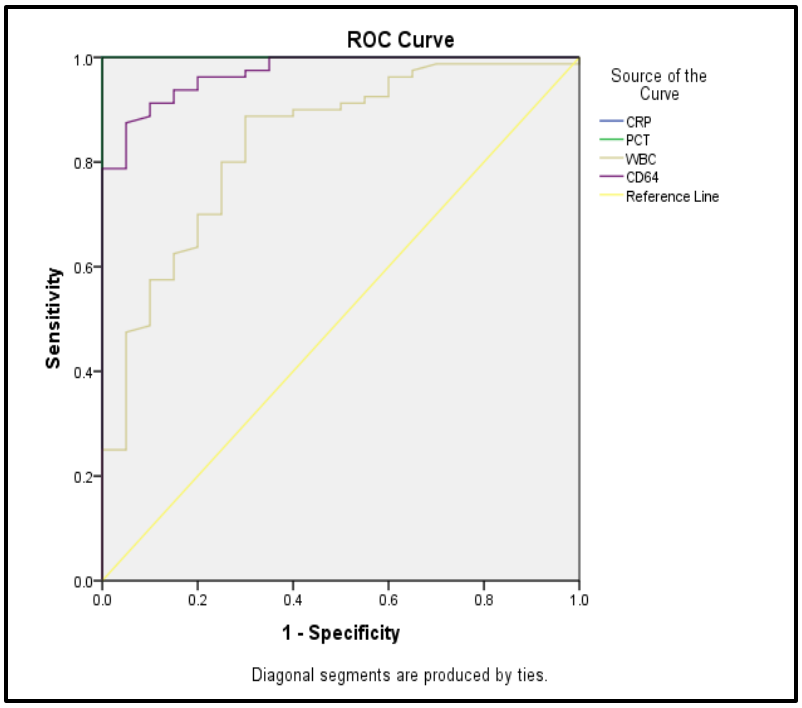

Figure 3.2: Comparison of the nCD64, PCT, CRP, and WBC receiving operating characteristic (ROC) curves as markers for diagnosis of neonatal and infantile sepsis.

Receiver Operating Characteristic (ROC)

The Receiver Operating Characteristic (ROC) for CD64 as a diagnostic marker to detect neonatal and infantile sepsis in(case and control)and area under the curve (AUC) were (0.971) this indicates an excellent distinctive nature of WBC count as a classifying index .as in figure (3-3).

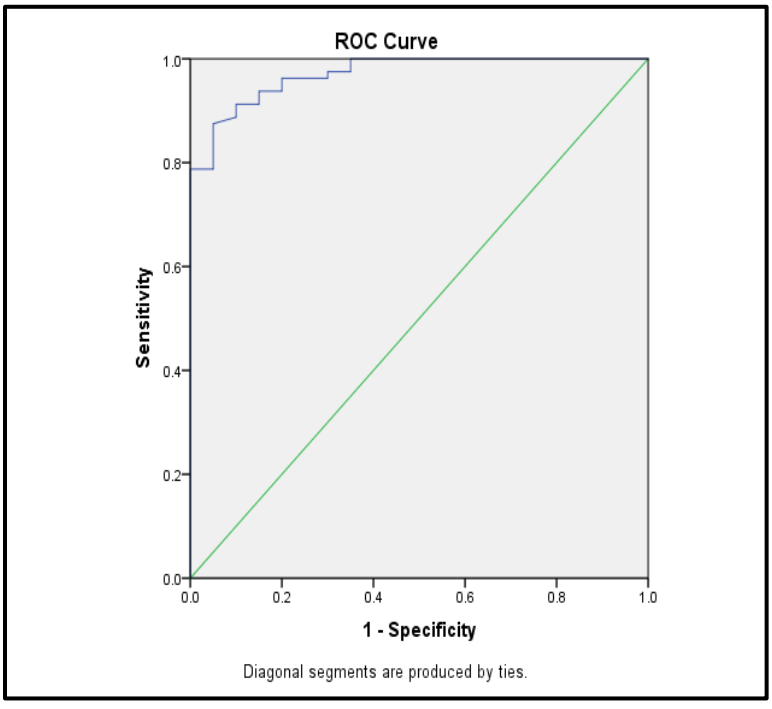

Figure 3.3: The Receiver Operating Characteristic for CD64 as a diagnostic marker to detect neonatal and infantile sepsis in (case and control)among the total sample (n=100).

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The Receiver Operating Characteristic (ROC) for PCT as a diagnostic marker to detect neonatal and infantile sepsis in(case and control)and area under the curve(AUC) were (1.00) this indicates an excellent distinctive as a classifying index. as in figure (3-4).

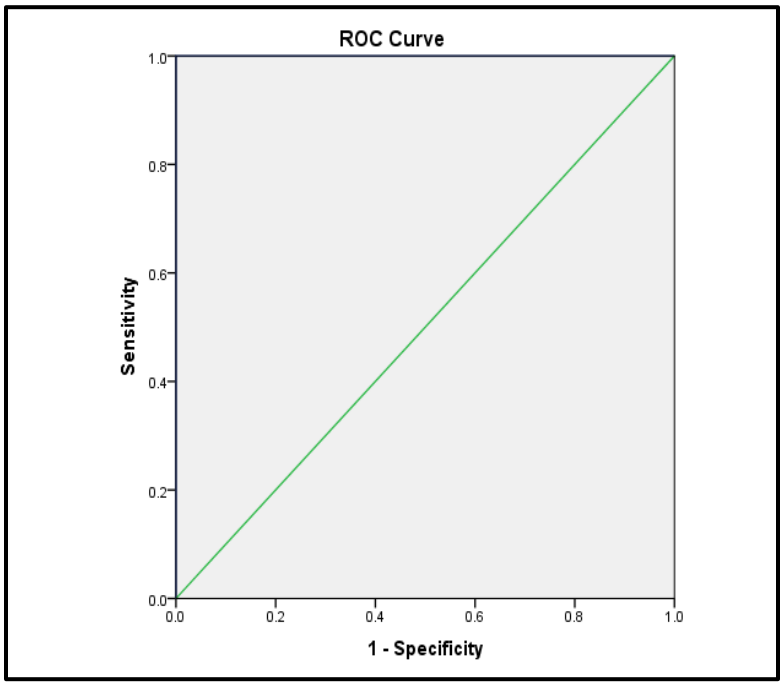

Figure 3.4: The Receiver Operating Characteristic for PCT as a diagnostic marker to detect neonatal and infantile sepsis in (case and control) among the total sample (n=100).

Discussion

Neonatal sepsis (NS) could be defined both clinically(Child et al., 2005).Neonatal sepsis can be categorized according to the time of occurrence of the disease: early-onset (EOS) and late-onset (LOS). The prevalence of neonatal sepsis reported ranges from 7.1(Pahang and Aziz, 1995)to 38(Tallur et al., 2000)per 1000 live births in Asia. In this study, the Male to female ratio of sepsis group=44/36=1.22. A study in China found that the Male to female ratio of sepsis group=31/29=1.06.In our study, we did not perform blood culture because our research did not depend on blood culture, it depends on the diagnosis of neonatal and infantile septicemia by many markers such as (PCT andCD64).In this study, the mean PCT for neonates of the sepsis group was (0.52±2.76)ng/mL, and the control group was (0.48±0.72) ng/mL. A study in Germany found that the mean PCT for neonates of the sepsis group was (0.39±0.96)ng/mL which is similar to the figure in the study. In this study, The mean PCT for infants of the sepsis group was(1.82±3.26)ng/mL, and the control group was(0.61±0.85)ng/mL. A study in Turkey found that the mean PCT for infants of the control group(0.08±0.39)ng/mL.

In this study using PCT≥(0.81)ng/ml as the cut-off value by using ROC curvesis the range of statistical findings recorded as follows, PCT sensitivity in the diagnosis of neonatal and infants sepsis (100%), specificity(50%), PPV (88.8%) and NPV (100%).In this study, We discovered that nCD64 had the largest AUC (0.971), and its sensitivity(88.6%), specificity value were (75.6%), PPV (79.5%), and NPV(86.1%),Which was capable of distinguishing infected neonates and infants from non-infected.

Neutrophil CD64 (nCD64) is the best individual marker for bacterial sepsis in infants, while in neonates the highest diagnostic correctness at the time of suspected sepsis. In our research, nCD64 is a highly sensitive marker for the diagnosis of suspected neonates and infants with sepsis, nCD64 as a diagnostic marker, and may be helpful in combination with other biomarkers of inflammation, such as PCT, CRP, when active. In this study, The mean CD64 for neonates of the sepsis group

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was(53.90±18.49), and the control group was (14.79±19.65). A study in China found that the mean CD64 for neonates of the sepsis group was (8.64±4.51) and the control group was (2.15±1.57).In this study, We discovered that nCD64 had the largest AUC (0.971), and its sensitivity (88.6%), specificity value were (75.6%), PPV (79.5%), and NPV (86.1%), Which was capable of distinguishing infected neonates and infants from non-infected. Neutrophil CD64 (nCD64) is the best individual marker for bacterial sepsis in infants, while in neonates the highest diagnostic correctness atthe time of suspected sepsis.

Conclusions

As compared to CRP, PCT and nCD64 are better diagnostic biomarkers for early detection of neonatal and infantile sepsis. The combination of these biomarkers could increase sensitivity for the diagnosis of suspected early and late-onset neonatal sepsis based on common serum biomarkers with the aid of optimal cut-off value based on ROC curve analysis.

Recommendations

Use CRP, PCT, and nCD64 to diagnose neonatal and infantile septicemia. Use nCD64 is a useful biomarker by flow cytometry is more specific for diagnosis the sepsis.

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