



# Comparative Analysis of Procalcitonin and C-Reactive Protein as An Early Biomarker of Neonatal Sepsis in a Tertiary Care Hospital in Central India

<sup>1</sup>Dr. Snehal Korde, <sup>2</sup>Dr. Girish Nanoti

<sup>1</sup>Assistant Professor, Department of Paediatrics, NKP Salve Institute of Medical Sciences and Research Centre and Lata Mangeshkar Hospital, Nagpur, Maharashtra, India.

<sup>2</sup>Professor and Head, Department of Paediatrics, NKP Salve Institute of Medical Sciences and Research Centre and Lata Mangeshkar Hospital, Nagpur, Maharashtra, India.

(Received: 11 June 2024

Revised: 16 July 2024

Accepted: 10 August 2024)

## KEYWORDS

Procalcitonin, C-reactive protein, Neonatal sepsis, Sensitivity, Specificity

## ABSTRACT:

**Introduction:** Background - Neonatal sepsis is a significant contributor to newborn morbidity and mortality, especially in developing nations. Neonatal sepsis presents a diagnostic challenge due to its nonspecific presentation and low sensitivity with time-consuming nature of bacterial cultures. Therefore, relying on sepsis indicators is essential to prevent unnecessary NICU stays and antibiotic therapy. C-reactive protein (CRP) and Procalcitonin (PCT) are commonly used biomarkers of sepsis which are easily available and have the highest sensitivity and specificity rates. **Objective -** The main objective of the study was to determine the diagnostic accuracy of PCT as an early marker of neonatal sepsis in comparison with CRP.

**Methodology –** A cross-sectional study was conducted in the Neonatal Intensive Care Unit (NICU) Department of Paediatrics at N.K.P. Salve Institute of Medical Sciences and Research Centre & Lata Mangeshkar Hospital, Nagpur. A nonprobability sampling technique was used for the selection of neonates. Fifty neonates showing clinical signs and symptoms and fulfilling inclusion criteria were included in the study. Clinical and laboratory parameters including PCT and CRP were recorded. Data was analysed using SPSS version 21.

**Results –** Among the 50 neonates who met all of the inclusion criteria 4 had clinical sepsis, 7 had suspected sepsis, and 39 had confirmed sepsis. 37 neonates had positive PCT(>0.5ng/dl) 29 had positive CRP. Blood culture was positive in 39 neonates with the commonest organism being *Klebsiella pneumoniae*. The sensitivity, specificity, positive predictive value, and negative predictive value of procalcitonin (82.05%, 70%, 91.43%, and 50%) were better than CRP in predicting sepsis early.

**Conclusion -** In the early diagnosis of neonatal sepsis, procalcitonin scores significantly higher than CRP due to its higher sensitivity and positive predictive value.

## 1. Introduction

Neonatal sepsis is a significant contributor to newborn morbidity and neonatal deaths, especially in developing nations<sup>1</sup>. Neonatal sepsis is a clinical illness that manifests in the first month of life as systemic signs and symptoms of infection with or without concomitant bacteraemia. Sepsis that develops within the first 72 hours of life is known as early-onset sepsis (EOS), and sepsis that develops after that time is known as late-onset

sepsis (LOS). EOS is frequently caused by organisms acquired following delivery through mother's vaginal canal while, LOS is commonly brought on by organisms obtained from nosocomial or community sources<sup>2</sup>. Newborn sepsis frequently presents with a non-specific clinical presentation. Blood culture though "gold standard" delays the time for diagnosis. It is sensitive in identifying relatively low bacterial loads; nevertheless, they frequently result in false-positive results because of sample contamination and false-negative results



following antibiotic usage by the mother<sup>3,4</sup>. The use of real-time polymerase chain reaction for pathogenic microorganisms in clinical settings is not common due to its high cost. Due to growing concerns of antibiotic resistance, it is necessary to rely on biomarkers of sepsis to prevent unnecessary antibiotic usage. There are many biomarkers of sepsis like C-reactive Protein (CRP), Procalcitonin (PCT), serum amyloid A, hepcidin, Interleukin (IL)-1, IL-6, IL-8, CD 64, CD 11b, tumor necrosis factor, prepepsin.<sup>5,6</sup> Among all CRP and PCT are commonly used biomarkers of sepsis. CRP is a late-rising inflammatory marker so its use in the early diagnosis of sepsis and initiation of antibiotics is limited. On the other hand, PCT a peptide precursor of hormone calcitonin has its peak at 6-12 hour after infection and with short half-life of 20-24 hours which enables rapid detection of sepsis<sup>7</sup>. Therefore, current study was aimed to compare the diagnostic accuracy of Procalcitonin and C-reactive protein in neonatal sepsis.

## Methodology

Study area- Department of Paediatrics, N.K.P. Salve Institute of Medical Sciences and Research Centre & Lata Mangeshkar Hospital Nagpur

Study design- A cross sectional study

Sample size- Consecutive type of non-probability sampling was used for selection of cases

Study duration- Period of 10 months

Inclusion criteria

1. Neonates at risk of early onset sepsis with risk factors in mother like Prolonged labour, Prolonged premature rupture of membrane, Intrapartum fever, urinary tract infection, chorioamnionitis.

2. Neonates showing clinical signs and symptoms sepsis like temperature instability, apnoea, bradycardia, tachycardia, hypotension/hypoperfusion, feeding intolerance, abdominal distension, necrotizing enterocolitis.

Exclusion criteria

1. Neonates who received antibiotics prior to blood culture sample

2. Neonates with major congenital anomalies

Informed written consent was taken from the parents of all neonates included in the study. Total of 50 neonates were included in our study group by consecutive non probability sampling technique. The demographic data like name, sex, age at onset of sepsis, gestational age,

birth weight was recorded. Detailed clinical assessment including various symptoms and signs was done. Before starting the antibiotics, blood specimens were taken from each neonate. The blood specimens included total leukocyte count (TLC), absolute neutrophil count (ANC), the ratio of immature neutrophils to total neutrophil count (I/T ratio), platelet count, blood culture, antibiotic sensitivity, PCT and CRP estimation. Blood serum PCT was measured by using a Quantitative method using combination of Up-converting Phosphor Technology (UPT) with sandwich immunochromatography. PCT level >0.5 was considered as positive. Serum CRP level was measured by Latex/Turbidimetric method. CRP >6mg/dl was taken as positive.

Neonates were classified into three categories

**1. Group I (Confirmed sepsis):** clinical signs and symptoms of sepsis with positive blood culture.

**2. Group II (Suspected sepsis):** Clinical signs and symptoms with at least 2 positive septic screen parameters of the following but negative blood culture

a. TLC < 5000 or > 20000 per mm<sup>3</sup>

b. Low ANC as per Monroe chart<sup>8</sup> for term neonates and Mouzinho chart<sup>9</sup> for VLBW neonates

c. I/T ratio > 0.2

e. CRP > 6 mg/L

**3. Group III (Clinical sepsis):** Clinical signs and symptoms suggestive of sepsis with negative blood culture and negative septic screen.

**Ethical issues-** Ethical approval was obtained from Institutional Ethics Committee N.K.P. Salve Institute of Medical Sciences and Research Centre & Lata Mangeshkar Hospital Nagpur (No. NKPSIMS &RC and LMH/ IEC/5/2021 dated 26/03/2021).

## Statistical analysis

Categorical and nominal data like gender, gestational age, birth weight, onset of sepsis and various signs and symptoms of neonatal sepsis were expressed in terms of numbers and percentage. Various markers of sepsis like CRP, PCT, TLC, Platelet counts were expressed in terms of mean, standard deviation and ANOVA test was applied. The distribution was also obtained according to confirmed sepsis, suspected sepsis and clinical sepsis.



Using blood culture as the gold standard, the sensitivity and specificity, PPV, NPV, and PCT values were computed. ROC curve was plotted for both PCT and CRP.

## Results

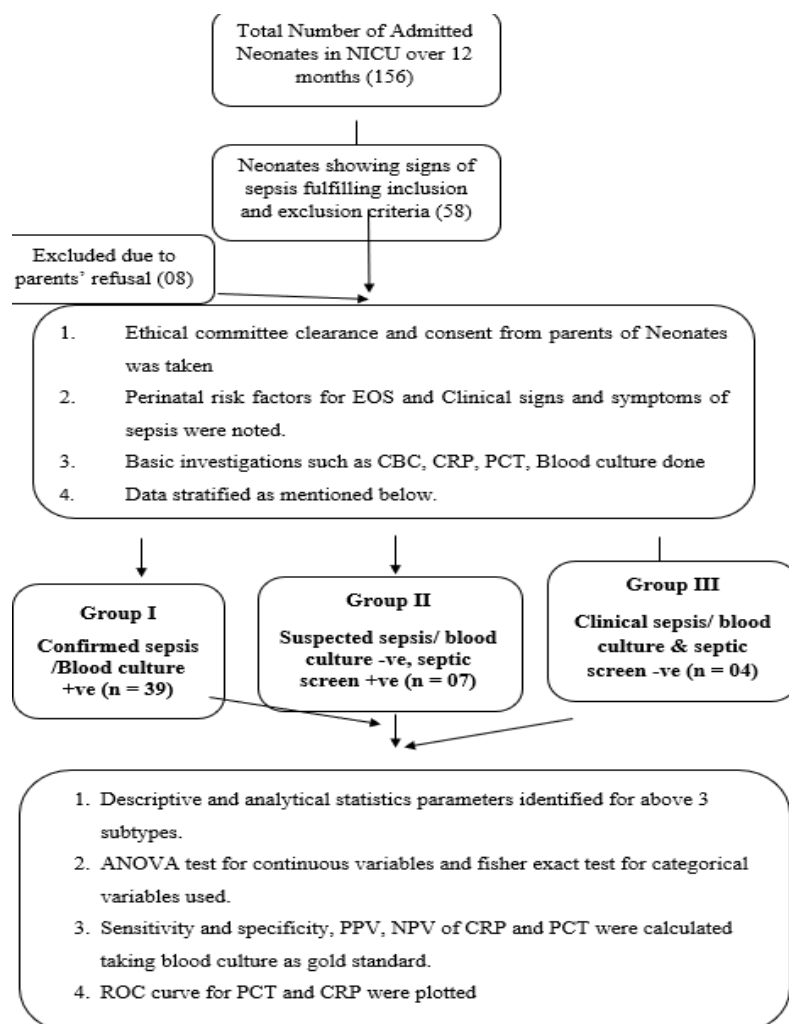
Among the 156 neonates admitted to NICU, 50 neonates had fulfilled the inclusion and exclusion criteria and were divided into three groups (Figure 1).

In this study, males (62%) were more than females (38%). In preterm and term neonates, confirmed sepsis was more (79.4% & 72.7%) than suspected and clinical sepsis (20.5% and 27.3% respectively). This study

showed that neonates with birth weight <1.5 Kg were most affected with sepsis (75% in confirmed sepsis group, 20.8% in suspected sepsis group, and 4.2% in clinical sepsis group). Late onset sepsis (90%) was more common than early onset sepsis (10%) (Table 1).

In this study, temperature instability was noted in 72% of the cases, followed by respiratory distress and delayed capillary refill time (CRT) in 46% and 42% of the cases respectively (Table 2). There were 39 culture positive sepsis. *Klebsiella pneumoniae* (14/39) was the most common organism isolated in blood culture, followed by candida (11/39), *Pseudomonas aeruginosa* (4/39) (Table 3).

**Figure 1: Study Profile: Patient Flow Chart**



**Table 1: Demographics of the neonates**

Variables		Group I (39) Confirmed Sepsis	Group II (7) Suspected Sepsis	Group III (4) Clinical Sepsis	Total (50)	Fisher Exact Test p value (clinical sepsis vs Suspected and confirmed sepsis)
Gender	Male	24 (61.5%)	5 (71.4%)	2 (50%)	31 (62%)	0.99
	Female	15 (38.5%)	2 (28.6%)	2 (50%)	19 (38%)	
Gestational age	< 34 weeks	24 (61.5%)	5 (71.4%)	0 (0)	29 (58%)	0.91
	34-37 weeks	7 (17.9%)	2 (28.6%)	1 (25%)	10 (20%)	
	>37 weeks	8 (20.6%)	0 (0)	3 (75%)	11 (22%)	
Birth weight	<1500 gms	18 (46.2%)	5 (71.4%)	1 (25%)	24 (48%)	0.62
	1500-2500 gms	16 (41%)	1 (14.3%)	0 (0)	17 (38%)	
	>2500 gms	5 (12.8%)	1 (14.3%)	3 (75%)	9 (18%)	
Onset of sepsis	Early onset sepsis	3 (7.6%)	2 (28.6%)	0 (0)	5 (10%)	0.6
	Late onset sepsis	36 (92.4%)	5 (71.4%)	4 (100%)	45 (90%)	

**Table 2: Distribution of study subjects according to various signs and symptoms**

Signs and Symptoms	N out of 50	Percentage
Apnea	20	40%
Respiratory distress	23	46%
Temperature instability	36	72%
Fever	06	12%
Feeding intolerance	12	24%
Delayed CRT	21	42%
Convulsion	08	16%
Lethargy	07	14%
Bleeding manifestations	11	22%

**Table 3: Distribution of study subjects according to Organism isolated in Blood culture**

Organism	Number	Percentage
Klebsiella	14	28
Candida	11	22
Pseudomonas	04	8
Non-fermenter	01	2



E. coli	03	6
B. Cepacia	01	2
MRSA	02	4
CONS	03	6
No growth	11	22
<b>Total</b>	<b>50</b>	<b>100</b>

**Table 4: Distribution of study subjects according to mean value of markers of sepsis and sepsis groups**

Sepsis group Diagnostic Markers	Clinical sepsis Mean $\pm$ SD	Suspected sepsis Mean $\pm$ SD	Confirmed sepsis Mean $\pm$ SD	ANOVA test P value
CRP (mg/L)	2.4 $\pm$ 3.23	24.73 $\pm$ 34.24	24.54 $\pm$ 30.33	0.37
PCT (ng/ml)	0.77 $\pm$ 1.06	4.00 $\pm$ 4.84	10.49 $\pm$ 18.23	0.44
WBC (/mm <sup>3</sup> )	8535 $\pm$ 2937	6351.42 $\pm$ 8186.56	9391.20 $\pm$ 5591.06	0.45
Platelets(/mm <sup>3</sup> )	323500 $\pm$ 15627	104714.28 $\pm$ 43553	138564.10 $\pm$ 86985	<b>0.006*</b>

**Table 5: Comparison of validity tests of CRP and PCT.**

Validity tests	CRP	Pro-calcitonin
Sensitivity	<b>58.97%</b>	<b>82.05%</b>
Specificity	<b>45.45%</b>	<b>70%</b>
Positive Predictive value	<b>79.31%</b>	<b>91.43%</b>
Negative Predictive value	<b>23.81%</b>	<b>50%</b>
Diagnostic accuracy	<b>56%</b>	<b>79.59%</b>

**Table 6: Comparison of mean PCT values in bacterial and fungal sepsis**

Type of sepsis	Mean PCT values	T test P value
Bacterial	16.78 $\pm$ 22.15	<b>0.03*</b>
Fungal	1.83 $\pm$ 2.70	

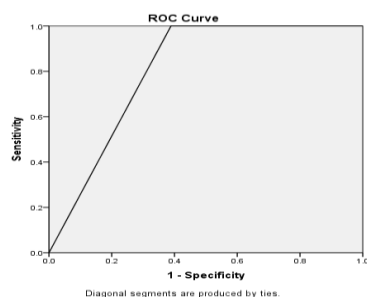


The mean values of CRP ( $p=0.37$ ) and PCT( $p=0.44$ ) in different types of sepsis were not found to be statistically significant but mean value of PCT was higher in confirmed sepsis group as compared to clinical sepsis group. While mean values of platelets were found to be statistically significant in different sepsis group. ( $p$  value=0.006) (Table 4).

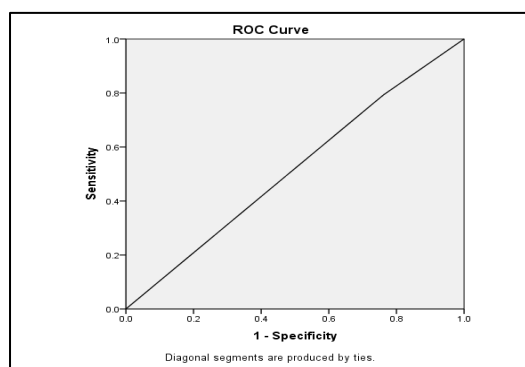
Overall, as the early marker of sepsis, procalcitonin has sensitivity of 82.05% and specificity of 70% as compared CRP with sensitivity of 58.97% and specificity of 45.45%. The PPV of PCT (91.43%) is also more as compared to CRP (79.31%) (Table 5).

When values of PCT in bacterial and fungal sepsis were compared, it was found to be statistically significant. ( $p$  value=0.03) (Table 6).

The ROC analysis showed the area under the curve was higher for PCT (0.806 with 95% confidence interval [CI], 0.569-0.952) than for CRP (0.516), showing effectiveness of PCT over CRP as an early diagnostic marker of neonatal infection (Figure 2,3).



**Figure 2: ROC curve of PCT with reference to gold standard Blood culture**



**Figure 3: ROC curve for CRP with reference to gold standard Blood culture**

## Discussion

In developing countries, major cause of neonatal mortality is sepsis. Early diagnosis and early initiation of antibiotics can improve the overall outcome of neonates. Because of time consuming nature of blood culture early diagnosis of sepsis cannot be made<sup>4</sup>. Accurate biomarkers are still required for the prompt and precise detection of neonatal sepsis. CRP and PCT are the important early markers in neonatal sepsis. As compared to PCT, CRP elevates 4-6 hours later after the beginning of inflammation and stays high for at least 1-2 days and then decline slowly over 5 days in spite of treatment with antibiotics<sup>6</sup>.

In present study, male newborns (73%) were more affected with sepsis than females (27%). Sepsis was found more in preterm (52%) neonates collectively than term (48%) in all three-sepsis group, which is similar to the results of the study by Hakeem A *et al*<sup>14</sup> but not with the study by Bharti *et al*<sup>10</sup> in which term (52%) were more affected than preterm (48%). Similar to research by Lim WH *et al*<sup>17</sup> where the majority (93.7%) of neonates had late-onset sepsis, 90% of babies in our study had late-onset sepsis and 10% had early-onset sepsis.

Also, babies with very low birth weight (46%) were diagnosed with sepsis more as compared to babies of normal birth weight (18%) as seen in study by Bharti *et al*<sup>10</sup> in which 69% low birth weight babies had sepsis than normal birth weight. Similar findings were found by Hakeem *et al*.<sup>14</sup>

Temperature instability (72%) was the most common sign followed by respiratory distress (46%) which was inconsistent with Bharti *et al*<sup>10</sup> in which refusal to feed was the most common complaint due to nonspecific presentation and different distribution of study population.

Mean PCT levels were high in confirmed sepsis group i.e.  $10.49 \pm 18.23$  ng/dl in confirmed sepsis group;  $4.00 \pm 4.84$  ng/dl in suspected sepsis group and  $0.77 \pm 1.06$  ng/dl in clinical sepsis group indicating severity of infection though; results were not statistically significant ( $p < 0.44$ ). This study findings were comparable to the study conducted by Bharti *et al* and Koksai N *et al*<sup>18</sup>.



Author	Parameters	Sample size	Sensitivity	Specificity	PPV	NPV
Sucilathangam G et al <sup>15</sup> 2012	PCT>0.5ng/ml CRP >6mg/l	50	92.8% 50.0%	75.0% 69.4%	59.0% 38.8%	96.0% 78.1%
Mamdouh M. Esmat et al <sup>16</sup> 2012	PCT1>19ng/ml CRP1≤48mg/l	61	70% 90%	54.9% 37.3%	27.4% 25.9%	88.2% 93.9%
Emad et al <sup>11</sup> 2020	PCT≥0.5ng/ml CRP>10 mg/dl	50	97.6% 89.5%,	89% 66.7%	97.6% 92.5%	88.9% 60%
Bharti et al <sup>10</sup> 2020	PCT>2 ng/ml CRP >10 mg/l	100	87.2% 76.92%	72.13% 49.18%	66.7% 76.9%	89.8% 60.0%
Present study	PCT >0.5ng/ml CRP>6 mg/L	50	82.05% 58.97%	70% 45.45%	91.43% 79.31%	50% 23.81%

Numerous studies have been conducted on neonates to assess PCT and CRP's sensitivity and specificity. However, there is a significant amount of variation in the outcomes across the research assessing several biomarkers of newborn sepsis, which can be accounted for by varying threshold values for the biomarkers. In this study the sensitivity and specificity of PCT were 82.05% and 70% respectively which were comparable with Bharti *et al*<sup>10</sup>. Sensitivity and specificity of CRP in this study were 58.97 % and 45.45% which were comparable to Sucilathangam G *et al*<sup>15</sup> but sensitivity of PCT was higher. The PPV and NPV of PCT in this study were 91.43% and 50% which is comparable to study by Emad *et al*<sup>11</sup> in PPV but not in NPV. A meta-analysis comparing diagnostic accuracy of PCT and CRP by Pontrelli *et al*<sup>13</sup>. also showed that PCT is better marker for diagnosing neonatal sepsis.

In the ROC analysis, the area under the curve was higher for PCT (0.806 with 95% confidence interval [CI], 0.659-0.952) than for CRP (0.516), showing that PCT is more precise than CRP as early diagnostic marker of neonatal sepsis as seen in the study by Bharti *et al*<sup>10</sup> and Liu *et al*<sup>12</sup>. In this study, mean values of PCT in bacterial sepsis (16.78±22.15) and fungal sepsis (1.83±2.70) were statistically significant. Thus, PCT was useful in distinguishing bacterial and fungal sepsis. Similar findings were seen in study by Liu *et al*<sup>12</sup>.

### Limitations

Because of nonhomogeneous population and small sample size, the results of the study cannot be generalized.

### Conclusion

We conclude that PCT is better early diagnostic marker of neonatal sepsis both in term and preterm neonates as compared to CRP. PCT with clinical parameters increases the precision of diagnosing neonatal sepsis thus, limiting unnecessary use of antibiotics and thereby antibiotic resistance. PCT is also a good biomarker in distinguishing bacterial sepsis from fungal sepsis but further studies are required on this topic.

### References:

1. Shane AL, Sanchez PJ, Stoll BJ. Neonatal sepsis. *Lancet* 2017;390:1770-80.
2. Singh M, Alsaleem M, Gray CP. Neonatal Sepsis. 2021 Mar 3. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. PMID: 30285373
3. Celik IH, Hanna M, Canpolat FE, Mohan Pammi. Diagnosis of neonatal sepsis: the past, present and future. *Pediatr Res.* 2022 Jan;91(2):337-350. doi: 10.1038/s41390-021-01696-z. Epub 2021 Nov 2. PMID: 34728808; PMCID: PMC8818018.
4. Yadav P, Yadav SK. Progress in Diagnosis and Treatment of Neonatal Sepsis: A Review Article. *JNMA J Nepal Med Assoc.* 2022 Mar



- 11;60(247):318-324. doi: 10.31729/jnma.7324. PMID: 35633256; PMCID: PMC9226748.
- Cantey JB, Lee JH. Biomarkers for the Diagnosis of Neonatal Sepsis. *Clin Perinatol.* 2021 Jun;48(2):215-227. doi: 10.1016/j.clp.2021.03.012. PMID: 34030810.
  - Gude SS, Peddi NC, Vuppalapati S, Venu Gopal S, Marasandra Ramesh H, Gude SS. Biomarkers of Neonatal Sepsis: From Being Mere Numbers to Becoming Guiding Diagnostics. *Cureus.* 2022 Mar 16;14(3):e23215. doi: 10.7759/cureus.23215. PMID: 35449688; PMCID: PMC9012212.
  - Eschborn S, Weitkamp JH. Procalcitonin versus C-reactive protein: review of kinetics and performance for diagnosis of neonatal sepsis. *J Perinatol.* 2019 Jul;39(7):893-903. doi: 10.1038/s41372-019-0363-4. Epub 2019 Mar 29. PMID: 30926891.
  - Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease. I. Reference values for neutrophilic cells. *J Pediatr* 1979;95:89-98
  - Mouzinho A, Rosenfeld CR, Sanchez PJ, Risser R. Revised reference ranges for circulating neutrophils in very-low-birth-weight neonates. *Pediatrics* 1994;94:76-82
  - Bharti, Abnish & Verma, Manoj & Gupta, Anuj & Mishra, Devendra. (2020). Role of procalcitonin in diagnosis of neonatal sepsis and procalcitonin guided duration of antibiotic therapy. *International Journal of Contemporary Pediatrics.* 7. 10.18203/2349-3291.ijcp20203088.
  - Emad A. Morad, Rehab A. Rabie, Mohamed A. Almalky, Manar G. Gebriel, "Evaluation of Procalcitonin, C- Reactive Protein, and Interleukin-6 as Early Markers for Diagnosis of Neonatal Sepsis", *International Journal of Microbiology*, vol.2020, ArticleID8889086, 9 pages, 2020. <https://doi.org/10.1155/2020/8889086>.
  - Liu C, Fang C, Xie L. Diagnostic utility of procalcitonin as a biomarker for late-onset neonatal sepsis. *Transl Pediatr.* 2020 Jun;9(3):237-242. doi: 10.21037/tp-20-127. PMID: 32775242; PMCID: PMC7347773.
  - Pontrelli G, De Crescenzo F, Buzzetti R, Jenkner A, Balduzzi S, Carducci FC, et al. Accuracy of serum procalcitonin for the diagnosis of sepsis in neonates and children with systemic inflammatory syndrome: a meta-analysis. *BMC Infect Dis* 2017; 17(1):302-14.
  - Hakeem A, Mohsen A, Kamel BA. Role of procalcitonin in the diagnosis of neonatal sepsis. *Int. J Adv Res Biol Sci.* 2015;2(3):48-54.
  - Sucilathangam G, Amuthavalli K, Velvizhi G, Ashihabegum MA, Jeyamurugan T, Palaniappan N. Early diagnostic markers for neonatal sepsis: comparing procalcitonin (PCT) and C-reactive protein (CRP). *J Clin Diag Res.* 2012 May;6(4):627-31.
  - Esmat MM, Hasan A, Moghazy HM, Sadek AA. Procalcitonin or C-reactive protein or both for diagnosis of neonatal sepsis. *J Appl Sci Res.* 2012;8:4615-23.
  - Lim WH, Lien R, Huang YC, Chiang MC, Fu RH, Chu SM, et al. Prevalence and pathogen distribution of neonatal sepsis among very-low-birth-weight infants. *Pediatr Neonatol.* 2012 Aug; 53(4):228-34.
  - Koksal N, Harmanci R, Çetinkaya M, Hacimustafaoglu M. Role of procalcitonin and CRP in diagnosis and follow-up of neonatal sepsis. *Turk J Pediatr.* 2007;49(1):21-9.