Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2019, 8(4): 54-60

The Association between Neonatal Sepsis and C-reactive Protein: A Cross-Sectional Study at Tertiary Care Hospital

Mohamed Abdul Fattah[1,2], Sara Abdullah Alaskar[1], Reem Ibrahim Alkhamis[1], Fatmah Othman[2,3]* and Tarig Karar[1,2]

[1] Clinical Laboratory Science Department, College of Applied Medical Sciences, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia

[2] King Abdullah International Medical Research Center, Riyadh, Saudi Arabia

[3] Research Unit, College of Applied Medical Sciences, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia

**Corresponding e-mail: Othmanf@ksau-hs.edu.sa*

ABSTRACT

Background: *Neonatal Sepsis is a blood bacterial infection that develops in neonates and it is considered as a serious and life-threatening disease. The aim of this study was to evaluate the diagnostic value (sensitivity and specificity) of CRP in the diagnosis of neonatal sepsis.* ***Methods:*** *A cross-sectional hospital-based study was carried out at King Abdulaziz Medical City (KAMC) in Riyadh, Saudi Arabia between 2016 and 2017. In this study, we included all neonates who have been diagnosed with neonatal sepsis during the study period. Based on the result of blood culture we divided our study population into confirmed neonatal sepsis cases and neonates with clinical manifestation of sepsis and negative blood culture results.* ***Results:*** *A total of 145 neonates were included, 51 were selected in the control group (normal neonates without any signs of sepsis), 46 neonates had sepsis with a positive blood culture and 48 neonates in clinical sepsis group (with clinical signs of sepsis but their blood culture was negative). The CRP level was significantly high in neonates in the sepsis group (with a mean=7.02 ng/ml) compared with neonates in the clinical sepsis group (mean=3.73 ng/ml) and in the control group (mean=0.4 ng/ml). The frequency of the respiratory problems and jaundice was higher among neonates in the sepsis group compared to clinical sepsis group ($p<0.01$). CRP has a high sensitivity (95%) and specificity (86%) at the cut-off point of 4.09 ng/ml.* ***Conclusion:*** *CRP has been found to each high level in neonatal sepsis and it has a relatively high diagnostic value when 4.09 ng/ml is used as a cutoff point for the diagnosis of the neonatal sepsis.*

Keywords: Septicemia, Neonates, C-reactive protein

INTRODUCTION

C-reactive protein (CRP) is a part of a protein group called acute phase reactants that is produced by the liver and is considered as an inflammatory marker [1,2]. In many cases such as cardiovascular disease, cancer, autoimmune diseases and infections the CRP levels increases [3]. Thus, CRP levels reach more than 1 mg/dl throughout the body in case of inflammation. Although the usefulness of CRP remains important to determine and monitor the inflammation levels in the body, however, it cannot locate the location or the cause of the inflammation [3-6].

Neonatal sepsis is a type of blood infection usually bacterial, that develops in neonates and complete blood count (CBC) is required along with a blood culture to diagnose septicemia [7,8]. Early onset septicemia happens as a result of transmission of some bacteria from the mother to the new-born before or during delivery which usually occurs within the first 3 days of life. Late-onset septicemia happens as a result of a hospital-acquired bacteria and it occurs within 90 to 120 days after birth [9,10]. Group B *Streptococcus*, coagulase-positive *Staphylococcus*, and gram-positive bacteria are the most common pathogens that have been linked to neonatal sepsis [11,12]. In developing countries, septicemia is a known cause of mortality in neonates [7]. Screening for bacteria during pregnancy and taking the proper medications helped to decrease the problem, still, 1 to 21 per 1,000 neonates' developed neonatal sepsis [6]. Many studies have identified risk factors that were associated with an increase in mortality among neonates

who developed septicemia. In one study, the mortality rates for low birth weight neonates were 4 times higher than the mortality in normal weight neonate [6]. Other studies also have reported that between 32% to 48% of their study population with very low birth weight (1000 to 1500 grams), developed neonatal sepsis [13,14].

CRP levels along with other clinical signs and symptoms have a major role in the diagnosis and prognosis of neonatal sepsis [6,15]. Thus, the pathophysiological pathway of this is related to the stimulation of macrophage and T-cells by the bacterial infection, resulting in stimulation of interleukin-6 (IL-6) which in turn stimulate hepatocytes to increase the release of C-reactive protein from the liver [6,16]. For early diagnosis of septicemia, interleukin-6 (IL-6) and C-reactive protein have been used, however, the level of CRP is affected by many factors such as the introduction of antibiotics [6]. During this process, the levels of CRP increases by 1000-fold [6,17-22]. The present study was formulated as there was a paucity of evidence with respect to the association between neonatal sepsis and CRP as a biomarker of neonatal septicemia. The expected benefit of this study would help the clinicians to fix the period of antibiotic treatment and medical management to reduce the liver damage due to antibiotic exposure, development of bacterial resistance and neonatal mortality. Majority of studies investigating the diagnostic efficacy of the chemical markers of infection calculate optimum cutoff values for every single biomarker through the use of a receiver operator characteristic (ROC) curve. ROC curve plots sensitivity against the false-positive rate (1-specificity) for a range of multiple potential diagnostic cutoff levels [23-27]. The figure below shows an example of a ROC curve. A test with no diagnostic value would be represented as a straight line from the corner at bottom left-hand to the corner at upper right-hand and is represented by the dotted line in Figure 1 [27]. An ideal test would be represented by a line starting at the bottom left-hand corner and sharply ascend upright to 100% sensitivity, following the y-axis closely. The point closest to the left uppermost corner (inflection point) represents the cutoff point with the highest combined specificity and sensitivity or the best diagnostic accuracy and is represented as point A in the below Figure 1. Accuracy can be defined as the highest number who are diagnosed correctly as having or not having neonatal sepsis divided by the total number studied.

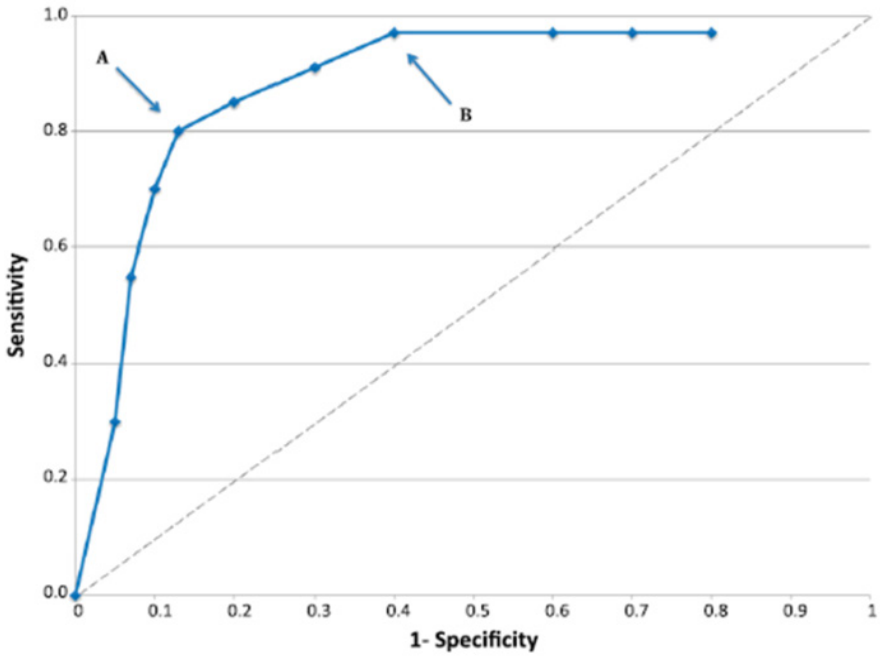

Figure 1 Example ROC curve

MATERIALS AND METHODS

We carried out a retrospective cross-sectional hospital-based study conducted at King Abdulaziz Medical City (KAMC) in Riyadh between 2013 to 2016. KAMC consider a tertiary care center that was located in the capital city of Saudi Arabia. The study population was all neonates who had recorded septicemia as a diagnosis using the ICD-10 diagnosis codes in the electronic medical file during the study period. Then the neonates were identified who had recorded diagnosis of septicemia with a positive blood culture to validate and confirm the diagnosis. According to the

hospital records, 48 neonates have a confirmed septicemia diagnosis with positive blood culture. Also, we included in our study population those neonates who have recorded diagnosis of septicemia with a negative blood culture to compare the level of CRP among the 2 groups. In order to enhance our sample, we included a control group who have no diagnosis of septicemia. Neonates with congenital malformations and congenital infections associated with the TORCH complex were excluded from the study.

For each patient, the following information was extracted: the demographic data, the clinical symptoms, the CBC results, and the level of CRP. Statistical analysis of study variables was carried out using SPSS software (version 20). Chi-square test was used for categorical variables if one or more of cells have an expected frequency of 5 or more, otherwise, Fisher's exact test was used, and we used an unpaired t-test for continuous variables. ROC curve was used to identify optimal cut-off point for CRP and diagnostic specificity and sensitivity were calculated accordingly. CRP is measured using Beckman Coulter IMMAGE 800. CRP reagent, when used in conjunction with the IMMAGE 800 Immunochemistry Systems and Calibrator 5 plus, was intended for the quantitative determination of C-reactive protein in human serum by rate nephelometry. It measures the rate of increase in light scattered from particles suspended in solution as a result of the antigen-antibody complex.

This study was approved by the Institutional Review Board (IRB) of the National Guard Health Affairs (NGHA) Riyadh, Saudi Arabia, protocol number SP17/223/R.

RESULTS

Table 1 showed the total number of subjects included in the study. A total of 145 neonates were included, 51 were selected in the control group (normal neonates without any signs of sepsis), 46 neonates had sepsis with positive blood culture, and 48 neonates were included in clinical sepsis group (with clinical signs of sepsis but their blood culture was negative). The gender distribution was similar across the groups.

Table 1 Demographic data of the study population

Variables	Sex		Total
	Male	Female	
	N (%)	N (%)	
Control	23 (45.1%)	28 (54.9%)	51
Clinical Sepsis	29 (60.4%)	19 (39.6%)	48
Sepsis	26 (56.5%)	20 (43.5%)	46

As shown in Table 2, respiratory problems and jaundice significantly increased in the proved sepsis group compared to clinical sepsis and control group ($p<0.01$). The percentage of abnormalities also increases with the approved sepsis group, 71% of neonates with sepsis had respiratory problems, 62.5% in clinical sepsis group and only 5.9% of the control group. For jaundice, 47.8% had it in approved sepsis group, 45.8% in clinical sepsis group and 7.8% in the control group.

Table 2 Clinical symptoms in study population

Variables	Control		Clinical Sepsis		Sepsis		p-value
	Normal (%)	Abnormal (%)	Normal (%)	Abnormal (%)	Normal (%)	Abnormal (%)	
Heart Rate	51 (100%)	0 (0%)	45 (93.8%)	3 (6.2%)	44 (95.7%)	2 (4.3%)	0.216
Respiratory Problems	48 (94.1%)	3 (5.9%)	18 (37.5%)	30 (62.5%)	13 (28.3%)	33 (71.7%)	0.000
Jaundice	47 (92.2%)	4 (7.8%)	26 (54.2%)	22 (45.8%)	24 (52.2%)	22 (47.8%)	0.000
Acidosis	51 (100%)	0 (0%)	43 (89.6%)	5 (10.4%)	41 (89.1%)	5 (10.9%)	0.054
Hyperglycemia	50 (98.0%)	1 (2%)	42 (87.5%)	6 (12.5%)	41 (89.1%)	5 (10.9%)	0.122
Skin Rash	49 (96.1%)	2 (3.9%)	46 (95.8%)	2 (4.2%)	44 (95.7%)	2 (4.3%)	0.994

For the level of WBC, Table 3 revealed that mean level of WBC was 12.9 cells/mm^3 ± 4.21 in control group, 13.3 cell/mm^3 ± 12.8 in sepsis group and 13.8 cell/mm^3 ± 8.5 in the clinical sepsis group.

Table 3 WBC levels in study population

Study Group	Mean cell/mm^3	Std. Deviation	Minimum cell/mm^3	Maximum cell/mm^3	p-value
Control	12.9518	± 4.2179	5.7	25.5	0.886
Sepsis	13.3535	± 12.8280	2.3	61.9	
Clinical Sepsis	13.8073	± 8.5834	2.2	29.1	

As presented in Table 4, the mean and SD of the CRP in control group was 0.456 ng/ml ± 0.78 and the sepsis group showed a higher value of CRP than the control and clinical sepsis groups with a mean of 7.02 ng/ml ± 1.9 with a maximum value of 12.1 ng/ml and minimum 3.6 ng/ml. The CRP results revealed a significant increase in the sepsis group compared with clinical sepsis and control group and were indicated by p=0.001.

Table 4 C-reactive protein (CRP) levels in study population

Study Group	Mean ng/ml	Std. Deviation	Minimum ng/ml	Maximum ng/ml	p-value
Control	0.4565	± 0.78724	0.02	4.71	<0.001
Clinical Sepsis	3.7323	± 0.74313	2.30	5.93	
Sepsis	7.0280	± 1.99545	3.60	12.17	

According to the ROC curve results shown in Table 5 and Figure 2, CRP has a high sensitivity (95%) and specificity (86%) at the cut of point of 4.09 ng/ml with an area under the curve of 0.979.

Table 5 ROC curve (receiver operating characteristic curve)

Variables	Cut point	Area Under the Curve (ROC)	Sensitivity	Specificity
CRP	4.090	0.979	95%	86%

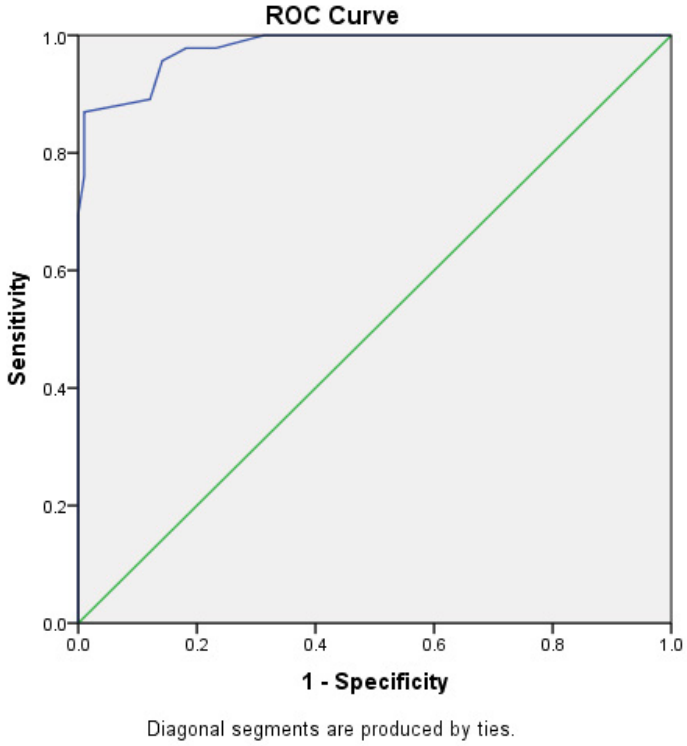

Figure 2 ROC curve of CRP

DISCUSSION

Bacterial infection in the neonate is one of the major causes of neonatal morbidity and mortality. The clinical manifestation of neonatal sepsis are not specific and usually occur in the late stages of the infection [28-31]. Therefore this study was conducted to evaluate the diagnostic value of CRP in the diagnosis of neonatal sepsis.

Abnormalities associated with sepsis include respiratory distress including tachypnea, grunting, nasal flaring, and retraction of respiratory muscles can be the sole manifestation of sepsis with or without pneumonia and can be confused with transient tachypnea of newborn initially. It was observed from the results of our study that respiratory problems had a strong association with neonatal sepsis which agreed with the results observed by previous studies that showed 96% of neonates with sepsis had respiratory problems [32,33]. Jaundice also showed a significant value which is consistent with the previous study done by Maamour, et al., [34].

WBC Levels in Study Population

Low WBCs and absolute neutrophil counts, as well as a high immature-to-total neutrophil ratio (I:T), are associated with an increased risk of infection and inflammatory disorders. Accordingly, an insignificant difference in WBCs count was observed between the study groups (p=0.88). Hence, it is an imprecise indicator for the bacterial neonatal sepsis. A study showed that complete WBCs count was not specific for the detection of neonatal sepsis or infections in general [35-37].

C-reactive Protein Levels in Study Population

However, CRP has been studied for diagnostic value in neonatal sepsis, the results of the present study showed a wide variation in the level of sensitivity and specificity [38,39]. Reviewing several studies of CRP, it has been reported that CRP level >1 ng/ml, as the cut-off point, has sensitivity ranged between 70% to 93% and specificity ranged between 41% to 98% [40,41]. In the present study, we attempt to determine the best cut-off point for CRP using ROC curve at which it can yield the best diagnostic sensitivity and specificity. A CRP level above 4.09 ng/ml has a sensitivity of 95% and specificity of 86%. The Area under the curve (AUC) was 0.979 which indicate high accuracy of the results. Mathai, et al., reported that there is an association between CRP levels and sepsis with an acceptable level in diagnostic value [42]. In previous studies, the sensitivity of CRP was ranged between 86% to 100% and specificity ranged between 70% to 100%. Diversity in different results concluded from multiple studies suggest that some physiological changes observed in the first few days of life and the effect of prenatal and postnatal antibiotic administration will affect the CRP level and functions as a cofounder in the relation between CRP and infection.

Our study has several limitations, one of these limitations is related to the observational nature of this research. Many factors such as antibiotic use and age, gestational age, maternal history of infecting were not recorded. Such factors would be helpful to examine such association in depth.

CONCLUSION AND RECOMMENDATION

It was concluded that our result agreed with our hypothesis and showed an association between high CRP levels and neonatal sepsis despite that CRP is a non-specific marker in inflammatory reactions; the relatively high specificity and sensitivity above 4.09 ng/ml level of CRP strengthen the use of this acute phase protein in the diagnosis of neonatal sepsis. However, it is recommended to test the diagnostic efficiency of CRP in a combination with other chemical markers to increase the specificity of the test.

REFERENCES

[1] Thompson, Darren, Mark B. Pepys, and Steve P. Wood. "The physiological structure of human C-reactive protein and its complex with phosphocholine." *Structure,* Vol. 7, No. 2, 1999, pp. 169-77.

[2] Lau, David CW, et al. "Adipokines: molecular links between obesity and atherosclerosis." *American Journal of Physiology-Heart and Circulatory Physiology,* Vol. 288, No. 5, 2005.

[3] Zhu, ShaoMing, et al. "Serum C-reactive protein predicts early mortality in hospitalized patients with HBV-related decompensated cirrhosis." *Medicine,* Vol. 96, No. 4, 2017.

[4] Ridker, P. M., P. Libby, and J. E. Buring. "Risk markers and the primary prevention of cardiovascular disease." *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. 11th ed. Philadelphia, PA: Elsevier* (2019).

[5] Clyne, B, and Olshaker JS. "The C-reactive protein." *Journal of Emergency Medicine*, Vol. 17, No. 6, 1999, pp. 1019-25.

[6] Hengst, Joan M. "The role of C-reactive protein in the evaluation and management of infants with suspected sepsis." *Advances in neonatal care: official journal of the National Association of Neonatal Nurses,* Vol. 3, No.1, 2003, pp. 3-13.

[7] MedlinePlus Medical Encyclopedia. "*Neonatal sepsis*." Medlineplus.gov, 2017. https://medlineplus.gov/ency/article/007303.htm.

[8] Russell, Alison R. Bedford. "Neonatal sepsis." *Pediatrics and Child Health,* Vol. 21, No. 6, 2011, pp. 265-69.

[9] MSD Manual Professional Edition. *Neonatal Sepsis*. MSD Manual Professional Edition. 2017. http://www.msdmanuals.com/professional/pediatrics/infections-in-neonates/neonatal-sepsis.

[10] MacDonald, Mhairi G., and Mary M. Seshia. Avery's Neonatology: pathophysiology and management of the newborn. Lippincott Williams and Wilkins, 2015.

[11] Fanaroff, Jonathan M., and Avroy A. Fanaroff. *Klaus and Fanaroff's Care of the High-Risk Neonate E-Book*. Elsevier Health Sciences, 2012.

[12] Simonsen, Kari A., et al. "Early-onset neonatal sepsis." *Clinical Microbiology Reviews,* Vol. 27, No. 1, 2014, pp. 21-47.

[13] Sobaih, B. Hasan, and H. Al-Mandeel. "Early and late-onset neonatal sepsis in very low birth weight infants in a tertiary center in Saudi Arabia." *Journal of Neonatal Biology,* Vol. 3, 2013, p. 159.

[14] Al-Nemri, Abdurrahman M., and Suliman H. Al-Fifi. "Cause-specific neonatal mortality at the armed forces hospital, southern region Khamis Mushayt, Kingdom of Saudi Arabia." *The Medical Journal of Cairo University,* Vol. 79, No. 2, 2011.

[15] Chiesa, Claudio, et al. "Diagnosis of neonatal sepsis: A clinical and laboratory challenge." *Clinical Chemistry,* Vol. 50, No. 2, 2004, pp. 279-87.

[16] Hisamuddin, Effat, et al. "Validity of C-reactive protein (CRP) for diagnosis of neonatal sepsis." *Pakistan Journal of Medical Sciences,* Vol. 31, No. 3, 2015, p. 527.

[17] Nibedita, P., B. Manasi, and M. Dipti. "Study of C-reactive protein in healthy neonates." *International Journal of Pediatric Research,* Vol. 3, No. 6, 2016, pp. 380-84.

[18] Landry, Alexander, et al. "Causes and outcomes of markedly elevated C-reactive protein levels." *Canadian Family Physician,* Vol. 63, No. 6, 2017, pp. 316-23.

[19] Chiesa, Claudio, et al. "C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection." *Clinical Chemistry,* Vol. 49, No. 1, 2003, pp. 60-68.

[20] Mahmood, A., K.A. Karamat, and T. Butt. "Neonatal sepsis: high antibiotic resistance of the bacterial pathogens in a neonatal intensive care unit in Karachi." *Journal of Pakistan Medical Association,* Vol. 52, No. 8, 2002, pp. 348-50.

[21] Kawamura, M., and H. Nishida. "The usefulness of serial C-reactive protein measurement in managing neonatal infection." *Acta Paediatrica,* Vol. 84, No. 1, 1995, pp. 10-13.

[22] Wagle, S., et al. "C-reactive protein as a diagnostic tool of sepsis in very immature babies." *Journal of Paediatrics and Child Health,* Vol. 30, No. 1, 1994, pp. 40-44.

[23] Parviz, Ayazi, et al. "Comparison of serum IL-1beta and C-reactive protein levels in early diagnosis and management of neonatal sepsis." *Le Infezioni in Medicina,* Vol. 4, 2014, pp. 296-301.

[24] Dagan, Ron, et al. "Identification of infants unlikely to have serious bacterial infection although hospitalized for suspected sepsis." *The Journal of Pediatrics,* Vol. 107, No. 6, 1985, pp. 855-60.

[25] Carr, Robert, et al. "Granulocyte-macrophage colony stimulating factor administered as prophylaxis for reduction of sepsis in extremely preterm, small for gestational age neonates (the PROGRAMS trial): a single-blind, multicentre, randomized controlled trial." *The Lancet,* Vol. 373, No. 9659, 2009, pp. 226-33.

[26] Delanghe, Joris R., and Marijn M. Speeckaert. "Translational research and biomarkers in neonatal sepsis." *Clinica Chimica Acta,* Vol. 451, 2015, pp. 46-64.

[27] Fattah, M. A., et al. "Utility of cytokine, adhesion molecule and acute phase proteins in early diagnosis of neonatal sepsis." *Journal of Natural Science, Biology, and Medicine*, Vol. 8, No. 1, 2017, p. 32.

[28] Horan, Teresa C., Mary Andrus, and Margaret A. Dudeck. "CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting." *American Journal of Infection Control*, Vol. 36, No. 5, 2008, pp. 309-32.

[29] Døllner, Henrik, Lars Vatten, and Rigmor Austgulen. "Early diagnostic markers for neonatal sepsis: comparing C-reactive protein, interleukin-6, soluble tumor necrosis factor receptors, and soluble adhesion molecules." *Journal of Clinical Epidemiology*, Vol. 54, No.12, 2001, pp. 1251-57.

[30] Prashant, Akila, et al. "Comparative assessment of cytokines and other inflammatory markers for the early diagnosis of neonatal sepsis-A case-control study." *PloS One*, Vol. 8, No. 7, 2013.

[31] Vigushin, David M., Mark B. Pepys, and Philip N. Hawkins. "Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease." *The Journal of Clinical Investigation*, Vol. 91, No. 4, 1993, pp. 1351-57.

[32] McArdle, Andrew J., et al. "Determinants of carboxyhemoglobin levels and relationship with sepsis in a retrospective cohort of preterm neonates." *PloS One*, Vol. 11, No. 8, 2016.

[33] Hermansen, Christian L., and Kevin N. Lorah. "Respiratory distress in the newborn." *American Family Physician*, Vol. 76, No. 7, 2007, pp. 987-94.

[34] Maamouri, Gholmali, et al. "Hyperbilirubinemia and Neonatal Infection." *International Journal of Pediatrics,* Vol. 1, No. 1, 2013, pp. 5-12.

[35] Simonsen, Kari A., et al. "Early-onset neonatal sepsis." *Clinical Microbiology Reviews*, Vol. 27 No. 1, 2014, pp. 21-47.

[36] Manroe, Barbara L., et al. "The neonatal blood count in health and disease. I. Reference values for neutrophilic cells." *The Journal of Pediatrics*, Vol. 95, No. 1, 1979, pp. 89-98.

[37] Mally, Pradeep, Jie Xu, and K. D. Hendricks-Muñoz. "Biomarkers for neonatal sepsis: recent developments." *Research and Reports in Neonatology*, Vol. 4, No. 157, 2014, p. e68.

[38] Monneret, Guillaume, et al. "Procalcitonin and C-reactive protein levels in neonatal infections." *Acta Paediatrica*, Vol. 86, No. 2, 1997, pp. 209-12.

[39] 39 Lapillonne, Alexandre, et al. "Lack of specificity of procalcitonin for sepsis diagnosis in premature infants." *The Lancet*, Vol. 351, No. 9110, 1998, pp. 1211-12.

[40] Caksen, H., et al. "The relationship between scoring systems and cytokine levels in neonatal sepsis." *Annals of the Academy of Medicine, Singapore*, Vol. 32, No. 3, 2003, pp. 418-20.

[41] Chiesa, Claudio, et al. "C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection." *Clinical Chemistry*, Vol. 49, No. 1, 2003, pp. 60-68.

[42] Mathai, Elizabeth, et al. "Is C-reactive protein level useful in differentiating infected from uninfected neonates among those at risk of infection." *Indian Pediatrics*, Vol. 41, No. 9, 2004, pp. 895-900.