Original Article ISSN (O):2395-2822; ISSN (P):2395-2814

Can CRP and Band Cell Be Protagonist in Neonatal Septicemia?

Sandeep Negi[1], Lakshmi Bala[2], Anand Jain[3], Manoj Joshi[4], Vinyl Kumar Pahuja[5]

[1]Research Scholar, Department of Biochemistry, Babu Banarasi Das University, Lucknow, U.P, India
[2]Professor & Head, Department of Biochemistry, Babu Banarasi Das University, Lucknow, U.P, India
[3]Professor, Dept. of Pediatrics, Veer Chandra Singh Garhwali Govt. Medical Science & Research Institute, Srinagar Garhwal, Uttarakhand, India.
[4]Microbiology Technologist, Dept. of Microbiology, Veer Chandra Singh Garhwali Govt. Medical Science & Research Institute, Srinagar Garhwal, Uttarakhand, India.
[5]Assistant Professor, Dept. of Microbiology, Veer Chandra Singh Garhwali Govt. Medical Science & Research Institute, Srinagar Garhwal, Uttarakhand, India.

Received: January 2021
Accepted: February 2021

ABSTRACT

Background: Neonatal sepsis in neonates is a common cause of death. It is a medical condition identified in less than 28 days of life by signs and symptoms of infection with or without accompanying bacteremia. Based on a variety of variables, occurrence varies among hospitals. Blood culture is considered the diagnostic gold standard but does not provide a fast outcome. Therefore, for assessing neonatal sepsis, there is a need to look for a surrogate marker. **Methods:** Study design: In the present study total of 372 neonates were studied for clinically suspected septicemia from March 2019 to February 2020. **Results:** At the end of the study we found neonatal septicemia was observed maximum in male neonates (62.63%). The majority of the cases (55.10%) had C-Section delivery. Cases of early-onset (48.65%) were seen slightly less than late (51.43%). The majority of risk factor was seen in Meconium Stained Liquor (MSL). Out of the total 372 samples only (128/372; 34.40%), blood culture showed positive results after analysis rest cultures were sterile. Out of total positive cultures, band cells were reported in 89.84% of samples. CRP test was also showed positive results in 90.62% cases out of all positive blood cultures. We obtained 69.53% bacterial and 39.30% fungal isolates from the positive blood culture samples. The final calculated value of sensitivity, specificity, positive predictive value (PPV), Negative predictive value (NPV) and diagnostic accuracy for Band cell was found to be 89.84%,79.09%,69.27%,93.68% and 82.79% respectively, while its value in case of CRP test was recorded 90.62%,43.03%,45.49%,89.74% and 59.40% respectively. Among bacterial isolates Klebsiella sp. and Staphylococcus aureus were found in the majority of cases. While in the case of fungal isolates Candida sp. was reported to be the main cause of neonatal sepsis. **Conclusion:** The Band cell and CRP test analysis against blood culture can be considered as a surrogate marker for rapid and early diagnosis of neonatal septicemia. These tests are also helpful for clinicians to take a quick decision during initiation or discontinuation of the antibiotic treatment.

Keywords: Septicemia, CRP, Blood culture, Band cells, Candida albicans.

INTRODUCTION

Neonatal sepsis accounts for over three million cases per year worldwide in which India has the highest number of incidences in the world (1,70,000 vs 22,020 per million live births). Sepsis is the second major cause of global neonatal mortality.The mortality rate in India ranges between 25%-65%, which is likely to be underestimated as Low- and Middle-Income Countries (LMICs) yet lack a consensus definition of neonatal sepsis, standardization of diagnostic criteria, and culture test facilities.[1] The criteria for the initial diagnosis of neonatal sepsis have been established by the WHO, but the sensitivity and specificity of clinical diagnosis can vary significantly. Neonatal septicemia is characterized as localized systemic infection or condition arising from an adverse reaction to the presence of an infectious agent or its toxins.[2] There are unspecific clinical symptoms of neonatal sepsis, so it is difficult to clinically diagnose sepsis, and laboratory assistance is required.[3] W.H.O guidelines are not applicable in many developing countries as many primary health centers in these countries are not equipped with blood culture facilities.Therefore to avoid deaths and complications due to septicemia, rapid and early diagnosis and appropriate treatment are necessary.[4] In diagnosing neonatal sepsis, blood culture remains a gold standard, but findings are usually obtained after three to five days and in different studies, its accuracy ranges between eight and 73 percent.[5,6] However, several screening measures (WBC counts, platelet counts, micro erythrocyte sedimentation rate (ESR), absolute neutrophilic count (ANC), C-reactive protein (CRP), (I/T) ratio, nitroblue teterazolium (NBT), serial interleukin-6 (IL-6) and pro-calcitonin) can predict sepsis within 6-8 Hrs.[7,8] The present study was done to detect an important diagnostic role of band cells and CRP test against blood culture test, collected from NICU in the

Name & Address of Corresponding Author
Dr. Vinyl Kumar Pahuja,
M.D. (Microbiology)
Department of Microbiology,
Veer Chandra Singh Garhwali Govt. Medical Science & Research Institute, Srinagar Garhwal, Uttarakhand, India.
Email: vinylpahuja2@gmail.com

suspected cases of neonatal sepsis admitted in Base Hospital of Govt. Medical College (VCSGGMSRI), Srinagar, Uttarakhand.

Study design

Blood samples were collected from 372 neonates admitted for clinically suspected neonatal sepsis in the NICU at Base Hospital of Govt. Medical College (VCSGGMSRI), from March 2019 to February 2020, data was analyzed prospectively.

Selection criteria for Subjects

Inclusion criteria:

1. Neonates of both sexes were included in this study.
2. Neonates that display signs and symptoms such as rejection as the refusal to feed, lethargy, fever, hypothermia, vomiting, diarrhea, abdominal distension, jaundice, respiratory distress, seizures, or any external evidence of sepsis-like umbilical cord infection, skin infection, etc. were taken up for study.
3. A sample was included in this study showing the growth of organisms of low pathogenicity, on repeat culture.

Exclusion criteria:

1. Neonates with the absence of signs of sepsis were excluded from this study.
2. Low pathogenic organisms like CoNS, Candida sp. unless grown on repeat culture were excluded.

MATERIALS AND METHODS

Blood culture

Using an aseptic procedure, each neonatal blood (1-2 ml) was collected and inoculated directly into the blood culture bottle, Brain Heart Infusion Broth (Himedia Laboratories), and transported for incubation and subsequent analysis to the Microbiology Laboratory, Base Hospital of Govt. Medical College (VCSGGMSRI), Srinagar.

Laboratory Techniques

The blood culture bottle was incubated aerobically at 37°C temperature after collection. Gram stain was done after 24 Hrs, followed by subculture on 5% sheep blood agar, chocolate agar, and MacConkey agar (Himedia Laboratories). If no growth was seen on the media plates, then blood culture bottles were further incubated and examined daily for seven days. In case growth on media, plates were observed, then the bacterial isolates were identified by conventional physiological and biochemical tests.[9] Various types of tests were conducted for identification of gram-positive bacteria and gram-negative bacteria, like gram stain, catalase, coagulase, hemolytic activity on a blood agar plate and CAMP test, colony characteristics found on media plates, triple sugar iron reaction, indole, mannitol motility, citrate, urease, development of H2S, VP test, etc.[10] Candida albicans and non-albicans candida sp. were differentiated with the help of a germ-tube test. In this test appearance of a germ tube in the wet mount was prepared by mixing 2-4 colonies of candida sp. in 0.5ml of rabbit serum up to 3-4 Hrs. at 37°C temperature, indicates a positive test that finally confirms candida albicans.[11]

For band cells (immature neutrophil cells) examination:-

Thin smears from blood were prepared over glass slide first, then stained with Leishman's stain and further analyzed microscopically. The band cells (%) were calculated by using the following formula.[12]

Band cells (%) =Total number of band cells/Total number of nuetrophils (mature+immature)*100

Using the CRP latex kit developed by Tulip diagnostics (P) Limited, the C-reactive protein was semi-quantitatively estimated. A serum CRP level of >= 6 mg/L was considered the lowest clinical significance concentration; the CRP latex reagent was standardized. In terms of micrograms per dl, the CRP amount can be determined by multiplying the highest dilution with clear-cut agglutination by a factor of 6.

Statistical analysis:

Results were analyzed using the Chi-square method for statistical significance.

Ethical Clearance:

Ethically approved study, along with consent form was filled and signed by parents/guardians of neonates who were informed about their participation in the study and also informed to maintain confidentiality about their details.

RESULTS

In the present study total of 372 neonates for clinically suspected septicemia. Male (233/372; 62.63%) preponderance was observed as compared to females (139/372;37.36%), as shown in figure 1(A). We noticed that neonates delivered by C-Section (LSCS) (205/372;55.10%) was greater in number than normal vaginal delivery (163/372; 43.81%) and other techniques were used, including instrumentation and vacuum-assisted delivery, (4/372;1.07%), as shown in figure 1(B). Early-onset of septicemia was observed clinically in (181/372;48.65%) neonates, out of which (49/181;27.07%) were blood culture positive. While (191/372; 51.34%) had a late onset of septicemia and (79/191; 41.36%) were blood culture positive, as displayed in [Table 1].

Table 1: Onset of neonatal septicemia

Onset	Blood Culture Negative	Blood Culture Positive	Total
Early	132	49	181
Late	112	79	191

Chi square =8.4079, P=0.00373 (Significant)

After analysis of different risk factors, we found meconium-stained liquor (MSL) dominated followed by premature rupture of membranes (PROM), mechanical ventilator support (MVS), and twin deliveries, as shown in [Table 2].

Table 2: Distribution of risk factors in neonates (n=372)

Risk Factor	Present in Neonates (%)	Absent in Neonates (%)
MSL	135(36.29%)	237(63.70%)
PROM	109(29.30%)	263(70.69%)
MVS	29(7.79%)	343(92.20%)
Twin Delivery	8(2.15%)	364(97.84%)

Chi square =197.789, P<0.00001 (Significant)

Blood cultures when analyzed showed that (244/372; 65.59%) cultures were sterile or negative while (128/372;34.40%) were recorded positive. Out of the blood culture-positive cases (128/372;34.40%), band cells were observed in (115/128; 89.84%) cases. As the band cell (%) increased the blood culture positivity also increased. A band cell ratio above 11% was found to correlate 100% with culture positivity, as shown in table 3.Out of the blood culture-positive cases (128/372; 34.40%), the CRP test was positive in (116/128;90.62%) cases, as illustrated in [Table 3].

Table 3: Comparison of Blood culture with Band cells and CRP

Band Cells (%)	Blood culture Positive (n=128)	Blood culture Negative (n=244)	p-value
Band cell Positive (%>11)	115 (89.84%)	51 (20.90%)	Chi square=161.486 P<0.0001 (Significant)
Band cell Negative (%<11)	13 (10.15%)	193 (79.09%)	
Total	128	244	
CRP Test	Blood culture Positive (n=128)	Blood culture Negative (n=244)	p-value
CRP Positive	116 (31.18%)	139 (56.96%)	Chi square=44.115 P<0.0001 (Significant)
CRP Negative	12(3.22%)	105 (43.03%)	
Total	128	244	

Table 4: sensitivity, specificity, PPV, NPV, and diagnostic accuracy of Band cell and CRP test

Name of test	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy
Band Cell	89.84%	79.09%	69.27%	93.68%	82.79%
CRP Test	90.62%	43.03%	45.49%	89.74%	59.40%

[Table 4] displays the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of the CRP and Band cell tests.

Out of positive blood culture (128/372; 34.40%), we found bacterial isolates in (89;69.53%) bacterial cultures and fungus in (39;30.46%) , as shown in [Figure 5]. The Distribution of bacterial isolates isolated from positive blood cultures was Klebsiella sp. (22/89;24.71%), followed by Enterobacter sp. (13/89;14.60), Escherichia coli (13/89;14.60%), Pseudomonas sp. (4/89;4.49%), Citrobacter sp. (1/89;1.12%), and Acinetobacter sp. (1/89;1.12%) from the gram-negative category, as shown in [Figure 6] (A), while Staphylococcus aureus (18/89;20.22), coagulase-negative Staphylococcus (15/89;16.85%) and Enterococcus sp. (2/89;2.24%) were found from the gram-positive group, as shown in [Figure 6] (B). Out of these fungal isolates (39;30.46%), we found (11;28.20%) were Candida albicans while others (28;71.79%) were non albicans Candida species, as shown in [Figure 7] (A).

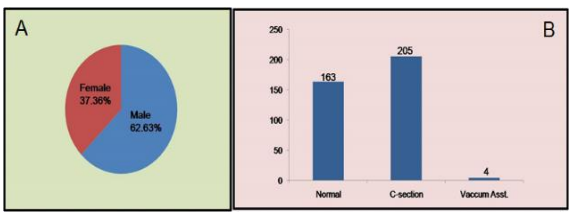

Figure 1: (A) Sex distribution of neonates (B) Distribution of delivery method

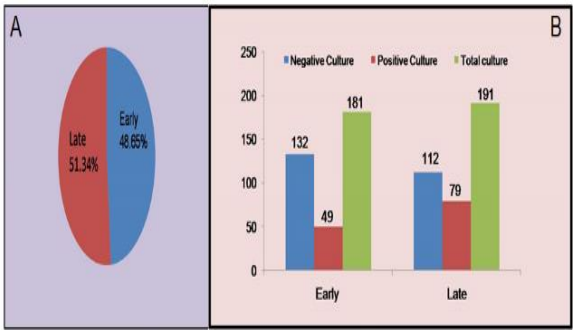

Figure 2: (A) onset of septicemia (B) Blood culture record in both early and late

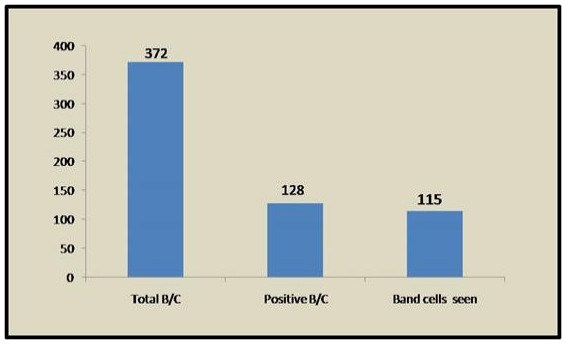

Figure 3: Total blood culture, positive blood culture and band cells seen

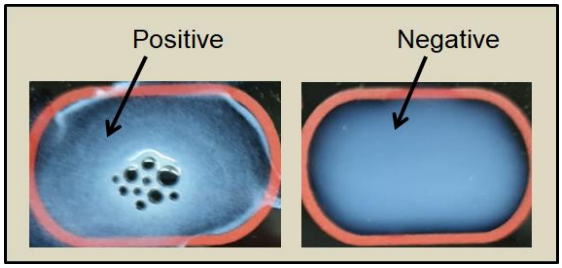

Figure 4: CRP test plate showing positive and Negative test

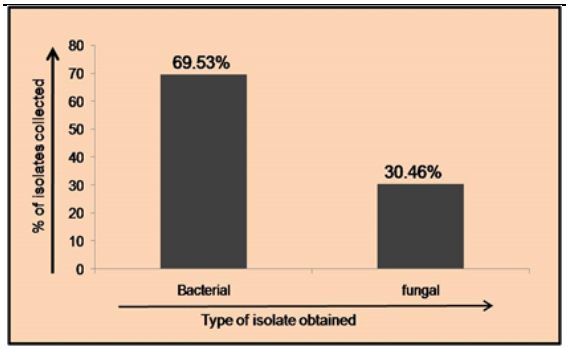

Figure 5: Distribution of isolates obtained in blood culture of neonates

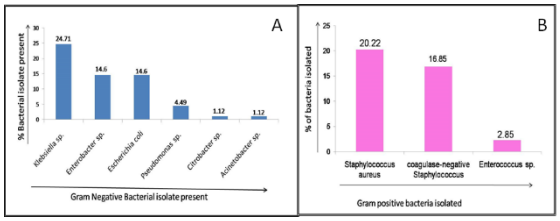

Figure 6: Distribution of bacterial isolates present in neonatal Blood culture (A) Gram Negative bacteria (B) Gram positive bacteria

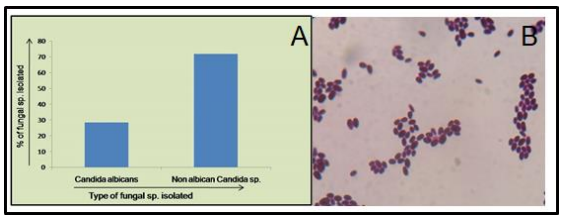

Figure 7: (A) Isolates of fungus sp. collected (B) Microscopic image of candida sp.

DISCUSSION

In the present study, we found neonatal septicemia was observed maximum in male (62.63%) as compared to female (37.36%) neonates. A recent study by Gupta et al (2020) also reported a similar type of sex distribution,[3] male (53%) and female(47%). The various other studies like Saboohi et al (2019),[12] Hisamuddin et al (2015),[13] also reported male preponderance in neonatal sepsis as compared to female. The higher incidence of sepsis in C-section delivery (205/372;55.10%) was reported in our study, this may be due to unhygienic conditions and possible nosocomial infections. The study by Kayange et al (2010),[14] and Yang AP et al (2015) reported neonates delivered by normal vaginal delivery were greater than C-section delivery. In the present study, we reported onset of early sepsis (48.65%) and late sepsis (51.34%) which was similar to studies like Kayange et al (2010),[14] which reported onset of sepsis in early (40%) and late (60%). In the present study blood cultures when analyzed showed that (128/372; 34.40%) were recorded positive. In recent study by Gupta et al (2020),[3] reported (35.81%) positive, Bunduki et al (2020),[15] reported (30.3%),Saboohi et al (2019),[12] reported (15.29%) and Hisamuddin et al (2015),[13] reported (29.25%). In the present study out of 128 positive blood culture samples, band cells were seen in 115 (89.84%) which indicates its higher sensitivity for the testing of neonatal septicemia. The results of sensitivity, specificity, PPV, and NPV of Band cells in the present study were similar to Saboohi et al (2019),[12] (83.82%, 76.47%, 54.16%, 93.44). These results are inlaying with other studies perform in other region of India.[20]

In our study out of 128 blood culture-positive samples,116 (90.62%) were positive for the CRP test which was similar to studies done by Gupta et al (2020),[3] reported (68.24%)and Hisamuddin et al (2015),[13] reported (70.74%) with a greater percentage of CRP positive in blood culture. In some studies like Bunduki et al (2020),[15] reported (41.2%), Saboohi et al (2019),[12] reported (32.94 %). This indicated the number of CRP positive tests in blood culture may vary with sample size taken or other factors. The results of sensitivity, specificity, PPV, NPV, and diagnostic reliability of CRP in the present study was similar to various other studies like Gupta et al (2020),[3] (86.7%,43%,45.5%,85% and 69%), Hisamuddin et al (2015),[13] (76.92%, 53.49%, 80%,48.94% and 70.07%), Bunduki et al (2020),[15] (95.7%, 82.4%, 70.2%, 97.8%).

The pattern of bacterial distribution in the current study which was isolated from positive blood cultures was Klebsiella sp. (24.71%), Enterobacter sp. (14.60), Escherichia coli (14.60%), Pseudomonas sp. (4.49%), Citrobacter sp. (1.12%), and Acinetobacter sp. (1.12%) from the gram-negative category, while Staphylococcus aureus (20.22), coagulase-negative Staphylococcus (16.85%) and Enterococcus sp. (2.24%) were found from the gram-positive group similarly in other studies like Jain A et al (2003),[16] and Yang AP et al (2015),[17] also found these isolates in their findings. Among gram-negative bacteria Klebsiella sp. and Staphylococcus aureus in gram-positive bacteria were reported in the majority by Jain A et al (2003),[16] same as in our present study. In this particular Garhwal Himalayan region recently Negi et al (2021),[18] pointed out the MDR strains of Klebsiella sp, which were resistant to many antibiotics. Their presence in the hospital environment may risk patients from life-threatening nosocomial infection, including neonatal sepsis. In our study among fungal isolates, we found (28.20%)

were Candida albicans while others (71.79%) were non albicans Candida species similarly Jain A et al (2003),[16] and Juyal et al (2013) also reported candida sp. during blood culture of neonates. In our study, we found Candida sp. is the main cause of neonatal sepsis due to fungal isolates in this particular Himalayan region. Rodwell hematological scoring system is not feasible in the limited setting of district hospitals of low income countries like India. These two tests may provide as sensitive indicator for the clinical sepsis in neonates.[20]

We study about the role of these two alternative test for early diagnosis of neonatal septicemia, as sensitivity of blood culture test is less and these tests are affordable, in low income countries like India. There is requirement of alternative test that are easily available and accessible in a small setup.

CONCLUSION

For rapid and early diagnosis of neonatal septicemia, the Band cell and CRP test can be considered as a surrogate marker. Both tests are useful for underdeveloped countries where blood culture assays are not readily available or where pediatricians have conditions waiting for blood culture report for a long duration of time. These tests are also helpful for clinicians to take a quick decision during initiation or discontinuation of the antibiotic treatment. It is a major health concern for the concerned authorities to take effective steps to minimize the spread of nosocomial infections, to preserve cleanliness and hygiene. Regular practice of hand washing and sanitization by doctors, staff, attendants, and patients can save many lives from life-threatening drug-resistant strains.

REFERENCES

1. Murthy S, Godinho MA, Guddattu V, Lewis LES, Nair NS. Risk factors of neonatal sepsis in India: A systematic review and meta-analysis. PLoS One. 2019 ;25 ;14 (4):e 0215683.doi: 10.1371/journal.pone.0215683. PMID: 31022223; PMCID: PMC6483350.
2. Singh M. Care of the new born revised 8ed (2017). CBS Publishers & Distributors Private Limited; 2017;30.
3. Gupta A, Rajput D, Sodani S, Mutha A. Comparison of CRP with blood culture in the diagnosis of neonatal septicaemia. Indian J Microbiol Res. 2020;15 :7(4):327-29.
4. The WHO Young Infants Study Group: Clinical predilection of serious bacterial infection in young infants in developing countries. Pediatr Infect Dis J .1999;18:s23-31.
5. Hornik CP, Benjamin DK, Becker KC, Benjamin Jr DK, Li J, Clark RH, Cohen-Wolkowiez M, Smith PB. Use of the complete blood cell count in early-onset neonatal sepsis. Pediatr Infect Dis J. 2012;31(8):799.
6. Marcdante K, Kliegman RM. Nelson essentials of pediatrics e-book. Elsevier Health Sciences. 2014;25.
7. Newman TB, Draper D, Puopolo KM, Wi S, Escobar GJ. Combining immature and total neutrophil counts to predict early onset sepsis in term and late preterm newborns: use of the I/T2. Pediatr Infect Dis J. 2014;33(8):798.
8. World Health Organization: Essential Newborn Care. In A report of a Technical Working Group WH0 Geneva; 1995
9. Collee JG, Miles RS, Watt B. Tests for identification of bacteria. Mackie and McCartney practical medical microbiology.1996;14:131-49.
10. Murray Baron, Pfaller Tenover: Manual of Clinical Microbiology. 6th edition. American Society of Microbiology Press,Washington DC;1999.
11. Sheppard DC, Locas MC, Restieri C, Laverdiere M. Utility of the germ tube test for direct identification of Candida albicans from positive blood culture bottles. J. Clin. Microbiol. 2008 ;1:46(10):3508-9.
12. Saboohi E, Saeed F, Khan RN, Khan MA. Immature to total neutrophil ratio as an early indicator of early neonatal sepsis. PaK J Med Sci.. 2019;35(1):241.
13. Hisamuddin E, Hisam A, Wahid S, Raza G. Validity of C-reactive protein (CRP) for diagnosis of neonatal sepsis. PaK J Med Sci.. 2015;31(3):527.
14. Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC pediatrics. 2010;10(1):1-9.
15. Bunduki, G.K., Adu-Sarkodie, Y. The usefulness of C-reactive protein as a biomarker in predicting neonatal sepsis in a sub-Saharan African region.BMC Res Notes.2020;13:194. https://doi.org/10.1186/s13104-020-05033-1
16. Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. Prevalence of extended-spectrum β-lactamase-producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital. J. Med. Microbiol..2003;1:52(5):421-5.
17. Yang AP, Liu J, Yue LH, Wang HQ, Yang WJ, Yang GH. Neutrophil CD64 combined with PCT, CRP and WBC improves the sensitivity for the early diagnosis of neonatal sepsis. Clin. Chem. Lab. Med. (CCLM). 2016;1:54(2):345-51.
18. Negi S, Joshi M, Pahuja VK, Bala L. Antibiotic Resistance Pattern of Klebsiella sp. at a Tertiary Care Centre from Garhwal Himalayan Region. Ann. int. med. dent. res..2021;7(1):6.
19. Juyal D, Sharma M, Pal S, Rathaur VK, Sharma N. Emergence of non-albicans Candida species in neonatal candidemia. North Am J Med Sci. 2013;5(9): 541.
20. Narasimha A, Harendra Kumar ML. Significance of Hematological Scoring System (HSS) in Early Diagnosis of Neonatal Sepsis. Indian J Hematol Blood Transfus. 2011 Mar;27(1):14-7. doi: 10.1007/s12288-010-0050-2. Epub 2011 Jan 1. PMID: 22379289; PMCID: PMC3102509.

How to cite this article: Negi S, Bala L, Jain A, Joshi M, Pahuja VK. Can CRP and Band Cell be Protagonist in Neonatal Septicemia?. Ann. Int. Med. Den. Res. 2021; 7(2):MB07-MB11.

Source of Support: Nil, **Conflict of Interest:** None declared