ISSN: 2573-9611

Research Article

Journal of Pediatrics & Neonatal Biology

Procalcitonin Levels in Neonatal Gram-Positive and Gram-Negative Bacterial Sepsis- a Differentiating Marker?

Samaha S. Mustapha[1*], Mukhtar Idris[2], Shamsudin Aliyu[2], Isa Abdulkadir[3] and William N. Ogala[1]

[1]*Department of Paediatrics Abubakar Tafawa Balewa, Bauchi.*

[2]*Department of Medial Microbiology Ahmadu Bello University Zaria*

[3]*Department Paediatrics Ahmadu Bello University, Zaria*

[*]Corresponding Author
Samaha S. Mustapha, Department of Paediatrics Abubakar Tafawa Balewa, Bauchi.

Submitted: 05 Mar 2023; **Accepted:**30 Mar 2023; **Published:**12 April 2023

Citations: Mustapha, S. S., Idris, M., Aliyu, S., Abdulkadir, I., Ogala, W. N. (2023). Procalcitonin Levels in Neonatal Gram-Positive and Gram-Negative Bacterial Sepsis- a Differentiating Marker?. *J Pediatr Neonatal Biol, 8*(2), 174-178.

Abstract

Neonatal sepsis diagnosis has remained challenging despite advances in neonatal care and scientists have sought the use of biomarkers in its diagnosis. Procalcitonin has shown the most promise amongst the biomarkers and has been proposed to even differentiate between gram negative and positive infection in adults. The aim of the study is to find out if procalcitonin can be used to differentiate between gram positive and negative infection in neonates as in adults. The study was carried out in a tertiary hospital in Zaria, Kaduna state, Nigeria over a period of ten months. Two hundred and forty-eight neonates with clinical features of sepsis were enrolled into the study and had their blood samples taken for investigation which include complete blood count, C-reactive protein, procalcitonin and blood culture. Common clinical features as it is found with sepsis were non-specific and include fever, poor suck, jaundice, and depressed primitive reflexes. Ninety-four neonates had confirmed sepsis with gram negative organism accounting for 52.3% of the isolates. Procalcitonin level differed between gram negative and positive infection, Enterobacteriaceae and non-fermentative gram-negative aerobes and between various isolates, but it was found to be statistically not significant using Kruskal Wallis-H test. Evidence as at now does not support our supposition therefore, more studies need to be carried out to substantiate or refute our finding.

Keywords: Neonatal Sepsis, Procalcitonin, Gram Positive Bacteria, Gram Negative Bacteria, Differentiating

Introduction

Neonatal sepsis (NS) is one of the leading causes of morbidity and mortality in the first 28-days of life. It contributes to 18% of neonatal deaths even with recent advances in perinatal and neonatal care [1]. This is largely because clinical features are non-specific, neonates have low level bacteraemia and blood culture, which remains the gold standard for its diagnosis, has inherent limitations; - it is cumbersome, has low yield and consumes time. Thus, this has created an impetus for the search for an ideal biomarker that can solve this diagnostic dilemma amongst which procalcitonin has shown the most promise [2]. Procalcitonin (PCT) is a 116-amino acid polypeptide precursor of calcitonin [2]. Increased levels of PCT at the onset of bacterial infection and sepsis was first demonstrated Assicot et al in 1993[3]. Inflammatory PCT is produced by parenchymal tissues and other differentiated cell types either directly via microbial toxins (endotoxin) or indirectly via a humoral or cell-mediated host response (IL-1b, TNF-α, IL-6). This induction can be attenuated by cytokines released during a viral infection (interferon γ) [2,4]. Its level demonstrates excellent correlation with bacterial load and the severity of the infection and provides indications of possible progression of infection-septic shock [2, 5]. hile there is a paucity of data in neonates, several studies have shown higher PCT concentration in adults with gram-negative than gram-positive bacterial infections and therefore, proposed it is discriminatory/ differentiating [5-10]. This study aims to determine if procalcitonin can be used to differentiate between bacterial gram-negative and gram-positive sepsis in neonates.

Materials and Methods

We conducted a cross-sectional study at the neonatal unit of Ahmadu Bello University Teaching Hospital (ABUTH) - a tertiary hospital located in Zaria, Kaduna state. The unit has a 30-bed capacity across its in-born and out-born wards and an average number of 900 admissions per year. The study was carried out over a 10-month period from October 2018-July 2019. All neonates who were admitted over the period of study with diagnosis of sepsis

were recruited at the point of admission in the neonatal unit. Their samples were taken for investigation including blood culture, complete blood count (CBC), C-reactive protein (CRP) and procalcitonin (PCT). The study was approved by the Health Research and Ethics Committee of the hospital.

Procalcitonin Determination

Procalcitonin level was measured in whole blood using the Finecare™ CRP/PCT (C-Reactive Protein/Procalcitonin) rapid quantitative test strip along with Finecare™ FIA Meter according to manufacturer's instructions. The working range and the detection limits of the CRP/PCT Test system were for CRP, 0.5-150 mg/L and 0.5mg/L respectively, and for PCT, 0.1-100ng/ml and 0.1ng/ml, respectively.

Bacterial Identification

Blood specimen (3mls) from recruited neonates were taken under aseptic condition and immediately transferred into blood culture bottle containing 50mls of Tryptic Soy Broth and incubated aerobically at 37oC for 24hours. Broth with signs of growth were immediately removed and sub-cultured on 5% Sheep blood and MacConkey agar plate and incubated in same condition as the broth above. Growths from both culture plates were examined and gram stained. Gram negative rods were again sub-cultured on Nutrient agar (NA) for further identification and biochemical testing. MicrobactTM 24E (Oxiod UK) was used to identify the organisms according to the manufacturer's instructions.

Statistical Analysis

Data were analysed using the Statistical Package for Social Sciences SPSS version 22. Values were expressed as count/percentages, mean/standard deviation, or median and interquartile range (IQR). Differences between groups was assessed by Kruskal-Wallis H non-parametric. A p-value of <0.05 was considered significant.

Results

Patient's Characteristics

Table 1 below shows that the period of the study there were more males than females M: F of 1.4:1 with the median age at admission of 96hrs (4days). A significant proportion (88.3%) of the neonates were admitted through the out-born ward. The mean gestational age of the cohort was 39.2 (±1.1) weeks by USS while the mean weight at admission was 2700 ± 60g. Common clinical features were non-specific and include fever, poor suck, jaundice, and depressed primitive reflexes

Table I. Characteristics of Studied Neonates

	Frequency (%)	**Mean(±SD)**
Age at admission (hours)	94(100)	96(46.8-252) *
Sex		
Male	55(58.6)	
Female	39(41.4)	
Mode of ward entry		
Outborn	83(88.3)	
Inborn	11(11.7)	
Anthropometry		
Weight at admission(g)	94(100)	2700 (60)
Length (cm)	94(100)	48.7 (3.4)
Occipito-frontal circumference (cm)	94(100)	39.0 (1.9)
Common clinical features		
Fever	56(59.6)	
Poor suck	54(57.4)	
Jaundice	50(53.2)	
Depressed reflexes	50(53.2)	
Abnormal tone	44(46.8)	
Convulsion	39(41.5)	
Respiratory distress	32(34.0)	
Lethargy	27(28.7)	
Pallor	20(21.3)	

*=median (interquartile range)

Median PCT. Levels According To Bacterial Blood Culture Isolates of Subjects

Table 2 below shows median PCT levels according to blood culture bacterial groups and isolates. Using the Kruskal Wallis H test the difference in median PCT level between subjects with gram-negative (3.04ng/ml) and gram-positive infection (1.93ng/ml), was not statistically significant χ^2= 1.549, p= 0.300. Even as median PCT levels differ between Enterobacteriaceae and non-fermentative gram-negative aerobes and, also between different isolates, this was similarly not found to be statistically significant χ^2= 26.706, p= 0.082 and χ^2= 18.672, p= 0.412 respectively, using Kruskal Wallis H test.

Table 2: Median PCT levels according to bacterial isolates from blood cultures

Bacterial isolate	Frequency (%)	Median PCT in ng/ml (IQR)
Gram positive bacteria (GPB)	**42(44.7)**	**1.93 (0.58, 5.11)**
CoNS	7 (7.4)	2.43 (0.66, 10.53)
Enterococcus spp	5 (5.3)	2.22 (1.52, 11.23)
MDR and MRSA Staph spp	2 (2.1)	3.91 (0.18, 7.64)
Staphylococcus aureus	9 (9.6)	1.18 (0.63, 10.94)
Staphylococcus aureus MRSA	17 (18.1)	1.26 (0.32, 11.66)
Others	2 (2.1)	1.39 (0.66, 3.21)
Gram negative bacteria (GNB)	**52 (55.3)**	**3.04 (0.96, 11.19)**
Enterobacteriacae	**43 (45.7)**	**2.78 (0.81, 7.92)**
Citrobacter spp	4 (4.3)	6.31 (1.24, 20.09)
Enterobacter agglomerans	8 (8.5)	2.45 (0.29,7.93)
Escherichia coli	7 (7.4)	7.45 (0.63, 17.47)
Klebsiella spp	12 (12.8)	2.88 (1.13, 6.78)
Proteus mirabilis	5 (5.3)	5.52 (2.78, 15.93)
Serratia marcescens	3 (3.1)	2.78 (2.20, 4.70)
Others	4 (4.3)	0.28 (0.22, 0.64)
Non fermentative G- aerobes (NFGA)	**7 (7.4)**	**28.99 (2.00, 69.79)**
Acinetobacter spp	3 (3.1)	69.8 (40.80, 84.90)
Pseudomonas spp	4 (4.3)	15.49(1.25, 54.87)
Not known	2 (2.1)	6.12 (0.87, 11.37)

CoNS (Coagulase negative staphylococcus), MDR (multidrug resistant) and MRSA (methicillin resistant staphylococcus aureus)
Levels of PCT by percentage according to common blood culture bacterial isolates in subjects

Figure1; below shows that all subjects with *Acinetobacter, Pseudomonas, and Serratia marcescens* isolates had PCT elevation. On the other hand, only 58.8% of subjects with Staphylococcus aureus MRSA sepsis have elevated PCT even though it was the most common isolate

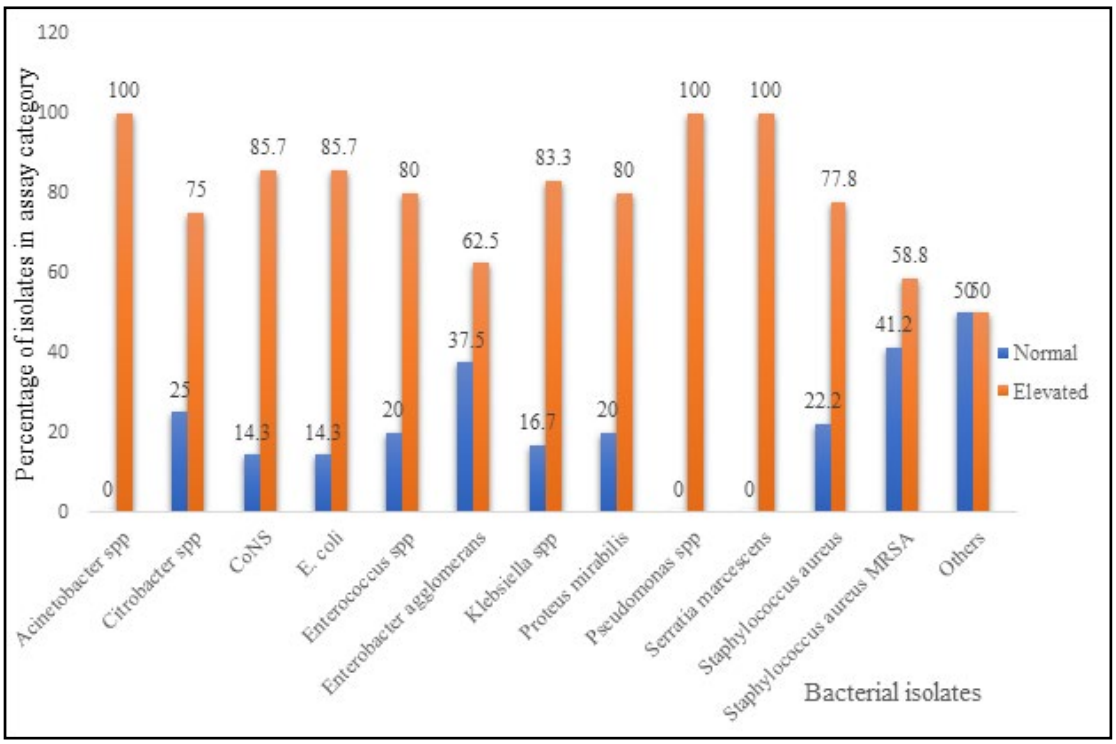

Figure 1: PCT levels by percentage according to common bacterial isolates

Discussion

Neonatal sepsis is a leading cause of morbidity and mortality even with timely and appropriate antimicrobial therapy, this is more so in developing countries. Prompt and early diagnosis is still a challenge to clinicians and contributes to the morbidity and mortality seen. Blood culture remains the gold standard for diagnosis and identification of organism but has several challenges. Procalcitonin among other biomarkers has shown potentials to enhance diagnosis of sepsis. In this study, PCT level was found to be greater in neonates with gram-negative than gram-positive bacterial sepsis generally and their corresponding isolates. This is similar to findings by various studies.5-10,11 The reason for this finding may be explained by differences in the activation pathway and initiation of inflammatory cascades of the organism.2 Gram negative bacteria activates TLR4 while gram-positive bacteria activates TLR2 resulting in varying production of proinflammatory cytokines.12 The TLR4 is a more potent inducer of IL-6, IL-1β, and TNF-α which are in turn potent inducers of PCT release. Therefore, GNB induces faster and greater inflammatory response at a lower infecting dose than GPB.12 The peptidoglycan layer of GBP is an insoluble continuous macromolecule and its ability to stimulate inflammatory response is highly dependent on its dispersion into smaller particles. Therefore, GNB induces faster and greater inflammatory response at a lower infecting dose than GPB [1,2].

This study found a greater PCT level in NFGA than Enterobacteriaceae, similar to what Found, but however in contrast to observations by Guo et al [5,6,7,10]. The greater level of PCT in NFGA may be explained by the fact that an organism such as Pseudomonas activates both TLR2 and TLR4 depending on acylation of its LPS. It activates TLR2 better than other Enterobacteriaceae and possibly the TLR2 ligand is suspected to be more potent [1,2]. Perhaps the strain found in this study was the tetra or penta-acylated LPS that stimulates TLR2 leading to a potent inflammatory and PCT response. Furthermore, some strains of Acinetobacter additionally produce an exo-polysaccharide which protects it from the host's defence, thereby increasing its [1,3]. An earlier study by [1,4]. Showed high levels of PCT following Acinetobacter iatrogenic infection. Even though PCT levels differ in gram negative and positive bacterial sepsis, Enterobacteriaceae and non-fermentative gram-negative sepsis, and infection with different isolates, interestingly, it was not found to be statistically significant. We wondered whether differences in neonatal and adult physiology could explain this finding. We are alluding to that fact that neonatal responses are not quite as high as that of adult likely because of their immature immune system/responses and perhaps our relatively small sample size when compared to other studies? So far, this may the first report of such study in neonates.

Conclusion

PCT level differ in gram-negative and gram-positive sepsis, in infection with Enterobacteriaceae and non-fermentative gram-negative aerobes and with different isolates even though these differences were not significant. More evidence will be required to validate its use to differentiate between gram-negative and positive organism infections.

References

1. Chirico, G., & Loda, C. (2011). Laboratory aid to the diagnosis and therapy of infection in the neonate. Pediatric reports, 3(1), e1.
2. Christ-Carin, M. (2005). Procalcitonin in bacterial infec-

tions-hype, hope, more or less?. Swiss medical weekly, 135(3132), 451-451.
3. Assicot, M., Bohuon, C., Gendrel, D., Raymond, J., Carsin, H., & Guilbaud, J. J. T. L. (1993). High serum procalcitonin concentrations in patients with sepsis and infection. The Lancet, 341(8844), 515-518.
4. Linscheid, P., Seboek, D., Nylen, E. S., Langer, I., Schlatter, M., Becker, K. L., & Müller, B. (2003). In vitro and in vivo calcitonin I gene expression in parenchymal cells: a novel product of human adipose tissue. Endocrinology, 144(12), 5578-5584.
5. Brodská, H., Malíčková, K., Adámková, V., Benáková, H., Šťastná, M. M., & Zima, T. (2013). Significantly higher procalcitonin levels could differentiate Gram-negative sepsis from Gram-positive and fungal sepsis. Clinical and experimental medicine, 13, 165-170.
6. Leli, C., Ferranti, M., Moretti, A., Dhahab, A., Salim, Z., Cenci, E., & Mencacci, A. (2015). Procalcitonin levels in gram-positive, gram-negative, and fungal bloodstream infections. Disease markers, 2015.
7. Yan, S. T., Sun, L. C., Jia, H. B., Gao, W., Yang, J. P., & Zhang, G. Q. (2017). Procalcitonin levels in bloodstream infections caused by different sources and species of bacteria. The American journal of emergency medicine, 35(4), 579-583.
8. Gai, L., Tong, Y., & Yan, B. Q. (2018). Research on the diagnostic effect of PCT level in serum on patients with sepsis due to different pathogenic causes. Eur Rev Med Pharmacol Sci, 22(13), 4238-4242.
9. İnci, A. (2016). Investigation of differences in CRP, PCT, WBC and MPV in Gram-negative, Gram-positive and fungal bloodstream infections. Disease and Molecular Medicine, 4(4), 81-84.
10. Guo, S. Y., Zhou, Y., Hu, Q. F., Yao, J., & Wang, H. (2015). Procalcitonin is a marker of gram-negative bacteremia in patients with sepsis. The American Journal of the Medical Sciences, 349(6), 499-504.
11. Park, I. H., Lee, S. H., Yu, S. T., & Oh, Y. K. (2014). Serum procalcitonin as a diagnostic marker of neonatal sepsis. Korean journal of pediatrics, 57(10), 451.
12. Elson, G., Dunn-Siegrist, I., Daubeuf, B., & Pugin, J. (2007). Contribution of Toll-like receptors to the innate immune response to Gram-negative and Gram-positive bacteria. Blood, 109(4), 1574-1583.
13. Joly-Guillou, M. L. (2005). Clinical impact and pathogenicity of Acinetobacter. Clinical microbiology and infection, 11(11), 868-873.
14. FM, B. (1998). Kinetics of procalcitonin in iatrogenic sepsis. Intensive Care Med, 24, 888-892.