Journal of Medical & Surgical Pathology

Jacob et al., J Med Surg Pathol 2018, 3:3
DOI: 10.4172/2472-4971.1000163

Research Article Open Access

Evaluation of Various Diagnostic Markers for Early Detection of Neonatal Sepsis

Jerusha Samuela Jacob[1*], Febe Renjitha Suman D[1], Sai Shalini CN[1], Binu Ninan[2], Suresh Varadarajan[3] and Jesu Magdalene[1]

[1]*Department of Pathology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai, India*

[2]*Department of Neonatology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai, India*

[3]*Department of Community Medicine, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai, India*

[*]**Corresponding author:** Jerusha Samuela Jacob, Department of Pathology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai, India, E-mail: dr.jerusha@gmail.com

Received date: July 23, 2018; **Accepted date:** August 24, 2018; **Published date:** August 28, 2018

Abstract

Background: Prompt diagnosis of neonatal sepsis at an early stage is essential to initiate therapy as well as to avoid unnecessary usage of antibiotics. Therefore the search for an ideal marker including antigenic expression on inflammatory cells is still continuing. The aim of the study was to identify an ideal early laboratory marker for diagnosis of neonatal sepsis.

Methods: The samples sent for complete blood count were processed for Haematologic Scoring System (HSS) and flow cytometry for expression of CD64. The volume, conductivity and scatter of neutrophils and monocytes were retrieved from the analyzer. C Reactive protein (CRP) and culture reports were retrieved from Lab Information System. The samples were grouped as controls from normal babies, suspected sepsis with negative blood culture and proven sepsis with positive blood culture. Statistical analysis was done and compared among groups. A score of 3 and above HSS had sensitivity of 87% and specificity of 85%.

Results: Mean fluorescent nCD64 at a cut-off of >105 can be considered as an ideal marker for early diagnosis of neonatal sepsis. It showed a higher sensitivity (97%) and higher specificity (>95%) for detecting neonatal sepsis.

Conclusion: HSS and mean volume of neutrophils or a combination of mean scatter and conductivity of neutrophils may be utilized as markers when flow cytometry facility is not available.

Keywords: Neonatal sepsis; Blood; Haematologic scoring system; Flow cytometry; Septicaemia

Introduction

Neonatal mortality occurs in around 34 per 1000 live births in developed countries [1,2]. Septicemia and infections are the leading causes responsible for 30%-50% of neonatal deaths [1]. As the early warning signs are often subtle, prompt diagnosis at an early stage is essential to initiate therapy as well as to avoid unnecessary usage of antibiotics. Bacterial culture is the gold standard method of diagnosis; however, due to the prolonged turnaround time of bacterial culture, C Reactive Protein (CRP) is often considered as an early diagnostic marker. By contrast, although CRP is highly specific for neonatal sepsis, its sensitivity is low [3]. Despite the favourable claims, most diagnostic markers fail to meet the demands required for clinical practice; therefore, the search for an ideal diagnostic marker, or a battery of markers, for diagnosis of neonatal sepsis is continuing.

This study aimed to identify an early diagnostic marker for neonatal sepsis. In this study, the existing Haematology Scoring System (HSS) and the Cell Population Data (CPD) (volume, conductivity, and scatter of neutrophils and monocytes and the novel flow cytometric expression of CD64 on neutrophils and monocytes) were compared between sepsis groups and controls. The sensitivity and specificity were compared with the biochemical marker CRP.

Materials and Methods

Study setting

This study was carried out as a case control study in the Department of Pathology and Neonatology of our tertiary care hospital, Chennai between August 2014 and July 2016.

Study participants

All the participants who were born during the study period were selected for the study. The controls consisted of babies born during the study period without any clinical suspicion of sepsis or disease and those who were discharged within 3-5 days. The cases comprised of clinical suspected neonates of sepsis on whom CRP and blood culture were performed. These cases were further classified into culture positive and culture negative sub-groups based on the results of the blood culture.

Sample size and sampling technique

The participants were selected using convenient sampling. A total of 53 neonates were enrolled in the control group. In 97 neonates, culture was negative and in 86 neonates, the culture was positive.

Ethical approval and informed consent

Approval was obtained from the Institutional Ethics Committee prior to the start of the study. The parents of each prospective participant were explained in detail about the study and informed consent was obtained from the parents prior to data collection.

Data collection

Beckman coulter LH 780 (workstation software version IB3 revision 123391 Fullerton CA) was used for CBC and cell population analysis. A peripheral smear was stained with Leishman's stain. Flow cytometry for neutrophil CD64 (nCD64) was carried out with a FACSCalibur (Beckton Dickinson, USA 2008) using a monoclonal anti-mouse CD64 antibody conjugated with fluorescein isothiocyante (BD Biosciences Catalogue number #560970).

Haematology scoring system: The following seven parameters were scored one point each. Total white blood cell (WBC) count <5000/µl or >25,000/µl, increase in the immature Polymorphonuclear Cell (PMN) count, increase in the ratio of immature to total PMN count: immature to mature PMN of >0.3, degenerative changes in PMN, platelet count of <1,50,000/µl and when no PMN is seen, 2 score points were allotted. The minimum and maximum scores were 0 and 8, respectively [4].

CPD: The VCS data available from the haematology analyser expressed the CPD for neutrophils and monocytes as mean ± Standard Deviation (SD).

CD64 expression: Flow cytometry for CD64 was carried out on 60 samples, 4 of which were from normal controls and used for standardization. The 56 samples were blindly selected during the last part of the study period, excluding samples received during the holidays. Samples were received for CBC in evacuated tubes with EDTA as anticoagulant; samples were utilized for flow cytometry and were processed within 4 h of collection. The expression level of CD64 was measured as the geometric Mean Fluorescence Intensity (MFI) on neutrophils and monocytes.

CRP and microbial culture: The values of CRP and the microbial results were retrieved from the laboratory information system.

Operational definition

A hematologic score of ≤ 2 predicts that sepsis is an unlikely event in a neonate; however, a score of ≥ 3 is indicative of sepsis.

Data analysis

Data were entered into Microsoft Excel 2008 and statistically analysed. Analysis of the HSS scores and the MFI nCD64 and monocyte CD64 (mCD64) between Groups 1-3 (normal controls, suspected sepsis and proven sepsis) was done with Package 'pROC' version 3.3.1, using a Kruskal Wallis test; a P value of <0.05 was considered to be statistically significant. A post hoc Dunn test was carried out for intergroup comparisons. Analyses of VCS and CRP between groups were performed using Med Calc for Windows, version 15.0 (Med Calc Software, Ostend, Belgium. Mann-Whitney T-test). To assess the diagnostic performances, Receiver Operator Curve (ROC) analysis was done.

Results

The bio-demography of the study population is shown in Table 1. The organisms isolated from Group 3 were *Staphylococcus aureus* (22.5%), *Klebsiella pneumoniae* (18.6%), *Acinetobacter* species (12.8%), *Pseudomonas aeruginosa* (8.1%), *Escherichia coli* (7%) and *Salmonella enteritidis* (2.3%).

Characteristics			Controls (Group 1)	Suspected (Group 2)	Proven sepsis (Group 3)
No. of neonates			53 (22.5%)	97 (41.1%)	86 (36.4%)
Gestational age		Term	23 (9.74%)	59 (25%)	49 (20.7%)
		Pre-term	20 (8.47%)	38 (16.1%)	37 (15.6%)
Male:Female			0.9:1	1.5:1	1.3:1
Age	<3 days		46	87	64
	>3 days		7	10	22

Table 1: Bio-demography of the study population.

HSS: The comparative evaluation of HSS among groups is shown in Table 2. A score of 3 and above had the highest sensitivity of 87% and specificity of 85% (area under the curve (AUC)=0.9 au).

Group	HSS (Mean ± SD)	HSS (Median)	Comparison	P value
1	1.7 ± 0.8	2	1 *vs.* 2	0.24
2	1.9 ± 0.7	2	2 *vs.* 3	<0.001
3	3.9 ± 1.3	4	1 *vs.* 3	<0.001

Group 1-Normal controls, Group 2-Suspected sepsis, Group 3-Proven sepsis

Table 2: Comparison of the Haematology Scoring System (HSS) among the three groups.

CPD: The analysis of the comparative data (mean ± SD calculated, two tailed probability P ≤ 0.05) showed a significant difference in the conductivity and scatter of neutrophils and the volume of monocytes between Groups 1 and 2. These parameters, in addition to neutrophil volume, showed significant differences between Groups 1 and 3 and also Groups 2 and 3. The cut-off for these parameters was defined by

ROC analysis with an AUC of 0.9 au. The sensitivity and specificity of the individual parameters are shown in Table 3.

Parameters	**1** *vs.* **2**	**1** *vs.* **3**	**2** *vs.* **3**
MN-V-NE	-	>152.8	>158.8
Sensitivity	-	96.9	96.9
Specificity	-	100	100
MN-C-NE	-	<154.5	<145.4
Sensitivity	-	96.9	67.2
Specificity		100	97.7
MN-S-NE	<134.6	<129.9	<129.2
Sensitivity	65.6	96.9	97.3
Specificity	91.3	95.7	67.8
MN-V-MO	>174	>177.1	>186.5
Sensitivity	87.4	96.9	87.5
Specificity	97.8	100	94.3

Group 1: Normal controls; Group 2: Suspected sepsis; Group 3: Proven sepsis; MN-V-NE: Mean volume of Neutrophils; MN-C-Ne: Mean Conductivity of Neutrophils; MN-S-NE: Mean Scatter of Neutrophils; MN-V-MO: Mean Volume of Monocytes.

Table 3: Sensitivity and specificity of the VCS parameters to predict sepsis

CD64 expression: The mean ± SD of MFI and the comparative analysis of the MFI of neutrophils and monocytes between the groups are shown in Table 4.

nCD64	**Mean ± SD**	**Median**	**p value**	**MFI**	**Sensitivity**	**Specificity**
Group 2	84.4 ± 18.3	83.4	<0.001	≥ 105	1	0.85
Group 3	193.5 ± 33.2	192.3		≥ 138.8	0.95	1
mCD64						
Group 2	112.6 ± 39.2	110	<0.049	153.3	0.97	0.89
Group 3	138.8 ± 56.4	129.2		157.9/161.4	0.97	0.95

Group 2: Suspected sepsis; Group 3: Proven sepsis

Table 4: Comparison of Mean Fluorescence Intensity (MFI) of Neutrophil CD64 (nCD64 and monocyte CD64 (mCD64)

CRP: At the value of >0.42, the comparison of CRP between Groups 2 and 3 showed a sensitivity of 85.9% and a specificity of 93.1%.

Comparison of various parameters: The various parameters analysed in the study with respect to sensitivity and specificity, turnaround time and economy are depicted in Table 5.

Parameter	**Sensitivity (%)**	**Specificity (%)**	**Time**	**Cost**
HSS	87	85	2 h	Moderate
CPD				
MN-V-NE	96.9	100	30 mins	Minimum
MN-C-NE	67.2	97.7	30 mins	Minimum
MN-S-NE	97.3	67.8	30 mins	Minimum
MN-V-MO	87.5	94.3	30 mins	Minimum
nCD64				
≥ 105	100	85	1-2 h	High
≥ 138.8	95	100	1-2 h	High
mCD64				
>157.9/161.4	97	95	1-2 h	High
CRP	85.9	93.1	1 h	Moderate

HSS: Haematology Scoring System; CPD: Cell Population Data; MN-V-NE: Mean Volume Of Neutrophils; MN-C-Ne: Mean Conductivity Of Neutrophils; MN-S-NE: Mean Scatter Of Neutrophils; MN-V-MO: Mean Volume Of Monocytes; nCD64: Neutrophil CD64; mCD64: Monocyte CD64; CRP: C Reactive Protein

Table 5: Comparative analysis of the parameters in sepsis

Discussion

Moreover, many parameters hold subjective variation; some earlier studies observed a higher sensitivity for HSS at a score of ≥ 3 or ≥ 4, but the specificity is low [5-7]. The feasibility, cost effectiveness, ready availability and certainty of sepsis with higher scores indicate that HSS can definitely provide a guideline to antibiotic therapy. From our study, we recommend the administration of antibiotics if the score is ≥ 3. Neonates with scores between 2 and 3 should be vigilantly watched while those with ≤ 2 should be investigated for other pathologies.

The mean volume of neutrophils (MN-V-NE) has a high sensitivity (96.9%) and specificity (100%) at the cut-off of >158.8. This increase in neutrophil volume may be due to the toxic changes and left shift. Although the sensitivity of our study was comparable to other studies, the specificity in our study is higher [8,9]. The conductivity (MN-C-NE) and scatter (MN-S-NE) of neutrophils were not very good parameters individually to predict sepsis; however, a combination of both can be used effectively as conductivity is more sensitive and scatter is more specific.

At a cut-off of >138.8, the sensitivity of MFI nCD64 was 95% while the specificity was 100%. Grenc et al. reported a lower sensitivity at a lower cut-off 109 [10]. Jain et al. had found up regulation during the infection, which had down regulated as the sepsis resolved [11]. CD64 surface upregulation is induced by granulocyte stimulating factor (G-CSF) and interferon V (INF-V), and may be increased within 1-4 h of infection [12,13]. Measurement of nCD64 expression is highly specific and can be performed for the diagnosis of neonatal sepsis using only a minimal volume of the blood sample collected for CBC. This marker, which is up regulated within an h of the onset of bacterial infection, requires a short turnaround time. Sophisticated equipment is essential but the methodology is simple in the hands of trained personnel. CRP at a cut-off of >0.42 showed 85.9% sensitivity and 93.1% specificity in distinguishing sepsis.

The numerical and subjective morphological parameter HSS, the objective morphological parameter CPD and the expression of cell surface marker CD64 were elevated in the diagnosis of sepsis. Considering the mortality and morbidity associated with neonatal sepsis, a diagnostic marker with a very high sensitivity approaching 100% is desirable because all septic infants with life threatening infection should be identified and treated without substantial disease. This competent diagnostic marker also needs to have a reasonably high specificity of more than 85% in order to minimize the unnecessary usage of antibiotics. nCD64 at a cut-off of >105 is characterized as an ideal laboratory marker. However each laboratory should define the MFI cut-off as it is variable according to staining and voltage characteristics.

In resource-constrained settings, HSS and MN-V-Ne, or a combination of MN-S-Ne and MN-C-Ne may be utilized.

Conclusion

nCD64 at a cut-off of >105 is characterized as an ideal laboratory marker for the early diagnosis of neonatal sepsis, but each laboratory should define the MFI cut-off; however, HSS and MN-V-Ne or a combination of MN-S-Ne and MN-C-Ne may be utilized as markers when a flow cytometry facility is not available.

Limitation

One limitation of the present study was that the parameters were not assessed during the course and at the end of the treatment.

Acknowledgments

Malini, Scientist, Staff haematology laboratory.

Conflict of Interest

There is no conflict of interest among the authors of this research article.

Funding

The authors of this article don't have any source of funding.

References

1. Costello A, Francis V, Byrne A (2001) The State of the World's Newborns. Washington, DC: Save The Children Fund.
2. Report of the National Neonatal Perinatal Database (National Neonatology Forum) (2003) NNPD Network; ICMR.
3. Mishra UK, Jacobs SE, Doyle LW, Garland SM (2006) Newer approaches to the diagnosis of early onset neonatal sepsis. Arch Dis Child Fetal Neonatal Ed 91: F208-F212.
4. Rodwell RL, Leslie AL, Tudehope DI (1988) Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr 112: 761-767.
5. Khalada BK, Mohammad AR , Tuhin S, Chandan KR, Quddusur RM, et al. (2010) Role of hematologic scoring system in early diagnosis of neonatal septicaemia. Bangabandhu Sheikh Mujib Medical University Journal 3: 62-67.
6. Coulter 3-D VCS technology (2013) Coulter LH 780 system helps 1B3. Copyright Beckman Coulter Inc.
7. Narasimha A, Harendra Kumar MLH (2011) Significance of Hematological Scoring System (HSS) in early diagnosis of neonatal sepsis. Indian J Hematol Blood Transfus 27: 14-17.
8. Raimondi F, Ferrara T, Capasso L, Sellitto M, Landolfo F, et al. (2010) Automated determination of neutrophil volume as screening test for late-onset sepsis in very low birth infants. Pediatr Infect Dis J 29:288.
9. Bhargava M, Saluja S, Sindhuri U, Saraf A, Sharma P (2014) Elevated mean neutrophil volume+CRP is a highly sensitive and specific predictor of neonatal sepsis. Int J Lab Hem 36: e11–e14.
10. Groselj-Grenc M, Ihan A, Derganc M (2008) Neutrophil and monocyte CD64 and CD163 expression in critically ill neonates and children with sepsis: comparison of fluorescence intensities and calculated indexes. Mediators Inflamm 2008: 1–10.
11. Jain P, Pradhan R, Warner N (2013) Role of flow cytometry in diagnosis and management of neonatal sepsis. Indian J Hematol Blood Transfus 29: 191-277.
12. Kakinoki Y, Kubota H, Yamamoto Y (2004) CD64 surface expression on neutrophils and monocytes is significantly up-regulated after stimulation with granulocyte colony-stimulating factor during CHOP chemotherapy for patients with non-Hodgkin's lymphoma. Int J Hematol 79: 55-62.
13. Nimmerjahn F, Ravetch JV (2006) Fcγ receptors: old friends and new family members. Immunity 24: 19-28.