DOI: 10.7860/IJNMR/2019/41233.2246

Original Article

Pathology Section

Diagnostic Utility of Haematologic Scoring System in Neonatal Sepsis

MODALI RISHITA SARMA, JINKALA SREE REKHA, SRIKANTA KANUNGO

ABSTRACT

Introduction: Sepsis is an important cause of neonatal mortality and morbidity. Numerous serological markers exist for diagnosis of sepsis which acts as adjuvants to the clinical signs. A Haematological Scoring System (HSS) for predicting sepsis in neonates which includes the total leucocyte count, immature neutrophil count, toxic changes in the neutrophils and various other parameters in the routine haemogram was used.

Aim: In the present study, authors evaluated the diagnostic utility of HSS in predicting neonatal sepsis in neonates admitted with clinical diagnosis/suspicion of sepsis.

Materials and Methods: This was a prospective observational study, conducted on neonates with clinical diagnosis/suspicion of sepsis. The various parameters included in the HSS for neonatal sepsis were analysed in all the smears. Statistical analyses were conducted; the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of each variable of HSS score was calculated. The comparison of HSS score between the culture positive sepsis and culture negative sepsis was carried out by using Independent t-test or Mann-Whitney U test.

Results: Seventy-eight samples were received from neonates with clinical diagnosis/ suspicion of neonatal sepsis. Among the variables in HSS score, Total Neutrophil Count (TNC) and Immature Neutrophil Count (INC) show high sensitivity; Immature to Mature neutrophil ratio (I:M ratio) and low platelet count showed high specificity; TNC and INC showed high PPV; toxic change and low platelet count showed high NPV in predicting neonatal sepsis. Presence of Schistocytes in the peripheral smear showed high specificity of 80% in predicting neonatal sepsis. Increased band count has a high specificity (91%) and NPV (72%) in predicting neonatal sepsis.

Conclusion: HSS score had high sensitivity and NPV of 91% in predicting neonatal sepsis. The prevalence rate ratio of neonates developing sepsis with high HSS score is 3.9 times more compared with neonates with low HSS score. HSS is a simple, quick, cost-effective and routine laboratory test which helps in predicting neonatal sepsis. The higher the HSS score, more is the probability of neonatal sepsis.

Keywords: Haematological parameters, Neonates, Neutrophils

INTRODUCTION

Neonatal septicaemia is a clinical syndrome characterised by signs and symptoms of infection with accompanying bacteraemia in first month of life [1]. Neonates are prone to sepsis even with minimal stress and infection which is related to immaturity of both the cellular and humoral immune systems at birth. Despite continuing advances in diagnosis and treatment, it remains as one of the important cause of high mortality and morbidity [2].

Various clinical and laboratory parameters serve as predictors of neonatal sepsis. Micro ESR, C Reactive Protein (CRP), Interleukin 6 (IL-6), IL-8, C11b and procalcitonin are all sensitive markers of neonatal infections [3]. Bacterial culture is the gold standard for diagnosis of neonatal sepsis, however, positive culture can be seen only in 10-60% of cases [4]. The Haematologic Scoring System (HSS) proposed by Rodwell RL et al., [Table/Fig-1] helps in prediction of neonatal sepsis which requires complete hemogram and peripheral smear examination [5]. The HSS score uses the data available in complete hemogram, which can be obtained in a couple of hours and is cost-effective. Hence, this study was undertaken to prospectively assess the diagnostic utility of HSS in predicting neonatal sepsis.

MATERIALS AND METHODS

This was a prospective observational study and was done under the ICMR Short term student Project (STS 2016) with approval

Criteria	Abnormality	Score
Total leucocyte count (TLC)	< 5000/mm^3	1
	>25000/mm^3 at birth	
	>30000/mm^3 at 12-24 hours	
	>21000/mm^3 from day 2 onwards	
Total neutrophil count (TNC) (Normal – 1800-5000/mm^3)	No mature neutrophils seen	2
	Increased/decreased	1
Immature neutrophil count (INC) (Normal-600/mm^3)	Increased	1
I:T ratio (Immature neutrophils : TLC)	>0.2 Increased	1
I:M ratio (Immature : Mature neutrophils)	>0.3	1
Degenerative changes in neutrophils	Toxic granules/cytoplasmic vacuoles	1
Platelet count	<1,50,000/mm^3	1

[Table/Fig-1]: Hematologic scoring system.

of the Institute Ethics Committee (JIP/IEC/2016/26/864). Inclusion criteria were, neonates admitted with clinical suspicion or diagnosis of sepsis in the neonatal care unit of a tertiary care centre, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India. Assuming that 40 cases per month were seen at the Neonatal Intensive Care Unit at JIPMER, convenient sampling was done and all consecutive neonates admitted to neonatology ICU with clinical suspicion of sepsis were included in the study over a period of two months (July and August 2016). Exclusion criteria were, neonates on therapeutic hypothermia, haemolysis secondary to blood group incompatibilities and neonates born to mother with HELLP syndrome/ Idiopathic thrombocytopenic purpura (primary and secondary).

An amount of 2 mL of blood sample was collected in EDTA vacutainers under aseptic conditions and processed in Sysmex XT-2000i haematology analyser for various haematological parameters. The peripheral smears made from these samples were stained with leishman stain and analysed. WBC differential count for 200 cells was done. The various parameters included in the HSS for neonatal sepsis were analysed in all the smears. Bacterial culture was taken as the gold standard for diagnosing sepsis.

HSS was applied on all the samples and each sample was placed in one of the three categories: Category 1: ≤2 sepsis unlikely, Category 2: 3-4 suspected sepsis and Category 3: ≥5 -likely sepsis (Maximum score: 9, Minimum score: 0)
Depending upon the culture result, the study group was divided into three groups as, Group A- culture positive, Group B - culture negative and HSS score of Category 2 and 3 and Group C culture negative and HSS score of category 1.

STATISTICAL ANALYSIS

Categorical data on gender, clinical characteristics, culture profile etc., were expressed as frequency and percentages. The continuous data such as age, birth weight, HSS score etc., were expressed as mean with SD or median with range. The sensitivity, specificity, PPV and NPV of each variable of HSS score were calculated. The comparison of the culture profile in relation to the categorical variables mentioned above were carried out by using chi-square test and Fischer's-exact test. The comparison of HSS score between the culture positive sepsis and culture negative sepsis was carried out by using Independent t-test or Mann-Whitney U test. The statistical analysis was done by using the Graph pad instat 3.10 software and IBM SPSS 20.0 software.

RESULTS

A total of 90 cases satisfied the inclusion criteria. Out of which, 78 cases had culture correlation and these were analysed. The mean age and birth weight of the neonates were 2.2±3.7 days and 1.69±0.7 kg respectively with a male to female ratio of 1.3:1. Prematurity, early onset sepsis and Intrauterine Growth Restriction (IUGR) was seen in 85%, 96% and 18% of neonates respectively. The baseline data of the study population is elaborated in [Table/Fig-2,3].

Sl. No	Categorical variable		Frequency (n=78)	Percentage (%)
1.	Gender	Male	47	60
		Female	31	40
2.	Gestational age	Term	12	15
		Preterm	66	85
3.	Onset of sepsis	EOS	75	96
		LOS	3	4
4.	IUGR	Present	14	18
		Absent	64	32
5.	HSS category	Category 1	22	28
		Category 2	38	49
		Category 3	18	23
6.	Culture profile	Positive	22	28
		Negative	56	72

[Table/Fig-2]: Base line data of study population.
EOS: Early onset sepsis; LOS: Late onset sepsis; IUGR: Intrauterine growth restriction; HSS: Hematologic scoring system

Sl. No	Continuous Variable		Mean with SD
1.	Age		2.2±3.7 days
2.	Birth weight (BW) (kg)	Normal BW	1.69±0.7
		Low BW (LBW)	1.7±0.68
		Very LBW	1.69±0.69
		Extreme LBW	1.7±0.7
3.	HSS score	For all the cases	3.06±1.61
		Culture positive	3.22±1.47
		Culture negative	1.33±1.68

[Table/Fig-3]: Mean of age, birth weight and HSS Score in study population.

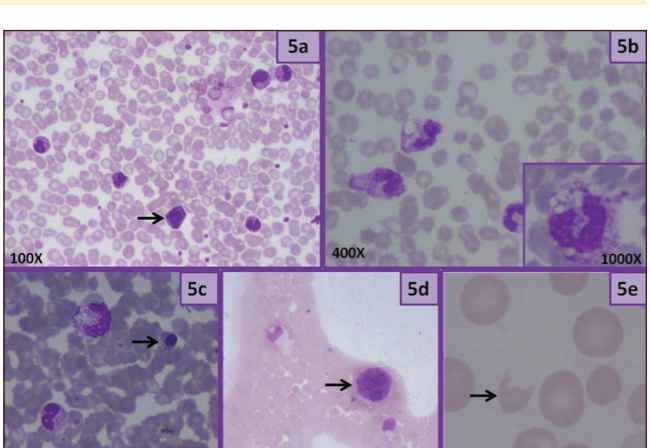
The percentage of neonates with HSS Category 1, 2 and 3 was 28, 49 and 23 respectively. The microbiological profile was identified in 28% and includes positivity for bacterial, fungal organisms and viral serology [Table/Fig-4]. As the present authors have predominantly bacterial culture positive cases, the microbiological profile is henceforth referred as culture positive and culture negative in this study. The mean HSS score in the culture positive and negative groups was 3.22±1.47 and 1.33±1.68 respectively. However, the median HSS score in both culture positive and negative groups was three with Interquartile Range (IQR) of 2-4. Neutrophilic left shift and toxic change seen in these smears are shown in [Table/Fig-5].

Sl. No	Microbiological profile (organism)	No. of cases (n=22)
1.	Coagulase negative *Staphylococcus*	6
2.	*Klebsiella pneumoniae*	4
3.	*Staphylococcus aureus*	3
4.	*Psuedomonas aeruginosa*	2
5.	*Enterobacter fecalis*	2
6.	*Acinetobacter* Sps (A. boumannii and A. iwofii)	2
7.	Gram negative non fermenting bacilli	1
8.	*Candida albicans*	1
9.	CMV Ig M positive and CMV induced hepatitis on liver biopsy	1

[Table/Fig-4]: Distribution of microbiological profiles.

INC (67.8%) has the maximum sensitivity followed by TNC (64.2%) and I:M ratio (68.18%), TLC (68%) and platelet count (80%) exhibited high specificity in predicting neonatal sepsis. TNC (75%) followed by INC (74.5%) and I:T ratio (74.4%) showed the maximum PPV. Platelet count and neutrophilic toxic/degenerative change had the highest NPV of 83.3% and 79% respectively [Table/Fig-6].

Majority of the cases were in Category 2 of HSS in both the culture positive and culture negative groups (73% and 39%)



[Table/Fig-5]: Leishman stained periperal smear (PS) showing 5a) neutrophilic leucocytosis and shift to left, arrow showing promyelocyte X 200; 5b) neutrophilic toxic change X 400, inset showing neutrophil with toxic granules and vacuolations X 1000; 5c) arrow pointing nucleated RBC and arrow head pointing band X 400; 5d) megakaryocyte in PS in a case of neonatal sepsis x 400; 5e) Schistocyte (arrow) in one of the cases

HSS Variable* (n=78)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Total WBC count	17.8	68	58.8	24.5
Total Neutrophil count (TNC)	64.2	45.4	75	33.3
Immature Neutrophil count (INC)	67.8	40.9	74.5	33.3
I:T Ratio (>0.12)	57	50	74.4	31.4
I:M ratio (>0.3)	26.7	68.2	68.2	26.7
Toxic/degenerative change	63.6	53.5	35	79
Platelet count	59	80	54	83.3

[Table/Fig-6]: Performance each variable of HSS score in predicting neonatal sepsis.
* Refer to [Table/Fig-1] for the ranges and scoring of HSS variables

[Table/Fig-7]. Comparison of the HSS categories with the culture profile by Fishers-exact test was statistically significant with a p-value of 0.02 and prevalence rate ratio of 3.9. The HSS score also showed high sensitivity and NPV of 91% [Table/Fig-8]. The power of the study is 87% assuming the Type I error of 0.05 calculated using power and sample size software version 3.1.6.

In addition to these, low platelet count showed statistical significance with the culture positivity. Schistocytes were seen in 10 (13%) cases and showed a specificity of 80% in predicting neonatal sepsis. Band count was not statistically significant between the culture positive and negative groups, however, it showed a high specificity (91%) and NPV (72%) [Table/Fig-9].

HSS categories (n=78)	Culture positive (n=22)	Culture negative (n=56)
Category 1 (HSS score ≤2, sepsis unlikely)	2	20
Category 2 (HSS score 3-4, Probable sepsis)	16	22
Category 3 (HSS score ≥5, likely sepsis)	4	14

[Table/Fig-7]: Distribution of HSS categories in relation to culture profile.

HSS category	Culture positive (n=22)	Culture negative (n=56)	
Category 2 and 3	20	36	**Sensitivity – 91% Specificity – 35.7% PPV – 35.7% NPV – 91% p value – 0.02 Prevalence risk ratio– 3.9**
Category 1	2	20	

[Table/Fig-8]: Performance of HSS score in predicting neonatal sepsis.

Variable		Culture positive (n=22)	Culture negative (n=56)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p value
Band count	>15	3	5	13	91	37	72	0.68
	≤15	19	51					
Schistocytes	Present	4	6	10.7	82	60	26.4	0.45
	Absent	18	50					
Platelet count (lakhs/mm^3)	≤1.5	13	11	59	80	54	83.3	0.002
	>1.5	9	45					

[Table/Fig-9]: : Sensitivity and specificity of band count, schistocytes and platelet count.

Parameters	Comparision of the present study with two recent studies				
		Sn	Sp	PPV	NPV
Total Leucocyte Count (TLC)	Priyanka T et al., [9]	23.6	71.2	35.8	-
	Bhalodia MJ et al., [10]	66.7	74.5	48	87
	Present study	17.8	68	58.8	24.5
Total Neutrophil Count (TNC)	Priyanka T et al., [9]	52.8	91.1	80	74
	Bhalodia MJ et al., [10]	45.8	92.1	46	92
	Present study	64.2	45.4	75	33.3
Immature Neutrophil Count (INC)	Priyanka T et al., [9]	55.5	93	84.2	75.5
	Bhalodia MJ et al., [10]	-	-	-	-
	Present study	67.8	40.9	74.5	33.3
I:T Ratio (> 0.12)	Priyanka T et al., [9]	35.8	72.7	39.3	59.1
	Bhalodia MJ et al., [10]	91.6	92.1	92	93
	Present study	57	50	74.4	31.4
I:M ratio (>0.3)	Priyanka T et al., [9]	25.8	74.1	26.5	60.2
	Bhalodia MJ et al., [10]	93.7	94.2	93	94
	Present study	26.7	68.2	68.2	26.7
Toxic/degenerative change	Priyanka T et al., [9]	45.6	86.2	69.3	70
	Bhalodia MJ et al., [10]	-	-	-	-
	Present study	63.6	53.5	35	79
Platelet count	Priyanka T et al., [9]	34.6	78.7	52.5	63.9
	Bhalodia MJ et al., [10]	56.3	55.9	56	58
	Present study	59	80	54	83.3

[Table/Fig-10]: Comparision of HSS parameters in the present study with two recent studies.
I:T – Immature to total neutrophil ratio; I:M – Immature to mature neutrophil ratio; Sn- sensitivity; Sp- Specificity; PPV – Positive predictive value; NPV – Negative predictive value

DISCUSSION

The increasing morbidity and mortality associated with neonatal sepsis makes its early diagnosis very crucial in the management of these neonates. A high index of clinical suspicion is also necessary as the clinical manifestations are varied and non-specific. There is no single laboratory test which in isolation or independently predicts neonatal sepsis and therefore it is always a combination of laboratory tests which help in predicting neonatal sepsis with certainty.

The significance of HSS score in predicting neonatal sepsis has been validated over the years because of its simple, cost-effective methodology. In the present study, authors have found that HSS has high sensitivity and NPV in predicting neonatal sepsis. The prevalence rate ratio of neonates developing sepsis with high HSS is 3.9 times more compared with neonates with low HSS. The present study is in agreement with similar studies done in the previous literature [6-8]. The recently published studies on HSS by Priyanka T et al., and Bhalodia MJ et al., have been compared with the results of the present study and are tabulated in [Table/Fig-10] [9,10]. The present study is in consensus with Priyanka T et al., in finding a high sensitivity for TNC and INC; the present study also agrees with Bhalodia MJ et al., which shows high specificity for I:M ratio [9,10].

The present authors also found that thrombocytopenia and fragmented RBC or schistocytes in the peripheral smear have a high specificity in predicting neonatal sepsis. Thrombocytopenia in sepsis can be seen because of sequestration, suppression of megakaryocytes by bacterial toxins or due to peripheral destruction, as a part of consumptive coagulopathy,i.e., Disseminated Intravascular Coagulation (DIC) in these neonates which lead to presence of schistocytes and hence a Microangiopathic Hemolytic Anemia (MAHA).

Neutrophilic leucocytosis, increase in INC and toxic change predicts neonatal sepsis in majority of cases. However, neutropenia is also a better predictor of sepsis, but is not encountered frequently. The present authors have four such cases in the present study, in which the culture was positive but the HSS score was low in three patients due to neutropenia.

In the present study, increase in band count had a high specificity and NPV value in predicting neonatal sepsis. In spite of its inaccuracy, owing to subjective variation in identification and sampling errors as stated by Cornbleet J, the utility of band count still exists in current neonatal haematology practise in predicting sepsis [11].

LIMITATION

Limitations of this study were that correlation with laboratory data like C-reactive protein, micro ESR etc., could not be done due to some technical difficulties, which of done have increased the validity of the study.

CONCLUSION

Haematologic Scoring System (HSS) system is a simple, quick, cost-effective and routine laboratory test which helps in predicting neonatal sepsis. The higher the HSS score, there is more probability of neonatal sepsis. Though there are many serological markers available, HSS serves as a reliable predictor of neonatal sepsis.

ACKNOWLEDGEMENTS

The present authors thank ICMR for selecting the project and providing the scholarship for this project. We also thank Dr. Nivedita Mondal, Neonatologist at Department of Paediatrics, JIPMER for her support in designing the study.

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AUTHOR(S):

1. Modali Rishita Sarma
2. Jinkala Sree Rekha
3. Srikanta Kanungo

PARTICULARS OF CONTRIBUTORS:

1. MBBS Student, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India.
2. Associate Professor, Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India.
3. Senior Resident, Department of Preventive and Social Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Jinkala Sree Rekha,
Associate Professor, Department of Pathology,
Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry-605006, India.
E-mail:sree.path177@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS:

None.

Date of Publishing: **Apr 01, 2019**