Assessment of Hematological Scoring System (HSS) in Early Diagnosis of Neonatal Sepsis in Neonatal Intensive Care Units

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ABSTRACT
Although there have been several advancements in the diagnosis of neonatal sepsis, it still remains a major challenge, as there is no specific and sensitive marker for the timely diagnosis. Frequent incidence of noninfectious conditions that resemble to the clinical picture of neonatal sepsis, primarily in very low birth weight preterm infants, that complicates the identification of neonatal sepsis. The present study was undertaken to assess the significance of the hematological scoring system (HSS) for early detection of neonatal sepsis in total 42 cases with blood culture proven neonatal sepsis of 102 clinically diagnosed neonates admitted to the Neonatal Intensive Care Units (NICU) in El Menoufia and El Kalyoubia governorates hospitals versus 30 age matched healthy controls. The following HSS parameters were found to be significant for the early diagnosis of neonatal sepsis; Total WBC count, Total PMN count, Immature PMN count, I:T PMN ratio and Platelet count while the most sensitive parameters was degenerative changes and toxic granulation in PMNL.

Keywords: Neonatal sepsis; Hematologic Scoring System (HSS); BACT/ALERT automated blood culture system.

INTRODUCTION

Sepsis remains a major cause of morbidity and mortality during the first year of life. Sepsis is the sixth leading cause of death among neonates, and the eighth leading cause of death for infants during the first year of life **(Heron, 2015).** Neonatal sepsis is responsible for a high percentage of deaths in newborns. The fact that 75% of this mortality occurs in developing countries is due to under-recognition of illness **(Obiero et al, 2015).** Pathogens in blood culture are only detected in 25% of cases**(Al-Zahrani et al, 2015)** and culture results take more than 48 h**,(Tripathi & Malik, 2010**) thereby being less efficient as sepsis progression is rapid **(Kumar & Bhat, 2015).** Basic hematological tests, such as CBC, absolute neutrophil count (ANC), differential count, and immature to total leucocytes ratio (I/T), have been used for the diagnosis of neonatal sepsis although CBC has a poor predictive value **(Shah & Padbury, 2014).** As the signs and symptoms of neonatal sepsis are nonspecific, usually a complete blood count (CBC) is done to evaluate the likelihood of infection and need for antibiotics treatment **(Mukhopadhyay & Puopolo, 2012)** Normal neutrophil values peak (7800–14,500 cells/ mm^3) during 12–14 h of age and decline (2700–13,000 cells/ mm^3) during 72–240 h of age **(Simonsen et al, 2014).** Rather than specifying a given range (or cut-off values) for CBC, it is better to use age-specific likelihood ratios for CBC **(Newman et al, 2010).** I/T ratio of >0.2

suggests sepsis, whether I/T ratios greater than 0.30 were associated with EOS **(Kumar & Bhat, 2015).** When WBC count (<5000) and ANC (<1000) values were low, they were most predictive of infection (**Mukhopadhyay & Puopolo, 2012).** In Europe, every year, 157 000 people die for this systemic multi-organs failure as a consequence of bacterial or fungal infection **(Goto & Al-Hasan; 2013).** Group B *Streptococcus* (GBS) remains the leading cause of EOS in term neonates, although the overall national incidence has decreased by 87% with the implementation of intra-partum antibiotic (IPA) for the prevention of early-onset GBS sepsis (**Centers for Disease Control and Prevention, 2013**). After the initiation of IPA for GBS, *Escherichia coli* has emerged as the leading cause of EOS in preterm neonates **(Kumar & Bhat, 2015).**

After GBS, the other prevalent gram-positive organisms causing neonatal sepsis include *Staphylococcus aureus*, *coagulase- negative Staphylococcus* (CoNS), *Enterococcus, and Listeria mono- cytogenes.* Other than *E. coli*, the most common gram-negative organisms are *Klebsiella, Enterobacte*r, *Citrobacter,* and *Pseudomonas* **(Kumar & Bhat, 2015).**

MATERIAL AND METHODS

This study was conducted on 102 neonates suspected to have clinically diagnosed neonatal sepsis admitted to Neonatal Intensive Care Units (NICUs) in 4 distinct hospitals from Menoufia and El Kalyoubia governorates were enrolled. These NICUs and corresponding patients' number were:-

1- Neonatal intensive care unit (NICU), National Liver Institute hospital- Menoufia University, from which 24 cases were included.

2- Neonatal intensive care unit (NICU), Menoufia University hospital, from which 41 cases were included.

3- Neonatal intensive care unit (NICU), Benha University hospital, from which 30 cases were included

4- Neonatal intensive care unit (NICU), Berket El Saba central hospital – Ministry of Health and Population, from which 7 cases were included.

These patients were evaluated for the following seven CBC parameters; (HSS parameters):- total WBC count, total PMN count, immature PMN count, platelets count, degenerative changes in PMN leucocytes (toxic granules and cytoplasmic vacuoles), I/T ratio and I/M ratio.

The sepsis work up was done including routine blood counts along with the HSS value.

The clinical diagnosis of neonatal sepsis dependent on positivity of at least two of the following laboratory screening tests to identify neonates with clinical neonatal septicemia.

The investigations are:-

i.Total leucocytic count < 5000/mm3 or ≥ 25,000 at birth or ≥ 30,000—12–24 h or ≥ 21,000—Day 2 onwards.

ii. Band cell count ≥ 20%

iii. Micro ESR ≥ 15 mm/ 1st hour

iv. C-reactive protein > 6 mg/dL

v. Absolute neutrophil count <1500/mm^3

The clinically diagnosed cases with neonatal sepsis will be undergone conventional blood culture test with automated BACT/ALERT® 3D 60 instrument (BioMérieux, INC. USA) blood culture system to confirm the diagnosis of neonatal sepsis.

Table (1): Hematological scoring system.

Criteria	Abnormality	Score
Total WBC count	≤ 5,000/µl	1
	≥ 25,000 at birth	1
	≥ 30,000—12–24 h	
	≥ 21,000—Day 2 onwards	
Total PMN count	No mature PMN seen	2
	Increased/decreased	1
Immature PMN count	Increased	1
I:T PMN ratio	Increased	1
I:M PMN ratio	≥ 0.3	1
Degenerative changes in PMN	Toxic granules/cytoplasmic vacuoles	1
Platelet count	≤ 150,000/ mm3	1

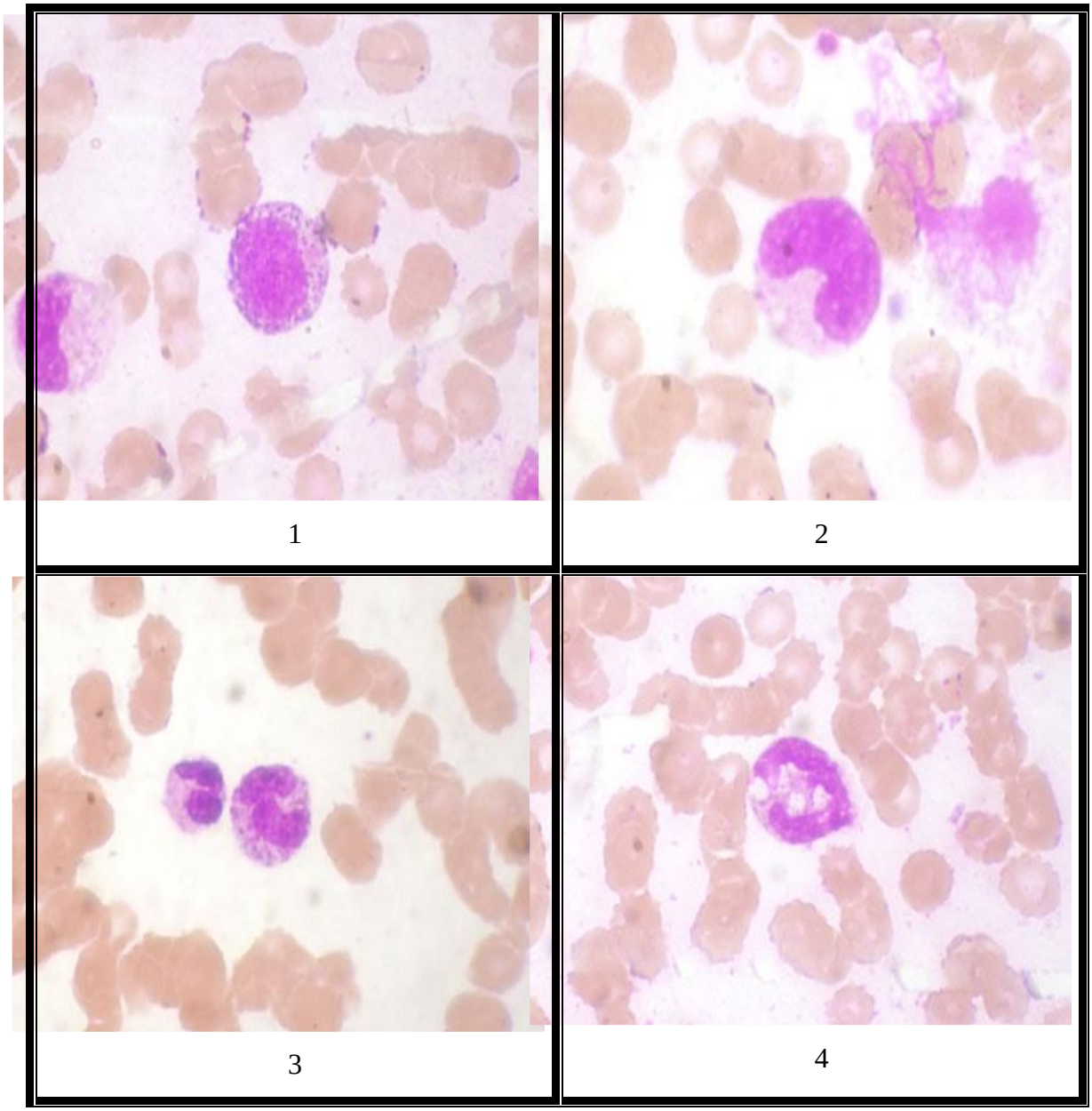
The normal values are

Total PMN count —1800–5400/mm3 Immature: Total PMN ratio— 0.120

Immature PMN count — 600 /mm3 Immature: Mature PMN ratio— ≥0.3

Table (2): Interpretation of hematological scoring system.

Score	Interpretation
≤ 2	Sepsis is unlikely
3 or 4	Sepsis is possible
≥ 4	Sepsis or infection is very likely



(Leishman, x1000)

Figure (1): Photomicrograph of neutrophils differentiation and abnormalities.

1- Immature neutrophils(Promyelocyte and Myelocyte) 3- Neutrophils with toxic granules

2- Band form

4- Neutrophil with cytoplasmic vacuoles

Results

In this study, from 102 clinically diagnosed neonatal sepsis, 42 cases were blood culture proven bacterial neonatal sepsis confirmed with the detection of bacterial growth with BACT/ALERT automated blood culture system. **Table (3)** represents types of bacteria detected with conventional blood culture method in 42 neonates out of 102 clinically diagnosed neonatal sepsis patients. **Table (2**) shows profile of HSS distribution in 42 blood culture proven neonatal sepsis patients.

Table (3): Bacteria and isolated with BACT/ ALERT automated blood culture system.

Organisms	No	percent
1- Bacterial		
Klebsiella	20	47.6%
Staphylococcus aureus	5	11.9%
Coagulase negative staphylococcus **(CoNs)**		
- *Staphylococcus epidermedis*	3	7.1%
- CoNS	2	4.8%
- *Staphylococcus lugdunensis*	1	2.4%
Streptococcus	1	2.4%
E coli	2	4.8%
Enterococci	3	7.1%
Enterobacter	4	9.5%
Acinatobacter	1	2.4%
Total	42	100%

Table (4): Profile of hematological scoring system in blood culture proven sepsis subgroup.

Criteria	Abnormality	score	Number of patients		Total abnormal
			Normal value	Abnormal value	
Total WBC count	**≤ 5,000/µl**	**1**		5	**5**
	≥ 25,000 at birth	**1**		15	**15**
	≥ 30,000—12–24 h				
	≥ 21,000—Day 2 onwards				
	Sum		22	20	**20**
Total PMN count	**No mature PMN seen**	**2**			
	Increased/decreased	**1**	3	39	**39**
Immature PMN count	**Increased**	**1**	22	20	**20**
I:T PMN ratio	**Increased**	**1**	34	8	**8**
I:M PMN ratio	**≥ 0.3**	**1**	41	1	**1**
Degenerative changes in PMN	**Toxic granules/cytoplasmic vacuoles**	**1**	42	0	**0**
Platelet count	**≤ 150,000/ µl**	**1**	25	17	**17**

Table (5): Statistics of hematological scoring system parameters in blood culture proven sepsis subgroup.

Parameter	Value	Cases (n=42)	Control (n=30)	Statistical significance
Total WBC count	Normal Abnormal	22 20	28 2	**P= 0.0002**
Mean± SD		17384 ± 9012	10811 ± 5206	**P = 0.0006**
Total PMN count (< 1800/µl or > 5400/µl)	Normal Abnormal	3 39	21 9	**P < 0.0001**
Mean± SD		10373±6673	5383±2928	**P < 0.0001**
Immature PMN count (> 600 /µl)	Normal Abnormal	22 20	29 1	**P = 0.0002**
Mean± SD		857 ±1024	209 ±163	**P = 0.001**
I:T PMN ratio (> 0.12)	≤ 0.12 > 0.12	34 8	29 1	**P = 0.04**
I:M PMN ratio (≥ 0.3)	< 0.3 ≥ 0.3	41 1	30 0	**P = 0.14**
Degenerative changes in PMN	Absent Present	42 0	30 0	**P = 0.00**
Platelet count (< 150000/ µl)	≥ 150000/ µl < 150000/ µl	25 17	29 1	**P = 0.0003**
Mean± SD		200761±137851	304500±123120	**P = 0.001**
HSS	< 3 ≥ 3	20 22	29 1	**P < 0.0001**
Mean± SD		2.5±1.29	0.47±0.68	**P < 0.0001**

Table (6): Sensitivity and specificity with positive and negative predictive values of hematological scoring system in blood culture proven sepsis subgroup.

Parameter	Cult, +ve (n=42)	Cult,-ve (n=60)	Sensitivity	Specificity	PPV	NPV
Total WBC count						
Abnormal count	20	24	45.45%	62.07%	47.62%	60%
Normal count	22	36				
Total PMN count (< 1800/µl or > 5400/µl)						
Abnormal count	39	44	46.99%	84.21%	92.86%	26.67%
Normal count	3	16				
Immature PMN count (> 600 /µl)						
Abnormal count	20	20	50%	64.52%	47.62%	66.67%
Normal count	22	40				
I:T PMN ratio(> 0.12)						
Abnormal	8	10	44.44%	59.52%	19.05%	83.33%
Normal	34	50				
I:M PMN ratio (≥ 0.3)						
Abnormal	1	6	14.29%	56.84%	2.38%	90%
Normal	41	54				
Platelet count (< 150000/ µl)						
Abnormal	17	27	38.64%	56.9%	55%	96.67%
Normal	25	33				
HSS						
≥ 3	22	24	47.8%	64.29%	52.38%	60%
< 3	20	36				

Table (5) represents statistical significance of HSS parameters in 42 blood culture proven neonatal sepsis patients. Which shows statistical significant difference between blood culture proven sepsis and control group in the following parameters including its mean ± SD:-

Total WBC count, total polymorph nuclear leucocytes (PMNL), immature polymorph nuclear leucocytes, platelets count and HSS. Whether statistical significance present between two groups in I:T PMN ratio (> 0.12). **Table (6**) represents sensitivity and specificity in addition to positive and negative predictive values for different HSS parameters.

Discussion

Hematologic scoring system (HSS) can improve the diagnostic accuracy of diagnosing neonatal sepsis **(Narasimha & Kumar, 2011).**

Our study was designed to detect the ability of hematological scoring system (HSS) in early diagnosis of neonatal sepsis. And try to prove that, HSS is a simple, quick, cost effective tool which can be used as a screening test for early diagnosis of neonatal sepsis. From 102 clinically diagnosed neonatal sepsis patients blood culture proven neonatal sepsis were detected in 42 cases. The bacteria which were isolated and its corresponding cases numbers were shown in **Table (1)**.

In this study, there were 7 hematologic parameters and indices were evaluated in HSS which include; total WBC count, total PMN count, immature PMN count, platelet count, degenerative changes in PMN cells (toxic granulations and cytoplasmic vacuoles), I/T ratio and I/M ratio. Total WBC count and its mean ± SD show statistical significance difference in blood culture proven sepsis group than control group. It has low sensitivity and PPV but to some extent moderate specificity and NPV. Low sensitivity for total WBC match the results of one study done by **Narasimha and Kumar** who found the sensitivity of total WBC count was 10.52% while the specificity was 91.66% the PPV and NPV in the same were 80% and 24.44% respectively for the diagnosis of neonatal sepsis **(Narasimha & Kumar,2011).** In another study by **Shirazi et al**., that evaluate the hematological profile of neonatal sepsis they found the sensitivity of total WBC count was 35% and specificity was 77 % for neonatal sepsis diagnosis **(Shirazi et al, 2010).** Total PMN count and its mean ± SD show statistical significance for the diagnosis of neonatal sepsis. It has a sensitivity of 46.99% for the diagnosis of neonatal sepsis and specificity of 84.21%. The PPV and NPV for PMN count for the diagnosis of neonatal sepsis was 92.86% and 26.67% respectively. **Narasimha and Kumar** reported high sensitivity and PPV for total PMN count in the diagnosis of neonatal sepsis; 89.47% and 75.55% respectively **(Narasimha & Kumar, 2011).** In accordance with large number of studies who have observed that the total leucocytic count or the total neutrophil count show no significant association with sepsis. It should not be deceived by neutrophil count alone without noting alteration in the ratio of mature and immature neutrophils **(Shirazi et al, 2010)**. Immature PMN count and its mean ± SD show statistical significance for the diagnosis of neonatal

sepsis. it has moderate specificity and NPV; 64.52% and 66.67% respectively. Immature circulating neutrophils, known as band cells, appear in peripheral blood in response to infection **(Bedford Russell, 2015). Narasimha and Kumar** reported high sensitivity and PPV for immature PMN count for the diagnosis of neonatal sepsis; 78.94% and 73.17% respectively **(Narasimha & Kumar, 2011).** Platelets count and its mean ± SD show statistical significance for the diagnosis of neonatal sepsis. it has high NPV for the exclusion of neonatal infections. Thrombocytopenia is a common feature of generalized infection and necrotising enterocolitis (NEC), but is also a feature of non-infective disorders such as hypoxic ischaemic encephalopathy **(Bedford Russell, 2015).**It characterized by poor sensitivity and specificity for the diagnosis of neonatal sepsis and are unreliable diagnostic parameter for initiating or discontinuing antibiotics **(Manzoni et al, 2009). Narasimha and Kumar** reported high specificity and PPV for platelets count in the diagnosis of neonatal sepsis; 75% and 85.71% respectively **(Narasimha & Kumar,2011).** Shirazi et al, found the sensitivity of platelets count was 61% and specificity was 82% for neonatal sepsis diagnosis **(Shirazi et al, 2010).** I/T ratio show statistical significance for the diagnosis of neonatal sepsis. It has high NPV for the exclusion of neonatal infection (83.33%). I/T ratio of > 0.2 is highly suspicious for neonatal sepsis **(Bedford Russell, 2015).** High immature-to-total neutrophil ratio (I:T ratio) were associated with increasing odds of infection,; however, the test sensitivities for detection of sepsis were low **(Hornik et al, 2012). Narasimha and Kumar** reported high PPV for I/T ratio for the diagnosis of neonatal sepsis 88.88 % **(Narasimha & Kumar, 2011).**
I/M ratio show no statistical significance for the diagnosis of neonatal sepsis. it has high NPV for the exclusion of neonatal infection (90%). **Narasimha and Kumar** reported high sensitivity and PPV for immature PMN count for the diagnosis of neonatal sepsis; 73.68% and 82.35% respectively **(Narasimha & Kumar, 2011)**.

Conclusions

In this study, the HSS was statistically significant test for the early diagnosis of neonatal sepsis. It is a useful preliminary screening test for early detection of neonates with sepsis with good negative predictive values.

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