DOI: 10.58665/NJISS

Original Article

Diagnostic Accuracy of Haematological Scoring System in Paired cord Blood and Peripheral Venous Blood for early Detection of Neonatal Sepsis – A Prospective Analytical Study

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Article History

Received On – 2022 Nov 19

Accepted On – 2023 Jul 03

Funding sources: None

Conflict of Interest: None

Keywords:
Haematological scoring system; High risk neonate; Neonatal sepsis; Peripheral venous blood; Umbilical cord blood;

Online Access

DOI: 10.60086/jnps483

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Abstract

Introduction: Sepsis is one of the major causes of neonatal morbidity and mortality. Early recognition and diagnosis of early onset neonatal sepsis (EONS) is difficult. Hence, there is a need for early predictive screening method for EONS, for which Haematological Scoring System (HSS) is used. It comprises of total leucocyte count, immature / total neutrophil ratio, total PMN count, immature PMN count, degenerative changes in PMN and platelet count.

Methods: 100 inborn neonates with two or more risk factors for EONS, chosen by sequential sampling method were included in this prospective analytical study. Blood samples were collected from umbilical cord and peripheral vein and analysed for haematological parameters. Blood cultures were performed as gold standard for diagnosing neonatal sepsis and sepsis screen was done to corroborate the diagnosis of EONS.

Results: Out of 100 neonates, 21 had sepsis, 14 had probable sepsis and 65 had no sepsis. Among the variables of HSS it was observed that elevated I:T ratio, thrombocytopenia, elevated I:M ratio and elevated immature neutrophil count have shown significant correlation with EONS with statistically significant p values (p value < 0.05), with raised I:T ratio being highly sensitive in identifying neonatal sepsis and degenerative changes in neutrophils being highly specific.

Conclusions: The HSS was found to be satisfactory in identifying EONS. It can be used as a simple, quick, cost effective and readily available screening test with decent sensitivity and high specificity, for detection of EONS.

Introduction

Neonatal sepsis is the commonest cause of neonatal mortality. Incidence of neonatal sepsis in India is 30 per 1000 live births (NNPD database).[1] Early onset neonatal sepsis (EONS) has subtle and varied clinical presentation in the initial stages. Aggressive approach to diagnosis and management is the principle determinant of the prognosis. Early diagnosis of EONS is the corner stone to reduce the case fatality rate.[2] EONS usually occurs in the first 72 hours of life, with 80 to 90% of cases presenting up to 48 hours after birth. EONS manifests as fulminant, multisystem illness usually acquired by vertical transmission from the mother with high case fatality rate.[2] The usual practice is to collect blood sample by venipuncture from the neonates and send for culture and haematology, which can induce pain or infection or iatrogenic anemia to the neonates. There are very few previous documented reports of early predictive screening methods for EONS by using cord blood sample.

The definitive diagnosis of EONS is made by a positive blood culture, which requires a

minimum of 48 to 72 hours and yields a positive result in 30 - 40% of cases. Blood culture remains the gold standard for diagnosis of neonatal sepsis, yet it is important to develop effective screening tools which can presumably diagnose or exclude EONS at the time of presentation.

As no single individual hematological parameter is superior in comparison to another in predicting EONS, a combination of these parameters in the form of (Haematological Scoring System) HSS has been recommended. HSS was introduced by Rodwell et al and[3] includes hematological parameters (Total leucocyte count, immature / total neutrophil ratio, total PMN count, immature / mature neutrophil ratio, immature PMN count, degenerative changes in PMN, platelet count). It has been shown that such score could accurately predict the presence or absence of infection and be reliable. The HSS has practical advantages - it is applicable to all infants, including those who have received antibiotic therapy prior to evaluation and simplifies the interpretation of hematologic profile. It is easily available, accessible, cheap, less time consuming and can be done in all the laboratories. However, this scoring has not been tested in our scenario and this study has been aimed check the accuracy of HSS in paired cord blood and peripheral venous blood for detection of EONS.

Methods

This prospective analytical study was carried out in nursery of tertiary care centre of Central India city over a period of one year from January 2020 to December 2020 after getting clearance from Ethics Review Committee. 100 term neonates with any two of the known risk factors like prematurity (< 37 weeks), preterm premature rupture of membranes, more than three vaginal examinations after rupture membranes / one unclean vaginal examination, history of maternal fever, foul – smelling liquor, chorioamnionitis, prolonged rupture of membrane (> 18 hrs), prolonged labour > 24 hours, perinatal asphyxia (Apgar score of < 4 in one minute) were included in the study by sequential sampling method. Neonates born with lethal congenital anomalies and outborns were excluded. Umbilical cord blood is collected at birth post stabilization of neonate. After clamping the cord, the placental end is wiped with 70% isopropyl alcohol and with a 22 Gauge syringe, 4 - 6 ml blood is collected from the placental end of umbilical artery or vein. 2 ml of cord blood is transferred to anticoagulant EDTA bulb for analysing haematological parameters, sepsis screen (CBC with ANC, toxic granules, I:T ratio. I:M ratio and CRP) and for peripheral smear for HSS. The remaining 2 - 3 ml of blood sample is sent for blood culture in BACTEC culture vials. Peripheral venous blood sample is also taken within six hours of birth for HSS, sepsis screen and blood culture before starting antibiotics according to NICU protocols. Demographic, birth and clinical details of all the subjects is recorded and tabulated on Microsoft excel spreadsheet and analysed as per SPSS version 20. The data was expressed in terms of rates, ratio and percentages. Effectiveness of HSS score and perinatal risk factors association with EONS were tested using Chi square test to compare or associate nominal data. A probability value (P value) of less than 0.05 was considered statistically significant. Using the above data diagnostic parameters – sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of UCB and PVB, HSS & its individual variables are calculated and compared. Diagnostic outcomes of all subjects were divided into three categories –

- Neonates with no signs of sepsis in next 72 hrs with PVBC sterile will be considered normal or no sepsis group
- Neonates with clinical signs of sepsis with sepsis screen +/- with PVBC sterile, were diagnosed as probable / clinical sepsis
- Neonates with clinical signs of sepsis and PVBC showing growth were grouped as proven sepsis4

Haematological scoring system as per rodwell et al.

CRITERIA	ABNORMALITY	
Total WBC Count	< = 5000/microliter	1
	> = 25000 at birth	1
	> = 30000 – 12-24 hr	1
	> = 21000 – day 2 onwards	1
Total neutrophil count	No neutrophil	2
	Increased / decreased	1
	Normal (1800 - 5400)	0
Immature neutrophil count	Increased (> 600 / microliter)	1
Immature:Total neutrophil ratio	> 0.2	1
Immature: Mature PMN ratio	> 0.3	1
Degenerative changes in PMN	Toxic granules / dohle bodies / cytoplasmic vacuoles	1
Platelet count	< 150000 / microliter	1

For the purpose of this study, we used Rodwell's HSS with 2 or less than 2 being negative and 3 or more than 3 being positive for sepsis.[3,5]

Results

Figure 1 depicts the distribution of diagnosis EONS in the study population. Table 2 represents the clinical features of EONS. Table 3 shows the HSS scores in UCB and PVB. Tables 4 and 5 compare the diagnostic accuracy of HSS scores and different hematological parameters in UCB and PVB respectively.

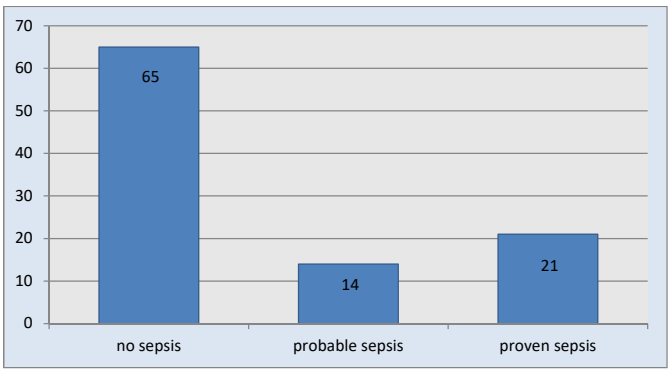

Figure 1: Diagnostic distribution for EONS

Table 1: Association of neonatal profile with EONS

Patient's Profile	No Sepsis (N = 65) No. (%)	Probable Sepsis (N = 14) No. (%)	Proven Sepsis (N = 21) No. (%)	Total No. (%)	p value
Gender:					
Male	30 (46.1)	5 (35.7)	6 (28.6)	41 (41.0)	
Female	35 (53.8)	9 (64.3)	15 (71.4)	59 (59)	
Gestational age:					
Preterm (< 37 weeks)	40 (61.5)	9 (64.2)	13 (61.9)	62 (62.0)	
Term (≥ 37 weeks)	25 (38.5)	5 (35.8)	8 (38.1)	38 (38.0)	
Birth weight:					
BW (< 2.5 kg)	38 (58.4)	9 (64.2)	15 (71.4)	62 (62.0)	
BW (≥ 2.5 kg)	27 (41.5)	5 (35.7)	6 (28.6)	38 (38.0)	
Mode of delivery:					
LSCS	28 (43)	5 (35.7)	7 (33.3)	40 (40.0)	
NVD	37 (57)	9 (64.3)	14 (66.7)	60 (60.0)	
Risk factors distribution:					
Prematurity (< 37 weeks)	40 (62.0)	6 (42.8)	13 (61.9)	59	0.0009
Maternal fever	19 (30.4)	8 (57.1)	14 (66.7)	41	0.002
Prolonged rupture of Membrane (> 18 hours)	23 (35.4)	5 (35.7)	9 (42.8)	37	0.03
Preterm premature rupture of membrane	20 (31.6)	4 (28.5)	7 (33.3)	31	0.04
3 or more vaginal examination after rupture of membrane	20 (30.4)	4 (28.5)	6 (28.5)	30	0.34
Meconium stained liquor	18 (27.8)	1 (7.1)	3 (14.3)	22	0.20
Birth asphyxia (Apgar score of < 4 in 1 minute)	12 (19.0)	4 (28.5)	2 (9.5)	18	0.30
Prolong labour (> 24 hours)	13 (19.0)	3 (21.4)	1 (4.7)	17	0.55
Foul smelling liquor	0 (0.0)	1 (7.1)	8 (38.1)	9	0.001
Chorioamnionitis	0 (0.0)	1 (7.1)	8 (38.1)	9	0.001

Table 2: Distribution of Clinical Features of EONS

Clinical features	Sepsis (Proven + probable) (N = 35)	
	No.	%
Respiratory distress (Tachypnoea, grunting, chest retraction)	24	68.57
Shock	20	57
Lethargy	16	45.7
Abdominal distension	13	37
Feeding difficulty / intolerance	11	31
Bleeding / altered orogastric aspirate	11	31
Seizure	10	28.6
Hypoglycemia	8	22.8
Jaundice	7	20
Fever	5	14

Table 3: HSS score in UCB and PVB

	Final diagnosis		
	Sepsis (Proven + probable) (N = 35)	No sepsis (N = 65)	Total (N = 100)
HSS in UCB			
Positive (score ≥ 3)	26	5	31
Negative (score < 2)	9	60	69
HSS in PVB:			
Positive (score ≥ 3)	22	7	29
Negative (score ≤ 2)	13	58	71
Total	35	65	100

Table 4: Diagnostic accuracy of different hematological parameters of HSS in UCB and PVB in predicting EONS

Haematological Parameters	Cord blood / peripheral venous blood	Sepsis (Proven +probable) (N = 35)	No sepsis (N = 6)	P value	Sensitivity (%)	Specificity (%)
Leucopenia ≤ 5000/ml or Leucocytosis ≥ 25000/ml	Cord blood	8	16	0.32	22.8	75.4
	Peripheral venous blood	5	17	0.42	14.3	73.8
Total neutrophil count < 1800 / ml or > 5400 / ml	Cord blood	16	20	0.06	45.7	69.2
	Peripheral venous blood	10	18	0.26	28.6	72.3
Immature neutrophil count > 600 / ml	Cord blood	18	12	0.02	51.4	81.5
	Peripheral venous blood	20	14	0.038	57.1	78.4
Immature: Total neutrophil ratio (I:T ratio) > 0.2	Cord blood	28	22	0.004	80	66.2
	Peripheral venous blood	31	24	0.002	88.6	63
Immature: Mature neutrophil ratio (I:M ratio) > 0.3	Cord blood	21	15	0.01	60	76.9
	Peripheral venous blood	23	16	0.006	65.7	75.4
Degenerative changes in neutrophils (Toxic granules, dohle bodies, cytoplasmic vacuoles)	Cord blood	9	10	0.28	25.7	84.6
	Peripheral venous blood	6	8	0.29	17.1	87.6

Table 5: Diagnostic parameters of HSS in UCB and PVB

	Sensitivity	Specificity	PPV	NPV
HSS in UCB	74%	92%	84%	87%
HSS in PVB	63%	88%	76%	82%

Discussion

For definite diagnosis of sepsis, blood culture results are gold standard which is time consuming and not readily available. The other recent available markers are sensitive but expensive. Hence they have limited use in financially constrained setup. Therefore, there is always a need for an infallible cost-effective test for early detection of EONS that could be easily performed. The importance of the HSS in such scenario is paramount. In this study we have evaluated the effectiveness and analyse the diagnostic accuracy of different haematological parameters of HSS in paired UCB and PVB in high risk neonates for detection of EONS.

Males, preterm and LBW babies predominantly developed sepsis in the present study, which is consistent with other studies from similar set ups in India.[4,6,7] Dutta NR et al attributed male predominance to globulin synthesizing factors on X chromosome resulting in more sepsis. Preterms and LBW babies have less effective phagocytosis and chemotactic activity and impaired defense mechanisms and low immunoglobulin M, resulting in sepsis predominance.[5,7] There was no significant correlation of sepsis status with NVD and LSCS delivered neonates just like other studies from our region.[4,6]

The present study illustrated the risk factors maternal fever, prematurity, prolonged rupture of membrane, premature rupture of membrane, foul smelling liquor, chorioamnionitis a statistically significant correlation with EONS with p value < 0.05. In the present study neonates with two or more risk factors were included. So, the neonates who were admitted were having multiple risk factors with some risk factors being more frequent than others, similar to other studies from our region.[6-9] Out of 35 sepsis (Proven + probable sepsis) cases, respiratory distress was the commonest clinical feature and majority had presented with more than two or three clinical features. These results are also corroborated well with other similar studies.[6,7]

The definitive diagnosis of sepsis is made by a positive blood culture, which requires a minimum of 48 to 72 hours, yields a positive result in 30 - 40% of cases, yet it is important to develop effective screening tools which can presumably diagnose or exclude neonatal sepsis at the time of presentation. Early diagnosis of neonatal sepsis is still a great challenge. For early diagnosis, HSS was introduced in the past by Rodwell et al.[3] In this study, we used HSS and its individual components for diagnosis of EONS. The advantages of HSS is that it is applicable to all infants, including those who have received prior antibiotic therapy, easily available, accessible, low cost, less time consuming and can be done in all the laboratories.

The parameters which showed a statistically significant correlation with EONS with p value < 0.05, were elevated I:T ratio, thrombocytopenia, elevated I:M ratio and elevated immature neutrophil count in HSS of UCB and PVB. Rest of the parameters did not show any statistically significant correlation with EONS. Similar findings were reported in other studies too.[4,6,7]

Neonates with sepsis develop thrombocytopenia, possibly because of DIC and the damaging effects of endotoxin on platelets causing increased platelet destruction, sequestration secondary to infections and failure in platelet production due to reduced megakaryocytes.[10] Total leukocyte count is found to be less reliable indicator of neonatal sepsis because of the wide variation in values.[10] The total PMN count has a limited role in sepsis screening as neutropenia has been more common in association with sepsis, compared with neutrophilia, probably because of increased adherence to altered endothelial cells and utilization at the site of infection.[11] The wide variation in the results of these parameters in different subjects might be also due to difference in the blood sampling time, exact method of test employed, the severity of infection, and the age of the neonates.[11]

Degenerative changes in PMN can also help diagnosing neonatal sepsis. The presence of toxic granules indicates the production of unusual PMN during infection and stress induced leukopoiesis. Activated PMN by bacterial infections can also give a microscopic overview as vacuoles or a large mass of blue at the edges of the cytoplasm called Dohle bodies. Their presence invariably indicates sepsis, but their count is not always increased.[12] Elevated I:T PMN ratio was found to be the most reliable indicator of sepsis in our study with highest sensitivity for sepsis whereas degenerative changes in neutrophils have highest specificity in both UCB and PVB.

Diagnostic parameters of HSS in UCB for detecting EONS are – sensitivity - 74%, specificity - 92%, PPV - 84%, NPV - 87%. Similar parameters of HSS in PVB for detecting EONS are – sensitivity - 63%, specificity - 88%, PPV - 76%, NPV - 82%. HSS score in UCB in our study has higher sensitivity as compared to PVB and comparable specificity. This may be attributed to more blood sample availability as UCB can be collected easily, effortlessly and easily accessible without causing any pain to the neonate. For neonates, blood sampling by venipuncture can be difficult. In routine clinical practice a large proportion of CBC reports were spurious because of the submission of an inadequate volume of blood and prevent repeated needle prick.[13] UCB sample is collected before instillation of antibiotics which also adds to its higher sensitivity and prevents iatrogenic anemia and introduction of infection.[14] Hence, HSS score in UCB can be used as a screening tool for detection of EONS.

We have to acknowledge our limitations. This is a single centric, small study which requires validation in further larger, multi centric studies in the future. This can be useful in reducing neonatal morbidity and mortality by identifying the neonatal sepsis early and providing guidance towards the decision-making for a rationale treatment. It also helps in avoiding unnecessary instillation of antibiotics and preventing development of antibiotic resistance.

Conclusions

The diagnostic parameters of various variables of HSS were found to be satisfactory in identifying EONS in high risk neonates. HSS score in UCB can be used as a simple, quick, cost effective and readily available tool with decent sensitivity and high specificity for detection of EONS. Elevated I:T PMN ratio was found to be the most reliable individual indicator of sepsis and TLC least reliable indicator of sepsis in both UCB and PVB.

References

1. Deorari AK. Changing pattern of bacteriologic profile in neonatal sepsis among intramural babies. J Neonatol. 2006;20(1):8–15. DOI:10.1177/0973217920060103

2. Hansen AR, Eichenwald EC, Stark AR, Martin CR, editors. Cloherty and stark's manual of neonatal care. 8th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2016.

3. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr. 1988;112(5):761–7. DOI: 10.1016/s0022-3476(88)80699-1

4. Makkar M, Gupta C, Pathak R, Garg S, Mahajan NC. Performance evaluation of hematologic scoring system in early diagnosis of neonatal sepsis. J Clin Neonatol . 2013;2(1):25–9. DOI: 10.4103/2249-4847.109243

5. Narasimha A, Harendra Kumar ML. Significance of hematological scoring system (HSS) in early diagnosis of neonatal sepsis. Indian J Hematol Blood Transfus . 2011;27(1):14–7. DOI: 10.1007/s12288-010-0050-2

6. Pramana KP, Kardana IM, Nilawati GAP. Diagnosis accuracy of hematological scoring system in early identification of neonatal sepsis. Bali Med J . 2016;5(3):139. DOI: 10.15562/bmj.v5i3.310

7. Rani Dutta N, Medhi P, Hazarika R. Haematological scoring system (hss) in early diagnosis of neonatal sepsis: A study from tertiary care hospital from North East India. J evol med dent sci . 2016;5(52):3359–66. DOI: 10.14260/jemds/2016/777

8. Mandot S, Gandhi J. Umbilical cord blood culture versus peripheral venous blood culture in early onset neonatal sepsis. Int J Contemp Pediatr . 2017. DOI: 10.18203/2349-3291.ijcp20164302

9. Meena J, Charles MVP, Ali A, Ramakrishnan S, Gosh S, Seetha KS. Utility of cord blood culture in early onset neonatal sepsis. Australas Med J. 2015;8(8):263–7. DOI: 10.4066/AMJ.2015.2460

10. Arif SH, Ahmad I, Ali SM, Khan HM. Thrombocytopenia and bacterial sepsis in neonates. Indian J Hematol Blood Transfus. 2012;28(3):147–51. DOI: 10.1007/s12288-011-0118-7

11. Al-Gwaiz LA, Babay HH. The diagnostic value of absolute neutrophil count, band count and morphologic changes of neutrophils in predicting bacterial infections. Med Princ Pract . 2007;16(5):344–7. DOI: 10.1159/000104806

12. Hutchison RE, Abraham NZ. Henry's clinical diagnosis and management by laboratory methods. McPherson RA, Pincus MR, editors. Philadelphia, USA: Elsevier; 2021.

13. Schelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM, Ascher DP. Volume of blood required to detect common neonatal pathogens. J Pediatr. 1996;129(2):275–8. DOI: 10.1016/s0022-3476(96)70254-8

14. Connell TG, Rele M, Cowley D, Buttery JP, Curtis N. How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. Pediatrics. 2007;119(5):891–6. DOI: 10.1542/peds.2006-0440