Section: Paediatrics

Original Research Article

TO ESTIMATE THE USEFULNESS OF RED CELL DISTRIBUTION WIDTH TO PLATELET RATIO IN DIAGNOSING SEPSIS AT EARLY STAGE IN TERM NEONATES

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Abstract

Background: Sepsis is a major preventive cause of neonatal morbidity and mortality in developing countries. Red cell distribution width to platelet ratio can be used as adjunct to C- reactive protein (CRP) in early diagnosis of sepsis along with clinical correlation. Primary objective of study is to estimate the usefulness of red cell distribution width to platelet ratio(RPR) in diagnosing sepsis at early stage in term neonates. Secondary objectives are to assess the sensitivity and specificity of red cell distribution width to platelet ratio in neonatal sepsis and compare with CRP and Neutrophil lymphocyte ratio and other complete blood count parameters. **Materials and Methods:** This Prospective observational study was done at NICU, Govt.Raja Mirasdar hospital, Thanjavur among 100 Term Neonates for a period of 12 months. 50 neonates with clinical suspicion of sepsis and 50 normal neonates without any risk factors are investigated with sepsis screen, blood culture and RPR in complete blood count. CRP of all neonates taken after 6 hours. Results were compared and data were analysed using descriptive statistics and subgroup analysis was done with sepsis and normal neonate groups. **Result:** Mean RPR value in sepsis absent group is 0.075 with SD 0.05 and in sepsis group Mean RPR value is 0.21 with SD 0.21 with p value of < 0.0001 which is statistically significant. Sensitivity and specificity of RPR was 74% and 76% respectively. Sensitivity of RPR was higher than CRP(60%) and equivalent to NLR(74%) in predicting sepsis earlier. Specificity of RPR was less than CRP(100%) and less than that of NLR(46%). **Conclusion:** Red Cell distribution width to platelet ratio is significantly increased in term neonates with sepsis. It can be used along with CRP with clinical suspicion of sepsis to identify cases and treat neonatal sepsis earlier with rational empirical antibiotics.

INTRODUCTION

Sepsis is a life- threatening condition and a leading cause of Morbidity and mortality in neonates, especially in developing countries. With effective prevention of sepsis, timely recognition, antimicrobial therapy and supportive care prevent sepsis- related mortality. Neonatal sepsis is a clinical syndrome characterized by signs suggestive of infection, with or without accompanying bacteremia, occurring in the first 1 month of life.[1] Blood culture, though a gold standard test in the diagnosis of neonatal sepsis requires a long turnaround time and comes out positive in less than a third of suspected neonates. C-Reactive Protein is an acute phase protein mostly produced by liver following the onset of inflammation, but it increases after 12hour onset of sepsis and noninfectious inflammatory disease.[2]

Red Cell Distribution (RDW) is the quantitative assessment of the variation in red cell volume and it corresponds to the microscopic analysis of the degree of anisocytosis. RDW can be expressed either as Standard deviation (SD) in femtolitre or as the coefficient of variation (CV) of the measurements of the red cell volume in percentage.

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RDW indicates whether the size of red cell volume is uniform or not. Some studies have observed that red cell distribution with (RDW) varies significantly in pathological conditions associated with inflammation and infection.[3] RDW is increased in sepsis because of the effect of proinflammatory cytokines on red cell production. The markers of inflammation like RDW-associated interleukins-6(IL-6), Tumor necrosis factor-alfa (TNF-α) and proinflammatory cytokines suppress the maturation process of RBCs, which increases their half lives with a resultant rise in the RDW. In addition, high oxidative stress can also reduce erythrocyte survival and increase the release of large premature erythrocytes into the circulation. Platelet plays a key role in regulating inflammation and innate immunity. Platelet adhere to endothelium and mediates neutrophil chemotaxis, infiltration and secretion of proinflammatory cytokines in the process of acute inflammation Another important factor in inflammation, known as tissue factor, induces coagulopathy and leads to reduced platelet count. Platelet count decreases in severe diseases and is a predictor of mortality. A great number of the studies have reported that red cell distribution width to platelet (RPR) is as a useful systemic inflammatory marker and prognostic indicator of adult inflammatory diseases such as hepatic fibrosis in chronic hepatitis B, myocardial infarction,[4] and acute pancreatitis. There is a paucity of studies on RDW in the newborns and its association with neonatal sepsis. In the present study, the RDW and RPR parameters, which are parts of a complete blood count analysis, were compared to the traditional CRP report to investigate the potential to predict sepsis in neonates with risk of sepsis.

Sepsis is generally defined as "life threatening organ dysfunction caused by a dysregulated host response to infection. Globally, three million neonates are affected by sepsis annually (22/1000 live births) and among them 11% - 19% die.[5] Among countries having higher neonatal mortality (NMR > 45 PER 1000 live births), 50% of these neonatal deaths are due to infection. Almost one-fifth of the total live births and 27 % of the neonatal mortality globally, are contributed by India.[6]

Clinical manifestations of early onset sepsis (EOS) are usually apparent within first 72 h after delivery. EOS is usually due to vertical mother-to-infant transmission and may be acquired before or during delivery. The causative organisms are similar to those infecting/ colonizing the mother. Late onset sepsis (LOS) is due to microbes picked up after birth from the community or the hospital usually manifesting 72 h after delivery. Sometimes, organisms may be acquired during delivery but the clinical findings may be detected 72 h after delivery. Many extremely low gestational age and high risk term infants who are forced to stay in hospital for a longer duration due to various reason are also susceptible to infection.

Investigations

Since treatment should be initiated in a neonate suspected to have sepsis without any delay, only minimal and rapid investigations should be undertaken.

Indirect markers to diagnose sepsis: Among them commonly and widely used indirect markers of infection are Total leucocyte count, Absolute neutrophil count, Immature to total neutrophil ratio >0.2, C- Reactive Protein (CRP), Micro ESR. These studies are collectively called as "sepsis screen''

C- Reactive Protein (CRP): CRP is elevated in conditions of infections and inflammation. CRP more than 6mg/dl is considered significant in neonates.

Red cell distribution width

RDW is usually expressed as RDW-coefficient of variation. Reference intervals of RDW for neonates are higher than for older children and adults. At birth, the lower reference limit for term and late preterm neonates is 15.5%. The upper reference limit is 20% and is slightly higher and variable in preterm neonates. The mean normal range for RDW in preterm is 17.8+_ 2.1 and in term babies is 16.7+_ 1.6. The normal range for RDW values at 32-34 weeks is higher than at 35-36 gestational weeks.[7] RDW is highly variable in preterm neonates.

Platelet Count

Thrombocytopenia is classified as Mild - 100000 to 150000 cells/cu mm, Moderate - 50000 to 99000 cells/cu mm, Severe - < 50000 cells/cu mm. Mean platelet count are lower in preterm neonate than term or near- term neonate.[8] Incidence of thrombocytopenia is inversely correlated to the gestational age, reaching approximately 70% among neonates born with a weight <1000 gram.[9] Newer Investigations are Procalcitonin assay, Fibronectin, Alpha 1 antitrypsin, Alpha 1 chymotrypsin, Haptoglobulin etc

Aims and Objectives

Primary objective - To estimate the usefulness of red cell distribution width to platelet ratio (RPR) in diagnosing sepsis at early stage in term neonates. Secondary objective - To estimate the sensitivity and specificity of red cell distribution width to platelet ratio in detecting neonatal sepsis in study groups and to compare with CRP, NLR (Neutrophil leukocyte ratio) and other complete blood count parameters.

MATERIALS AND METHODS

Sample size: Based on previous study mean and standard deviations between 2 groups the sample size was calculated. And keeping the confidence interval 95% and power at 80% sample size was found to be total of 100 and divided into 2 groups.

Sample Method: Conventional sampling method

Materials and Methods: Study Design - Prospective Observational study, Study Setting -

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SNCU, RMH THANJAVUR, Study Period - From march 2021 to march 2022, Study Population - A total of 50 normal term neonates and 50 term neonates with the risk of neonatal sepsis and clinical features of sepsis admitted at Govt. Rajamirasdar Hospital NICU were taken for the study after IEC approval.

Inclusion criteria

Cases: Term Neonates admitted in NICU with clinical features / risk factors of sepsis

Control: Term neonates admitted in NICU other than suspected sepsis and those enlisted in exclusion criteria

Exclusion Criteria: Preterm neonates, Neonate with birth asphyxia, Meconium aspiration syndrome, Congenital malformations, Metabolic disease, ABO/Rh ISO immunization

Parameters Analysed: Red cell distribution width (RDW), Platelet count, NLR, RDW to Platelet ratio, CRP

Study Protocol: Detailed clinical examination will be done in all neonates enrolled in our study after getting consent from the parents. Blood samples will be done and analyzed for sepsis screen, blood culture, RPR before giving antibiotics. CRP taken in early onset sepsis only after 6 - 12 hours. Neonates with evidence of sepsis totally 50 numbers are investigated and followed up. 50 normal newborns admitted for reasons other than exclusion criteria are also investigated with complete blood count and CRP.

Statistical analysis: The statistical analyses were performed using SPSS version 20 trial software. Data were presented as mean with Standard deviation for normal distribution/scale data. Data were presented as frequency with proportion n (%) for categorical data. Fisher's exact test was used to compare the proportions between the groups. Unpaired 't' test was used to compare the mean between the two groups. Mann Whitney U test was used to compare the median range of two groups for data with non-parametric distribution. ROC curve was constructed for various parameters in predicting the occurrence of neonatal sepsis and cut-off value, sensitivity, specificity, PPV and NPV was measured. p value<0.05 were considered statistically significant.

RESULTS

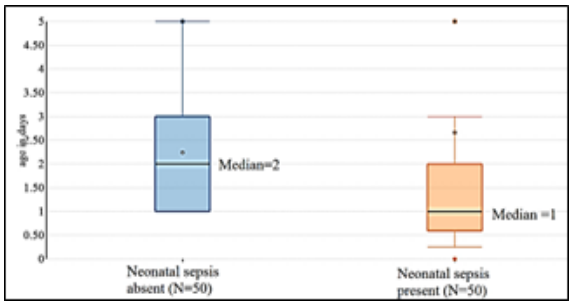

Figure 1: Comparison of neonatal age in days between the neonatal sepsis present and absent group

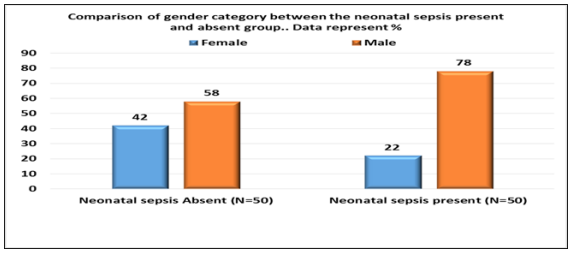

Figure 2: Comparison of gender category between the normal neonates and neonates with risk of sepsis

Data are expressed Mean with SD. Unpaired 't' test was used to compare the mean between the groups. Indicates p<0.05 and considered statistically significant.

Data are expressed as n (%). Fisher's exact test was used to compare the frequency between the two groups.

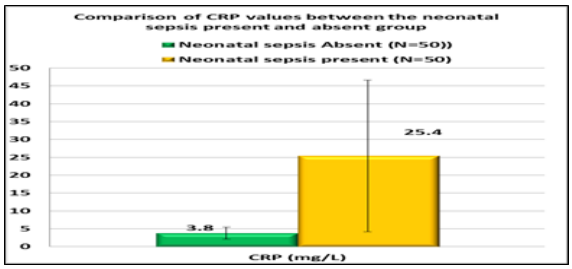

Figure 3: Comparison of CRP values between the neonatal sepsis present and absent group

Data are expressed Mean with SD. Unpaired 't' test was used to compare the mean between the groups.

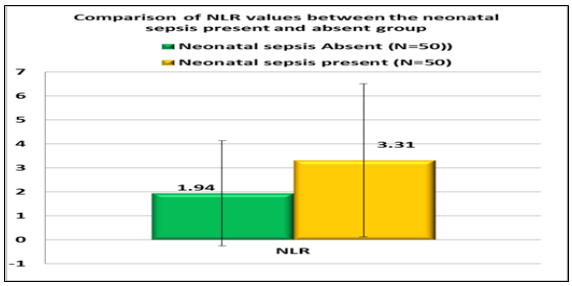

Figure 4: Comparison of NLR values the neonatal sepsis present and absent group

Data are expressed Mean with SD. Unpaired 't' test was used to compare the mean between the groups. *Indicates p<0.05 and considered statistically significant.

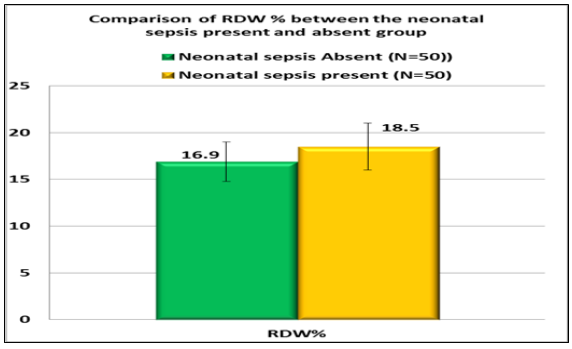

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Figure 5: Comparison of RDW values the neonatal sepsis present and absent group

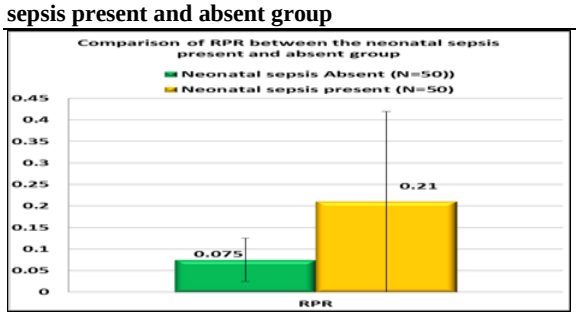

Figure 6: Comparison of RPR values the neonatal sepsis present and absent group

Data are expressed as n (%). Fisher's exact test was used to compare the frequency between the two groups.

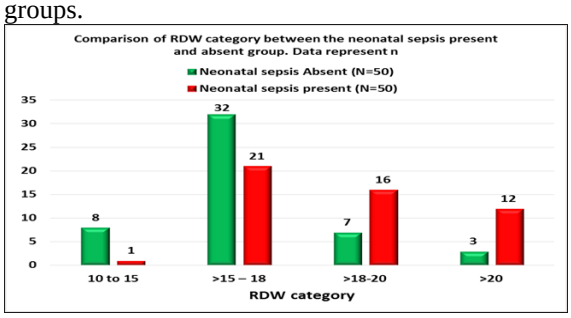

Figure 7: Comparison of RDW category between the neonatal sepsis present and absent group

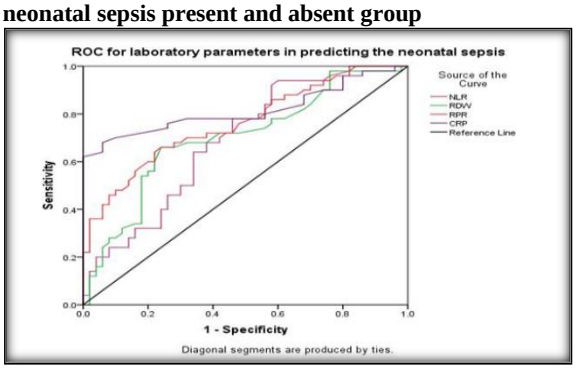

Figure 8: Description of ROC statistics for various laboratory parameters in predicting the neonatal sepsisstatu

Table 1: Comparison of neonatal age in days between the neonatal sepsis present and absent group.

S.No	Parameter	Normal Neonates (N=50)		Neonatal at risk of sepsis (N=50)		Mann whiteny U	Std error	P value
		Median	IQR	Median	IQR			
1	Age in days	2	1-3	1	0.5-2	710	141.7	<0.0001

Table 2. Comparison of gender category between the normal neonates and neonates with risk of sepsis

S.No	Gender	Normal Neonates (N=50)		Neonatal at risk of sepsis (N=50)		Chi square test	Df	P value
		n	%	n	%			
1	Female	21	42	11	22	4.59	1	0.053
2	Male	29	58	39	78			

Table 3: Comparison of CRP values between the neonatal sepsis present and absent group.

S. No	Lab parameters	Normal Neonate (N=50)		Neonatal with risk of sepsis present (N=50)		T value	Df	P value
		Mean	SD	Mean	SD			
1	CRP (mg/L)	3.81	1.7	25.4	21.2	7.21	98	<0.0001

Table 4: Comparison of various derived lab parameters between the neonatal sepsis present and absent group.

S. No	Lab parameters	Normal Neonate (N=50)		Neonatal with risk of sepsis present (N=50)		T value	Df	P value
		Mean	SD	Mean	SD			
1	NLR	1.94	2.27	3.31	3.2	2.48	98	0.015
2	RDW (%)	16.9	2.1	18.5	2.5	3.32	98	0.001
3	RPR	0.075	0.05	0.21	0.21	4.45	98	<0.0001

Table 5: Comparison of RDW category between the neonatal sepsis present and absent group.

S.No	RDW category	Normal Neonate (N=50)		Neonatal with risk of sepsis present (N=50)		Chi square value	Df	P value
		n	%	n	%			
1	10- 15	1	2	8	16	16.7	3	0.001
2	>15 – 18	21	42	32	64			
3	>18-20	16	32	7	14			
4	>20	12	24	3	6			

Table 6: Description of ROC statistics for various laboratory parameters in predicting the neonatal sepsis status.

S.No	Parameter	N	Area under curve	Std error	P value
1	NLR	100	0.688	0.053	0.001
2	RDW	100	0.699	0.053	0.001

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3	RPR	100	0.760	0.047	<0.0001
4	CRP	100	0.886	0.04	<0.0001

Total N= 100

Table 7: Validity indexes of various lab parameters with their cut off value for predicting the neonatal sepsis state.

S. No	Anthropometric parameter	Critical limit	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
1	NLR	1.22	74%	46%	50%	57.8%	60%
2	RDW	17.1	72%	68%	63%	67%	65%
3	RPR	0.08	74%	76%	68%	69%	69%
4	CRP	6	60%	100%	100%	71%	80%

Total N = 100.

Data are expressed Median with Interquartile range. Mann Whitney U test was used to compare the distribution across the group. indicates $p<0.05$ and considered statistically significant.
Data are expressed as n (%). Fisher's exact test was used to compare the frequency between the two groups.

Table 8: Comparison of various CBC parameters between the neonatal sepsis present and absent group.

S. No	Lab parameters	Normal Neonate (N=50)		Neonatal with risk of sepsis present (N=50)		T value	Df	P value
		Mean	SD	Mean	SD			
1	Hb (g/dL)	15.5	2.9	14.2	1.78	2.72	98	0.008
2	WBC (cells/cc) in thousand	13.5	4.39	14.4	8.3	0.604	98	0.547
3	MPV	9.92	0.94	9.83	1.15	0.428	98	0.671
4	Platelet count (cells/cc) in lakhs	2.96	1.62	1.81	1.31	3.89	98	<0.0001

DISCUSSION

Neonatal sepsis has various clinical features that can be nonspecific. These features overlap with other neonatal diseases and make diagnosis of Neonatal sepsis difficult, resulting in unnecessary antibiotic use. In some cases, clinical signs of neonatal sepsis can be subtle, and infants present them only in the late stage of the disease, leading to an increase in mortality and morbidity secondary to delay in diagnosis.[10] Golden standard of neonatal diagnosis is isolation of microorganisms in blood cultures. However, microorganism growth in blood culture takes approximately 48 h. Additionally, a negative blood culture does not exclude the diagnosis.[11] In order to diagnose neonatal sepsis and initiate early treatment prior to obvious clinical symptoms, some biomarkers can be detected. An ideal biomarker should have short half-life and high sensitivity, specificity, PPV, NPV, and cost- effectivity. However, a single biomarker with these features for sepsis diagnosis has not been identified yet.

Multiple studies investigated the role of WBC, absolute neutrophil count, I/T ratio, mean platelet volume (MPV), RDW, PDW, and neutrophil lymphocyte count parameters of complete blood count in the diagnosis of neonatal sepsis.

Currently, various parameters are used for diagnosis of neonatal sepsis, and the most frequently used ones are CRP and PCT. There is still not a biomarker that meets all the criteria of the ideal biomarker. Many studies have investigated biomarkers with high sensitivity, specificity, PPV, and NPV. Birol Karabulut & Baran Cengiz Arcagok is the first study to investigate the utility of RPR in neonatal sepsis. In our study we observed the following results

RPR and CRP: Birol Karabulut & Baran Cengiz Arcagok study observed that RPR and CRP has cut off value, sensitivity, specificity, negative predictive value and positive predictive value respectively as 0.052, 79.3%, 93.7%, 81.6%,92.6% and 7.2, 87.9%,71.3%,86.5%, 75.3%. the sensitivity, specificity, NPV and PPV values of RPR were higher than those of CRP. This study also observed there was no difference between groups regarding demographic (gestational age, birth weight, gender, mode of delivery) and perinatal data.[12] In our study, we observed that RPR and CRP has cut off value, sensitivity, specificity, negative predictive value, positive predictive value respectively as 0.08, 74%,76%, 69%, 68% and 6, 60%,100%, 71%, 100%. The sensitivity, specificity, NPV, and PPV values of RPR were lower than those of CRP. However, the values of RPR obtained in our study has statistically significant difference (p value < 0.0001) between study and control groups.

RDW: According to a study Deka A, Aravind P, Birol Karabulut & Baran Cengiz Arcagok study and Dr. Monu singh et al mean RDW range in normal neonate was 16.18±1.23, 16.9±1.3, 16.23±1.16, and in neonatal sepsis group was 18.40±1.18, 19.2±2.9, 21.31±3.08, has shown statistically significant difference between two groups.[12-14] In our study, the mean RDW range in normal neonate was 15.2±1.74 and in neonatal sepsis group was 17.1±1.12 also shown statistically significant difference (p value – 0.001)

NLR: Omran et al.'s study showed that both MPV and NLR showed a significant difference between septic and healthy neonates.[15] However, in our

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study, we observed that only Neutrophil-Lymphocyte Ratio with Groups by Unpaired t-test were t-value= 2.48, p=0.015, which shows a statistically significant difference between two groups. Other indices like Mean Platelet volume with Groups by Unpaired t-test which shows no statistically significant difference between cases and Groups, which was discordant with Omran et al.

Platelet: Arabdin M, Khan A, Zia S, et al. study showed platelet count difference between study and control group (p <0.001). In our study we also observed low platelet count in neonatal sepsis group with groups by unpaired t-test were t-value 3.89, p value <0.0001 which shows a statistically significant result. We also observed low Hb levels in neonatal sepsis group (p < 0.008) and shows statistically significant results

Limitations: sepsis is more common in preterm neonates. But in our study preterm neonates are not included because of high variability of RDW in preterm. Large samples in different gestational groups to determine definite cutoff value of RDW is needed so that further studies in preterm may find usefulness of RPR. The sample size was not large enough, given the routine availability of RDW. Moreover, we also did not compare RPR with procalcitonin, a valuable biomarker in neonatal sepsis. Lack of evaluation of the utility of RPR in differentiating infants with sepsis from those with other neonatal illnesses. RDW is also elevated in other conditions like iron, vitamin B12 and folate deficiencies and after blood transfusions. Serial RDW measurements to look for rise or fall in levels after the initiation of antibiotics is not done in our study. Follow up after the discharge of septic infants to study the morbidity is not done in our study.

CONCLUSION

Sepsis is a common cause of morbidity and mortality in developing countries. Early identification of maternal and fetal factors can help triaging neonates with risk of sepsis so that early initiation of empirical antibiotics can be done. Hand washing and hygienic practices in Newborn unit also prevent sepsis. In resource limited settings only complete blood count can be used to diagnose sepsis in neonates. Our study provides evidence for the usefulness of RPR as a reliable indicator in identifying sepsis and initiating treatment easier without waiting for time-consuming blood culture reports. RPR is cost-effective, easily calculated, can be tested with small amount of blood sample, easy to perform, and has as high or higher sensitivity, specificity, PPV, and NPV values as other commonly used markers. Further large trials are needed to prove the usefulness of this simple test which can have a large impact in reducing the morbidity and mortality of neonatal sepsis. Trials should also include premature neonates having inherent risk for sepsis.

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