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Original Article

Diagnostic Accuracy of Clinical Tool 'STOPS' and Serum Procalcitonin for Optimizing Antibiotic Therapy in Neonates Born at ≥ 28 Weeks of Gestation with Neonatal Sepsis

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Abstract. ***Background:*** **Antibiotic therapy is initiated in neonates on suspicion of sepsis. Optimizing therapy is a felt need of clinicians as prolonged injudicious use increases mortality and morbidity risk.**
Objective: **To evaluate the diagnostic accuracy of clinical tool 'STOPS' and serum procalcitonin (PCT) for identifying neonates with early-onset neonatal sepsis (EONS) or late-onset neonatal sepsis (LONS) and early discontinuation in those with no sepsis.**
Methods: **The study had a prospective analytical design conducted at a tertiary care hospital. Consecutively admitted neonates with suspected EONS or LONS were enrolled. The 'STOPS' tool comprising sensorium, temperature, oxygenation, perfusion, skin color, and blood sugar was applied at 6 and 12 hours of enrollment. Serum PCT was sent at 12 hr. The sensitivity, specificity, positive and negative predictive value (PPV and NPV), positive and negative likelihood ratio (PLR and NLR) were estimated.**
Results: **The study enrolled 380 neonates, of which 330 were given antibiotics for EONS and 50 for LONS. Temperature disturbance in the EONS group at 12 hr showed a PPV of 100% and a PLR of 9.1 (7.7 – 18). Perfusion assessment at 12 hr had a PPV of 77% and PLR of 8.25 (2.3 – 29). Skin color assessment at 12 hr had a PPV of 100% and PLR of 13.5 (9.7 – 27). The diagnostic accuracy of PCT in the EONS group was unremarkable. In the LONS group, skin color at 12 hr had a PPV of 100% and PLR of 11.2 (8.6 – 19.5). The diagnostic accuracy of PCT in the LONS group showed a PPV of 82% and PLR of 7 (1.7 – 29).**
Conclusion: **Identifying abnormal STOPS parameters was superior to PCT alone in EONS and as good as PCT in LONS. The 'STOPS' tool allows early identification of neonates with no sepsis, thereby optimizing antibiotic use.**

Keywords: Antibiotic; Clinical tool; Diagnosis; Neonatal sepsis; Procalcitonin; STOPS.

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Introduction. Neonatal sepsis imposes a high burden on healthcare services in developing nations. Prompt diagnosis and treatment are crucial to prevent severe morbidity and mortality.[1,2] The limited repertoire of

clinical features in neonates with sepsis overlaps with non-infectious conditions, and low sensitivity and positive predictive value of biomarkers lead to antibiotic therapy initiation based on clinical suspicion alone.[3] Prolonged empirical antibiotics in extremely low birth weight neonates for sepsis is associated with increased risk of death, necrotizing enterocolitis,[4] and intestinal dysbiosis with long-term health effects.[5] Optimizing antibiotic therapy and minimizing injudicious use is a felt need of neonatal clinicians and researchers. Blood culture remains the gold standard for diagnosing sepsis despite the limitation of availability of results by 48-72 hours. Even then, blood culture does not identify most infected neonates with frequent false-negative results due to neonatal sepsis's pauci-bacterial nature, a small volume of blood available for inoculating culture bottles, and prior maternal antibiotic exposure in early-onset neonatal sepsis (EONS) setting.[6,7] Several acute phase reactants have been evaluated for optimizing antibiotic usage but do not have high sensitivity if measured early in the course of sepsis.[8] Among the biomarkers in clinical application, serum procalcitonin (PCT) is useful in reducing antibiotics' duration in suspected EONS.[9] Cost and availability constraints in developing countries do not allow repeated testing with decision making based mainly on clinical signs.[10] For reliable identification of sepsis, a bedside clinical tool, quick, repeatable, not laboratory dependent, inexpensive, and demanding minimal training, is needed. Our unit has been using a combination of clinical signs in a simple bedside assessment tool, which includes sensorium, temperature, oxygenation, perfusion, skin color, and blood sugar (STOPS) for early identification of neonatal sepsis and optimizing antibiotic therapy. This study evaluated the accuracy of the 'STOPS' tool and serum PCT, individually and in combination for diagnosing early and late-onset neonatal sepsis (LONS) for early discontinuation of antibiotics in those with no sepsis.

Materials and Methods. The study was an observational analytical study conducted prospectively over 19 months at the neonatal unit of a tertiary care teaching hospital in Southern India. The study was approved by the institutional ethics committee. All neonates with maternal risk factors for EONS and asymptomatic at birth, all symptomatic neonates at birth or within 72 hours of birth with or without maternal risk factors for EONS, and all neonates with suspected LONS were eligible for enrollment. Consecutively admitted neonates were enrolled after informed consent from the parents and started on empirical intravenous (IV) antibiotics for maternal risk factors for early-onset sepsis or presence of clinical signs indicating EONS or LONS. Maternal risk factors included prolonged rupture of membranes in term gestation (PROM >24 hrs), maternal fever (>38˚C) from the onset of labor to delivery, spontaneous preterm (< 37wks) onset of labor (SPTOL), preterm (<37wks) pre-labor rupture of membranes (pPROM), maternal sepsis or urinary tract infection within past seven days, and clinical chorioamnionitis defined as fever >38˚C, maternal tachycardia (>100 beats/min), fetal tachycardia (>160 beats/min), uterine fundal tenderness, foul-smelling liquor, maternal leukocytosis (TLC >15000/mm^3) or positive C-reactive protein (CRP) with no other site of infection. Given the inclusion of a very preterm population (28-32 weeks gestation) and high EONS incidence due to gram-negative Enterobacteriaceae, empiric antibiotics were initiated.[11] Neonates below 28 weeks gestation and 1000 grams birth weight, those with congenital anomalies, confirmed sepsis, or multi-organ dysfunction were excluded as multiple morbidities in this population result in symptom overlap with sepsis, precluding the use of clinical prediction models.[12] Clinical signs prompting antibiotic therapy included fever (>38°C), respiratory distress (tachypnea, retractions, grunting, oxygen requirement), persistent tachycardia (>180 per min), excessive cry/irritability, depressed sensorium, poor feeding, recurrent apnea, seizures, abdominal distention, vomiting, gastrointestinal or per rectal bleeding, or signs of local infection such as omphalitis. The aim of our study was to estimate the diagnostic accuracy of the clinical tool 'STOPS,' serum PCT, a combination of 'STOPS' tool with PCT in identifying neonates with EONS or LONS for early discontinuation of antibiotics in neonates with no sepsis. Following initial stabilization for one hour, all enrolled neonates initiated on IV antibiotics for suspect sepsis underwent clinical assessment of 'STOPS' twice at six hourly intervals within the first 12 hour period of enrolment. The 'STOPS' tool has been developed, applied, and refined for over a decade in our unit by process of meticulous bedside observation, participative discussion of stakeholders, and revisiting the clinical parameter for its consistency, reliability, and easy assessment. Description of the 'STOPS' parameters and the score allotted is as given (**Table 1**). During the first three months of the study, 'STOPS' was recorded independently by the neonatal fellows and nurses, confirmed by the principal investigator, and the inter-observer reliability was evaluated. The neonatal intensive care unit (NICU) temperature was kept at 26ºC, and the temperature in skin mode of servo-controlled radiant warmers was set at 36.5 ºC. The neonates' skin temperature was cross-checked by temperature recording using a digital thermometer placed in the axilla. Temperature <36.5 °C was taken as hypothermia, and >38 °C was used to define fever. Oxygenation was assessed by Downes score and a fraction of inspired oxygen requirement (FiO2) for maintaining oxygen saturation (SpO2) between 91-95%. Downes score of ≤3 was normal, 4-6 suggested respiratory distress, and ≥7 indicated an impending

Table 1. Clinical tool 'STOPS'.

	Score allotted		
STOPS parameters	**0**	**1**	**2**
Sensorium	Arouses spontaneously, remains alert and has good tone and cry	Presence of Irritability or Poor response to touch or Reduced spontaneous movements or Weak cry or Poor feeding for 4 hr over a 6 hr observation period	Lethargic or No response to touch or Absence of spontaneous movements or No cry or Poor sucking for ≥ 4 hr over a 6 hr observation period or presence of single episode of seizures
Temperature	Euthermic (36.5-37.5 ºC) with warm peripheries	Cold hands and feet (felt by dorsum of hands) for 30 min or more despite clothing/ rewarming adequately observed during the 6 hr period	Single record of hypothermia < 36 ºC with cold hands and feet despite rewarming / or Fever with > 38 ºC with cold extremities
Oxygenation	No respiratory distress and no need for oxygen	Tachypnea (RR 60-80 per min) and/or mild grunting and/or minimal chest retractions and/or need for FiO2 ≤ 30% for more than 1 hr over a 6 hr observation period	Tachypnea (RR > 80 per min) and/or audible grunting and/or marked chest retractions and/or increase in need for FiO2 > 0.3 and/or need for CPAP and/or need for ventilator support and/or recurrent apnea requiring PPV
Perfusion	CRT ≤ 3sec, HR 100-180 per min and normal mean arterial pressure	Presence of CRT > 3 sec and/or tachycardia (HR>180 per min) and/or bradycardia (HR <100 per min) while at rest for 1 hr with normal mean arterial pressure during the 6 hr observation period	Arterial hypotension and presence of prolonged CRT > 3 sec with cool extremities or mottled skin or oliguria despite initial stabilization during the 6 hr observation period
Skin color	Pink	Pale/ bluish extremities despite rewarming observed during the 6 hr period	Dusky /off-color with or without normal SpO2
Blood Sugar	Blood sugar ≥ 45 mg/dl and ≤ 180 mg/dl	Two consecutive values of < 45 mg/dl or >180 mg/dl despite receiving appropriate dextrose concentration	-

CPAP: Continuous positive airway pressure; CRT: Capillary refilling time; FiO2: Fraction of inspired oxygen; HR: Heart rate; hr: hours; PPV: Positive pressure ventilation; RR: Respiratory rate; SpO2: Oxygen saturation by a pulse oximeter.

respiratory failure. Blood glucose estimation was done using heel prick estimation by a Glucometer (OneTouch, LifeScan Inc). Blood glucose <45 mg/dl was defined as hypoglycemia, and >180 mg/dl indicated hyperglycemia. Serum PCT estimation was done once between 12-14 hours of enrolment using the Enzyme-Linked Fluorescent Assay (ELFA) technique (VIDAS BRAHMS automated PCT bioMerieux). A cut-off of > 2 ng/ml was considered as a positive test.[13,14]

The case definitions of definite, clinical, and no sepsis in the study used as the reference standard[15,16] were adapted for our setting and were based on a combination of clinical signs, serum CRP and blood culture (**Table 2**). Serum PCT estimation was not used for this purpose. Serum CRP assays were done at 48 hours and repeated at 72 hours. Quantitative determination of CRP was done on Roche/Hitachi Cobas C systems on plasma using particle enhanced immune turbidimetric assay. A cut-off of ≥ 15 mg/L was considered as a positive test.[14] In asymptomatic neonates with sterile blood culture and negative CRP at 48 and 72 hours, antibiotic therapy was stopped. Symptomatic neonates with a positive blood culture received antibiotics for 7 days, and if asymptomatic by the 7th day with a negative CRP, antibiotics were discontinued. Lumbar puncture (LP) for cerebrospinal fluid (CSF) examination for meningitis

Table 2. Case definition (reference standards) for neonatal sepsis.

Case definitions	Clinical signs[#]	CRP[$]	Blood culture	Follow-up in 7 days^	Duration of antibiotics
No sepsis	Asymptomatic	Negative	Negative	Asymptomatic	≤ 5 days
	Asymptomatic	Negative	Positive*	Asymptomatic	≤ 5 days
Definite sepsis	Symptomatic	Positive	Positive	-	> 5 days
	Symptomatic	Negative	Positive	-	> 5 days
	Asymptomatic	Positive	Positive	Asymptomatic	≤ 5 days
Clinical sepsis	Symptomatic	Negative	Negative	-	> 5 days
	Symptomatic	Positive	Negative	-	> 5 days
	Asymptomatic	Positive	Negative	Asymptomatic	≤ 5 days

#: Clinical signs as described in **Table 1.** $: Quantitative CRP done at 48 and 72 hours – anyone or both positive (≥ 15 mg/L). ^: Follow-up within 7 days necessitating treatment with antibiotics. *: Considered as a possible blood culture contaminant.

in neonates with EONS was done only in those with a positive blood culture (definite sepsis), while in LONS, it was done in all neonates with definite or clinical sepsis.

The BACTEC or BacT/Alert microbial detection system was used to detect aerobic and facultative anaerobic microorganisms from the blood. Vitek-2 automated sensitivity and identification system was used for the identification of organisms. Pediatric blood culture bottles were inoculated with approximately 1 ml of the blood sample as per study protocol. Positive blood culture reports were alerted as soon as indicated by the microbial detection system, and a final report was made available by 48 hours as positive or negative.

All mothers with risk factors for EONS received IV Cefuroxime before delivery. EONS was defined as the onset of symptoms within 72 hrs of age (< 72 hrs), while LONS was defined as the onset of symptoms after 72 hrs of age (≥ 72hrs).[14,17]

Sample size calculation and statistical methods. We estimated the prevalence of definite EONS initiated on IV antibiotics to be 0.3/1000 hospitalized neonates based on a study conducted at our unit earlier.[18] Using the nomogram for sample size calculation proposed by Carley et al.[19] with a required confidence interval of 0.05 and desired sensitivity and specificity of 95%, the requisite sample size was 300 neonates. The data were entered in a Microsoft excel sheet, and the results were analyzed using statistical software SPSS version 17. The index test variables were changed to dichotomous variables and were tested using the Chi-square test or Fischer exact test with the reference standard. All p values reported are two-tailed, and a p-value of ≤ 0.05 is considered statistically significant. The sensitivity, specificity, PPV, NPV, PLR, and NLR were calculated to find the index test's diagnostic accuracy with the reference standard. An NLR of <0.2 was considered relevant. A PLR of 5-10 was taken as moderately useful and >10 as very useful. The area under the receiver operating characteristic (ROC) curve (AUC) was calculated for diagnostic accuracy of PCT in EONS and LONS. Kappa statistics were used to find out the agreement between observations recorded by the neonatal fellows and nurses.

Results. The total number of neonates initiated on IV antibiotics during the study period was 545, of whom 165 were excluded. There were 38 neonates < 28 weeks/ < 1000 gram birth weight, 75 neonates with surgical anomalies, 19 with proven sepsis, 9 with multi-organ dysfunction, 12 discharged before completion of the study, and 12 missed cases amongst those excluded. Three hundred eighty neonates were enrolled in the study, of whom 330 neonates were started on IV antibiotics for suspected EONS; 330/380 (86.8%). The mean gestation of neonates in the EONS group was 34.15 ± 2.8 weeks, and birth weight was 2203 ± 711 gm. In the EONS group, 48/330 (14.6%) had definite sepsis, and 47/330 (14.2%) had clinical sepsis. There were 203/330 (61.5%) neonates in the EONS group delivered with maternal risk factors for EONS, with preterm premature rupture of membranes (pPROM) being the most frequent risk factor [123/203 (60.5%)], followed by the spontaneous preterm onset of labor (SPTOL) [66/203 (32.5%)]. There were 226/330 (68.5%) neonates in the EONS group, who were symptomatic, and 104/330 (31.5%) who were

Table 3. Clinical characteristics of neonates in EONS and LONS groups.

Characteristic	EONS group, n (%)	LONS group, n (%)
Period of gestation (weeks)		
• 28-30	46 (13.9)	5 (10)
• 31-33	74 (22.4)	10 (20)
• 34-36	133 (40.3)	7 (14)
• ≥ 37	77 (23.3)	28 (56)
Birth weight (grams)		
• 1000-1500	68 (20.6)	10 (20)
• 1501-2000	64 (19.4)	6 (12)
• 2001-2500	91 (27.6)	9 (18)
• 2501-3000	67 (20.3)	11 (22)
• ≥ 3000	40 (12.1)	14 (28)
Symptoms at birth		
• Symptomatic	226 (68.5)	50 (100)
• Asymptomatic	104 (31.5)	0 (0)
Sepsis classification		
• No sepsis	235 (71.2)	30 (60)
• Definite sepsis	48 (14.6)	13 (26)
• Clinical sepsis	47 (14.2)	7 (14)
Blood culture positive,		
• Definite sepsis	10	6
• Clinical sepsis	0	0

Table 4. Diagnostic accuracy of STOPS and PCT in EONS.

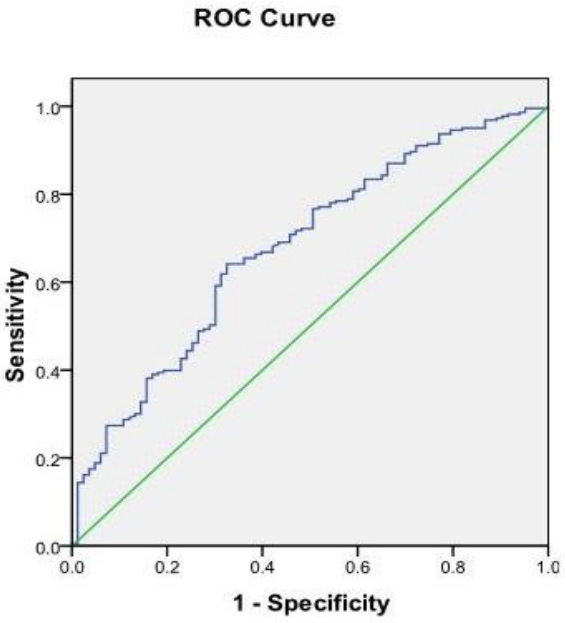
Index test	Time of assessment	Index test positive, n	No sepsis, n=235	Definite sepsis, n=48	Clinical sepsis, n=47	Sensitivity, %	Specificity, %	PPV, %	NPV, %	PLR	NLR
Clinical tool 'STOPS'											
Sensorium	6th hour	50	23	16	11	28	90	54	76	2.9	0.79
	12th hour	31	13	11	7	19	94	58	74	3.43	0.86
Temperature	6th hour	13	7	4	2	60	97	46	72	2.12	0.97
	12th hour	3	0	3	0	3	100	100	72	9.1 * (7.7-18)	0.97
Oxygenation	6th hour	169	96	37	36	77	59	43	86	1.88	0.39
	12th hour	137	68	36	33	73	71	50	87	2.51	0.39
Perfusion	6th hour	20	6	8	6	15	97	70	74	5.77 * (2.2-15)	0.87
	12th hour	13	3	7	3	11	99	77	73	8.25 * (2.3-29)	0.91
Skin color	6th hour	20	6	11	31	14	97	68	74	5.77 * (2.2-15)	0.88
	12th hour	4	0	3	1	4	100	100	72	13.5 * (9.7-27)	0.96
Sugar	6th hour	37	30	3	4	7	87	19	70	0.58	1.06
	12th hour	8	5	2	1	3	98	38	71	1.48	0.99
Serum procalcitonin											
PCT positive	12th hour	215	145	38	32	84	35	33	86	1.3	0.45

*: Useful PLR; PCT= Serum procalcitonin, > 2 ng/ml taken as positive cut-off at 12 hours. EONS= Early onset neonatal sepsis; NLR= Negative likelihood ratio; NPV= Negative predictive value; PLR= Positive likelihood ration; PPV= Positive predictive value.

asymptomatic; of them, only 6/104 (5.8%) had evidence of definite (1/104) or clinical (5/104) sepsis. Bacteria were isolated on blood culture in 10/330 (3%) neonates in the EONS group (**Table 3**). There were no cases of meningitis in the neonates with definite EONS.

There were 50/380 (13.2%) neonates in the LONS group. Of these, 5/50 (10%) were between 28-30 weeks gestational age and 10/50 (20%) between 1000 to 1500 gram bodyweight category. The LONS group's neonates were heterogeneous, with median gestation of 37.5 weeks (IQR 32.5 – 38) and a birth weight of 2550 gm (IQR 1751 – 3105). In the LONS group, 13/50 (26%) had definite sepsis, and 7/50 (14%) had clinical sepsis. Bacterial growth on blood culture was seen in 6/50 (12%) neonates with LONS (**Table 3**). One neonate with definite LONS was diagnosed with meningitis on cytology and biochemistry examination of the CSF.

Diagnostic accuracy of temperature recording in the EONS group at 12 hr showed a PPV of 100% and a PLR of 9.1 (7.7 – 18). Perfusion assessment at 12 hr had a PPV of 77% and PLR of 8.25 (2.3 – 29). Skin color assessment at 6 hr had a PPV of 68% and PLR of 5.77 (2.29 – 15), while at 12 hr it had a PPV of 100% and PLR of 13.5 (9.7 – 27) (**Table 4**). The diagnostic accuracy of PCT in the EONS group done at 12 hr showed a PPV of 33% and an NPV of 86%. The PLR was 1.3, and the NLR was 0.45 (**Table 4**) (**Figure 1**). In the LONS group, skin color's diagnostic accuracy at 12 hr had a PPV of 100%, NPV of 61%, and PLR of 11.2 (8.6 – 19.5) (Table 5). The diagnostic accuracy of PCT in the LONS group showed a PPV of 82%, NPV of 74%, PLR of 7 (1.7-29),



Diagonal segments are produced by ties.

Area Under the Curve

Test Result Variable(s):PCT (12hrs) (ng/ml) in EONS

Area	Std. Error	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.679	.034	.000	.612	.745

b. Null hypothesis: true area = 0.5

Figure 1. ROC curve with AUC for Procalcitonin in early onset neonatal sepsis.

Table 5. Diagnostic accuracy of STOPS and PCT in LONS.

Index test	Time of assessment	Index test positive, n	No sepsis, n=30	Definite sepsis, n=13	Clinical sepsis, n=7	Sensitivity, %	Specificity, %	PPV, %	NPV, %	PLR	NLR
Clinical tool 'STOPS'											
Sensorium	6th hour	14	4	9	1	50	87	71	72	3.75	0.58
	12th hour	9	3	5	1	30	90	67	66	3.0	0.78
Temperature	6th hour	4	2	2	0	10	93	50	61	1.5	0.96
	12th hour	0	-	-	-	-	-	-	-	-	-
Oxygenation	6th hour	11	6	5	0	25	80	45	62	1.25	094
	12th hour	7	3	4	0	20	90	57	63	2.0	0.89
Perfusion	6th hour	7	2	3	2	25	93	71	65	3.75	0.8
	12th hour	6	2	2	2	20	93	67	64	3.0	0.86
Skin color	6th hour	4	1	3	0	15	97	75	63	4.5 * (3.2-11.4)	0.88
	12th hour	1	0	1	0	5	100	100	61	11.2 * (8.6-19.5)	0.95
Sugar	6th hour	2	1	0	1	5	97	50	60	1.5	0.98
	12th hour	0	-	-	-	-	-	-	-	-	-
Serum procalcitonin											
PCT positive	12th hour	11	2	8	1	50	93	82	74	7.0 * (1.7-29)	0.54

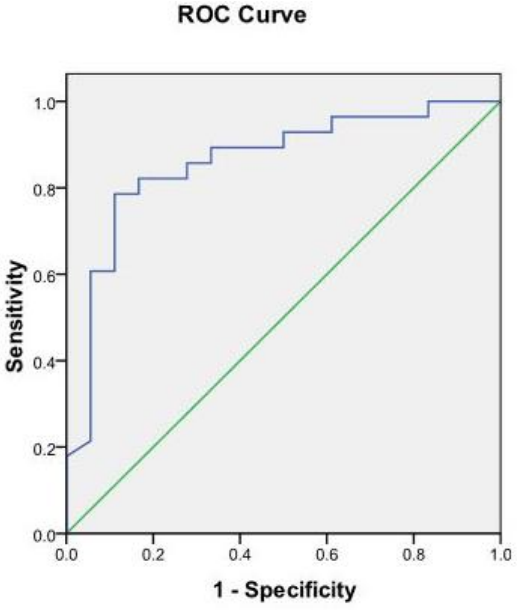
*: Useful PLR; PCT= Serum procalcitonin, > 2 ng/ml taken as positive cut-off at 12 hours. LONS= Late onset neonatal sepsis; NLR= Negative likelihood ratio; NPV= Negative predictive value; PLR= Positive likelihood ration; PPV= Positive predictive value.

and NLR of 0.54 (**Table 5**) (**Figure 2**).

In the EONS group, combining positive 'STOPS' and PCT at 12 hr revealed 289 (87.5%) neonates, with a positive index test indicating antibiotics use. Of these, there was definite sepsis in 48, clinical sepsis in 45, and no sepsis in 196 with a low miss rate of 2 neonates. In LONS, combining 'STOPS' and PCT at 12 hr showed 23 (46%) neonates, with a positive index test where antibiotics were indicated. Of these, 13 had definite sepsis, 7 clinical sepsis, and no sepsis in 3 with no missed case (**Table 6**).

Kappa statistics for the STOPS variables ranged between 0.810-1.0 showing perfect agreement with p=0.000.

Discussion. This study examined the diagnostic accuracy of the clinical tool 'STOPS,' lab estimation of serum PCT, and a combination of the 'STOPS' tool with PCT for identifying neonates with early or late-onset sepsis for early discontinuation of antibiotics in neonates with no sepsis. Our study found a significant number of neonates in the EONS group where antibiotics were indicated to have no infection (235/330; 71.2%). Only 10/95 (10.5%) neonates had a blood culture isolate, underscoring the importance of symptoms at or soon after birth and clinical signs in the form of abnormal 'STOPS' in identifying sepsis requiring continued antibiotic therapy (**Table 4**). All 'STOPS' parameters excluding oxygenation had a specificity of 95-100% for identifying EONS. Temperature disturbance, perfusion abnormalities, and skin color change were very or moderately useful based on the PLR (**Table 4**).



Diagonal segments are produced by ties.

Area Under the Curve

Test Result Variable(s):PCT (12hrs) (ng/ml) in LONS

Area			Asymptotic 95% Confidence Interval	
	Std. Error	Asymptotic Sig.[b]	Lower Bound	Upper Bound
.860	.058	.000	.746	.975

b. Null hypothesis: true area = 0.5

Figure 2. ROC curve with AUC for Procalcitonin in late-onset neonatal sepsis.

Table 6. Strategies for optimizing the use of antibiotics in neonatal sepsis.

EONS group, n=330	Index test positive – treatment indicated	Decrease in antibiotic use	No sepsis	Definite sepsis	Clinical sepsis	Unnecessary antibiotics	Missed cases
All neonates initiated on IV antibiotics	330	0%	235 (71.2%)	48 (14.6%)	47 (14.2%)	235	0/95
PCT positive (> 2 ng/ml) alone	215 (65%)	35%	145	38	32	145 (44%)	13 *
STOPS at 6th hour – any one sign abnormal	184 (55.7%)	44.3%	104	40	40	104 (31.5%)	15 *
STOPS at 12th hour – any one sign abnormal	145 (44%)	56%	75	38	36	75 (22.7%)	21 *
Strategy 1: Treat all symptomatic neonates and perform PCT at 12 hours	289 (87.5%)	12.5%	196	48	45	196 (59.7%)	2
Strategy 2: Treat all neonates with one abnormal STOPS parameter at 6 hours and perform PCT at 12 hours in neonates with normal STOPS at 6 hours	268 (81.25%)	18.75%	179	46	43	179 (54%)	6
Strategy 3: Treat all neonates with one abnormal STOPS parameter at 12 hours and perform PCT at 12 hours in neonates with normal STOPS at 12 hours	254 (77%)	33%	168	45	41	168 (51%)	9 *
LONS group, n=50							
All neonates initiated on IV antibiotics	50	0%	30 (60%)	13 (26%)	7 (14%)	30	0/20
PCT positive (> 2 ng/ml) alone	11 (22%)	78%	2	8	1	2 (4%)	1
STOPS at 6th hour – any two signs abnormal	8 (16%)	84%	1	6	1	1 (2%)	1
STOPS at 12th hour – any one sign abnormal	13 (26%)	74%	2	8	3	2 (4%)	1
Strategy 1: Treat all neonates with ≥ 2 abnormal STOPS parameters at 6 hours and perform PCT	19 (38%)	62%	2	12	5	2 (4%)	1
Strategy 2: Treat all neonates with ≥ 1 abnormal STOPS parameters at 12 hours and perform PCT with normal STOPS at 6 hours	23 (46%)	54%	3	13	7	3 (6%)	0

*: High miss rate; PCT= Serum procalcitonin, > 2 ng/ml taken as positive cut-off at 12 hours. EONS= Early-onset neonatal sepsis; IV: Intravenous; LONS= Late-onset neonatal sepsis.

Oxygenation did not perform well understandably, as a significant number of preterm neonates had respiratory distress syndrome (RDS). Serum PCT alone had a very low specificity of 35%, a sensitivity of 84%, and a PLR, which was not useful. It had an NPV of 86%, which was as good as any of the STOPS parameters (**Table 4**). The study shows that by combining the clinical tool 'STOPS' and PCT as a treatment strategy, i.e., treating all symptomatic neonates and performing PCT at 12 hours, a nearly 60% reduction in unnecessary antibiotics use could be achieved, and which was superior to either by using STOPS or using PCT (**Table 6**).

For diagnosing neonatal sepsis presence of clinical symptoms and signs are essential.[14] Only considering lab results may be erroneous as they may be incomplete (blood culture-negative sepsis) and misleading (positive CRP or hematological indices without clinical correlate).[17] We used the clinical parameters' STOPS', which were predefined, easy to learn, and interpret, with good inter-observer reliability. Most clinical studies evaluating sepsis have used similar clinical parameters for early sepsis diagnosis.[20-23] Ohlin et al. to evaluate the clinical signs most predictive of sepsis in neonates in the NICU, used, amongst others, altered sensorium, perfusion disturbance, abnormal skin color, and increasing oxygen requirement, which are similar to our 'STOPS' parameters. It showed a statistically significant association of perfusion disturbance and abnormal skin color in identifying neonatal sepsis, similar to our observation. Increasing oxygen requirement was not predictive, which is also similar to our observation.[23] Prediction tools for EONS as calculators have been studied by many authors and are the subject of a systematic review.[24] The review concluded that the neonatal EOS calculator is associated with a substantial reduction in empirical antibiotics for suspected EOS. However, there are several limitations to this approach in our setting. Firstly, all the included studies were from developed countries where the rate of EONS is lower compared to the developing countries. Secondly, it does not allow application in neonates below 34 weeks. In our study, we included neonates from 28 weeks gestation upwards. Maternal group-B streptococcus (GBS) status and GBS specific intrapartum antibiotics used for risk

prediction in the calculator are not relevant in our setting. The calculator allows the inclusion of clinical findings, including parameters related to sensorium, perfusion, and oxygenation similar to our 'STOPS.'

As regards LONS, our study found no sepsis in 30/50 (60%) neonates. All 'STOPS' parameters had specificity between 90-100% and PPV of 70%, with abnormal skin color having a valuable predictive ability (**Table 5**). Serum PCT alone had high specificity of 93% and was found to be moderately useful based on the PLR. Combining 'STOPS' and PCT did not further improve the diagnostic ability to exclude infection with surety and stop antibiotics (**Table 6**).

Several studies have evaluated clinical and laboratory parameters in LONS prediction models.[21,25,26] Arayici et al. introduced base excess for early diagnosis of neonatal sepsis in preterm newborns.[27] A retrospective study by Tollner[21] evaluated clinical and hematological parameters to create a scoring system. The study identified skin color changes, prolonged capillary refill time, and hypotonia (indicating altered sensorium) to be most predictive in the early part and later at the illness's peak. Another study by Singh et al. evaluating clinical signs in LONS for their predictive value included lethargy, temperature disturbances, and central cyanosis. The study found hyperthermia to have a specificity of 90% for definite or probable sepsis. The PPV for the 12 reported clinical signs was between 50 and 60%, except for grunting (75%) and intercostal retractions (100%). However, the study did not look at skin color changes, which in our study had the highest PLR.[25] A prospective case-control study describing a predictive model for LONS found clinical parameters of lethargy, poor feeding, temperature disturbance, abnormal heart rate, respiratory insufficiency, and hypoxemia to have high predictive ability. The adjusted odds ratio (OR) identified poor feeding and temperature disturbance to be the most useful.[26] Unlike earlier studies, we have included blood glucose estimation as it is routinely monitored in sick neonates, and both hyperglycemia and hypoglycemia are encountered in neonatal sepsis.[28,29]

Our study's strength is the easy interpretation of the 'STOPS' parameter, high inter-observer agreement, and optimum testing for ruling out sepsis by hematological indices, quantitative CRP, and blood culture. We have in our study proposed treatment strategies that allow early discontinuation of antibiotics. The most optimum strategy in EONS would be to treat neonates with one abnormal 'STOPS' parameter at 6 hours and perform PCT at 12 hours in those with normal STOPS at 6 hours. In LONS, the most optimum strategy would be to treat all neonates with two or more abnormal 'STOPS' parameters at 6 hours or a positive PCT test using a cut-off of > 2 ng/ml done at 12 hours. Being a single-center experience is a limitation of this study. Also, in temperature and skin color assessment at 12 hours in neonates with EONS, the high specificity and PPV of these parameters coupled with small-sample bias may have led to the high PLR estimate.

Conclusions. Our study has evaluated the diagnostic performance of a clinical tool, 'STOPS,' for identifying neonates with sepsis for early discontinuation of antibiotics in those with no sepsis. Our study has shown that abnormal 'STOPS' parameters are superior to PCT alone in EONS and as good as PCT in LONS. A simple bedside clinical tool incorporated in the NICU monitoring charts allows early identification of neonatal sepsis and optimizes antibiotic use.

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