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Hematological Parameteres in Neonatal Sepsis in Relation with Bacteriological Profile

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Abstract

Background: Septicaemia is one of the leading causes of mortality and morbidity in the neonates, especially in the developing countries like India.

Objective: To evaluate the usefulness of hematological parameters using hematological scoring system and CRP in the early diagnosis of neonatal sepsis and to study relationship between the hematological variables and bacteriological profile.

Methods and Materials: Documenting the data in the Performa, 106 cases were undergone for the measurement of haematological parameters according to haematological scoring system and followed by correlation with culture profile.

Results: The HSS score performance was analysed and that INC followed by I: M atio showed highest sensitivity. Platelet count had highest negative predicative value. Culture profile showed 30.6% of positive results.

Conclusion: The study of hematological parameters using hematological scoring system with blood culture in the early screening and diagnosis of neonatal sepsis aids in timely treatment, decreasing the neonatal morbidity and mortality.

Keywords: Haemotological parameters; Preterm; Blood culture.

Introduction

In developing countries like India, neonatal sepsis poses most common causes of health burden among the newborn and it is the most common reasons for admission to neonatal intensive care units in such countries. Sadly, the incidence of clinical neonatal sepsis in India (17,000/ 1, 00,000 live births) is the highest in the world with the case fatality rate of sepsis among neonates ranging from 25% to 65% in our country.[1] This number possibily under-recorded as many hospitals lack confirmatory method for sepsis and treatment is given only based on clinical grounds.

World-wide,neonatal sepsis is the second most common for the neonatal morbidity and mortality inspite of improvement in health care system leading to 750,000 annual deaths worldwide. According to the Global Sepsis Alliance, infections leading to sepsis are responsible for about one-fifth of the world's annual 2.7 million neonatal deaths.[2]

Neonatal sepsis by definition is systemic response to infection in the first month of the life characterised by bacteraemia presenting with systemic signs and symptoms of infection.[3] Neonatal sepsis is classified into early onset neonatal sepsis and late onset of neonatal sepsis with presentation <72 hr of life and >72 hr of life respectively. Early onset septicemia is usually caused most commonly by bacterial flora of maternal birth canal, while late onset septicemia represents nosocomial infections by organisms that colonize newborns.[4]

The mode of entry of the infective organism can be via blood, mucosa or umbilical cord. Bacteria access the bloodstream leading to generalised and multisystem involvement or less commonly localised to single organ like lungs, brain etc.

Neonatal sepsis poses a diagnostic challenge to the neonatologists due to a lack of objective evaluation.[5] The neonatal period is prone for infection even after improvement in health care because of host factors like fragile immune system and lack of single method of investigation which has high positive predictive value and at the same time this test is easy to perform, quick, low cost, easily available and acceptable. Unfortunately no such ideal test is available according literatures but it is found that combination of tests can give maximum diagnostic value which can avoid the unwanted empirical antibiotic treatment which burden the patients with antibiotic resistance, increased length of hospital stay along with financial stress to the family in developing country like India.

Looking at the options for diagnosis of neonatal sepsis, biomarkers like Surface markers, genomic study, levels of cytokines and chemokines, serum amyloid A can be costly, unavailable even if they promise higher positive predictive value.

Gold standard for confirmation of neonatal sepsis is Bacterial culture but the culture report come late requires at least 72 hour and culture reports can be false negative possibly because of low volume of blood collected and prior antibiotic treatment. Neonatologist cannot afford for late reports as cases of neonatal sepsis can deteriorate rapidly.

Rodwell R et al[6]., in 1998 followed a haematological scoring system (HSS) which included criteria of evaluating parameters like total leucocyte count (TLC), total neutrophil (PMN) count, immature PMN count, Immature to total PMN ration, Immature to mature PMN count, degenerative changes in PMN and platelet count. This scoring system was used in studies by Datta NR et al[7]., Khair KB et al[8]., on early onset neonatal sepsis, Bharadwaj R et al[4]., by dividing the cases based on Early onset neonatal septicemia and Late onset neonatal septicemia. Makkar M et al.[9] studied cases based on clinical findings and laboratory data which were classified into three categories: Sepsis, probable infection and normal.

Manucha V et al.[10] used part of Rodwell's haematological scoring system, excluded immature neutrophil count and degenerative changes study in neutrophil and also evaluated CRP levels in combination with the scoring system. Krishnamurthy et al.[11] used a modified haematological scoring system which included counting nRBC (Nucleated Red Blood Cell) along with Rodwell's haematological scoring system.

Priyanka T et al[12]., used Rodwells scoring system and culture studies on the cases which were divided into three groups- Group I- Rodwells score- <2 and culture negative, Group II- Rodwells score- 3 or 4 and culture positive or negative and Group III- Rodwells score >5 with culture positivity. C reactive protein, Micro-ESR and Procalcitonin levels were noted wherever available.

With having this background of knowledge about the options for early diagnosis of neonatal sepsis, our study aims at establishing the utility of haematological parameters in relation with bacteriological profile in neonates.

Aims and Objectives

1. To evaluate the usefulness of hematological parameters using hematological scoring system and C-Reactive Protein in the early diagnosis of neonatal sepsis.
2. To study relationship between the hematological variables and bacteriological profile.

Materials and Methods

The present study has been conducted in the department of pathology, Bidar Institute of Medical Sciences, Bidar, Karnataka, a tertiary care hospital.

Source of data: The data for prospective study was obtained from all the hematological samples received in Department of Pathology, Bidar Institute of Medical Sciences, from patients in BRIMS hospital admitted under department of paediatrics.

Place of study: Department of Pathology, Bidar Institute of Medical Sciences, Bidar.

Type of study: Prospective study.

Study period: Sep 2019 to March 2020.

Inclusion Criteria

Only hospital born neonates were included in the study

Neonates (<28 days of age) with clinically

suspected infection which included one or more of symptoms or risk factors:

Neonatal risk factors: low birth weight, prematurity, preterm babies, convulsions, cyanosis, not able to suck or sustain suckling, altered body temperature, nasal flaring, grunting, chest indrawing, respiratory rate >60 breaths/ min, jaundice, vomiting or coma.

Maternal risk factors: Gestational diabetes mellitus, premature rupture of membrane, prolonged labour, twin pregnancy, urinary tract infection, foul smelling meconium stained liquor, maternal fever at the time of delivery or pre-elampsia.

Exclusion Criteria

Neonates who have been given antibiotics already.

Neonates with congenital anomaly

Evident local infection at puncture site

Sample Size: minimum of 100.

Ethical consideration: The study was approved by Ethical committee of the institution and permission was also taken from the departments involved the study. After thorough review of the literature a Performa was made for questionnaire and data to be entered. Informed consent was taken from the parents of the cases enrolled for the study.

Method of collection of data: Relevant maternal and neonatal clinical information regarding age, clinical features and provisional diagnosis will be taken from data from neonatal intensive care unit, department of paediatrics.

Procedure: Strict aspectic precautions were followed for drawing the blood samples. Blood samples were collected before start of antibiotics. The peripheral vein puncture site was made sterile using ethyl alcohol and povidine iodine and site was dried for a minute. 3 ml of peripheral venous blood was taken within 24 hours of admission before initiation of antibiotic therapy. Out of 3 ml, 1 ml was sent for blood culture and 2 ml for peripheral smear- haematological parameters and CRP.

Haematological scoring system: Blood was processed for haematological parameters in Nihon Kohden haematology analyser from Celltac. The Leishman peripheral smears were made and WBC differential count for 200 cells were done.

Table 1: Hematological Scoring System.

Criteria	Abnormality	Score
Total leucocyte count(TLC)	< 5000/mm^3	1
	>25000/mm^3 at birth	1
	>30000/mm^3 at 12–48 hours	
	>21000/mm^3 from day 2 onwards	
Total neutrophil count (TNC)		
(Normal – 1800–5000/mm^3)	No mature neutrophils seen	2
	Increased/decreased	1
Immature neutrophil count (INC)		
(Normal–600/mm^3)	Increased	1
I:T ratio (Immature neutrophils : TLC)	>0.2 Increased	1
I:M ratio (Immature : Mature neutrophils)	>0.3	1
Degenerative changes in neutrophils	Toxic granules/cytoplasmic vacuoles	1
Platelet count	<1,50,000/mm^3	1

The cases are scored from 0 to 9 and categorised as:

Category 1: 2 or <2 – sepsis unlikely

Category 2: 3–4 – suspected sepsis

Category 3: 5 or >5- likely sepsis

Determination of C-Reactive protein: C-Reactive is measured by Latex agglutination slide quantitative method with >0.6 mg/dl is considered abnormal.

Blood culture: The blood for the blood culture is drawn before the start antibiotics. The cases with positive culture are considered as proved 'sepsis' and those with negative culture report was considered probable 'sepsis' as blood culture was considered as gold standard for diagnosis sepsis.

Statistical data: The data obtained was tabulated on Microsoft excel spreadsheet and analysed. Data was expressed as mean, frequency and percentages. Sensitivity, specificity, Positive Predictive Values (PPVs) and Negative Predictive Values (NPVs) were calculated for each parameter.

Results

A total of 3133 live births were recorded during the study period, out of which 106 cases were studied which satisfied the inclusion criteria. There were 88 cases for which had blood culture correlation.

During the study period, the mean age studied was 1.8 days and mean weight of the patient was 1.9 kg. There was male predominance among the cases, with male to female ratio being 1.25:1 (Table 2 and 3).

Early onset of sepsis was seen in 77.3% of cases. Intra uterine growth retardation and prematurity was present in 26.4% cases and 74% respectively, as shown in Table 1. The cases were allotted as HSS category 1, 2 and 3 based on the scoring of 0–2, 3–4 and 5 or more than five respectively. Among 106 study cases, HSS category 1 had (n=32)30.1%, category 2 had (n=48) 45.3% and category 3 had (n=26) 24.5% of cases.

Table 2: Baseline data of the study population.

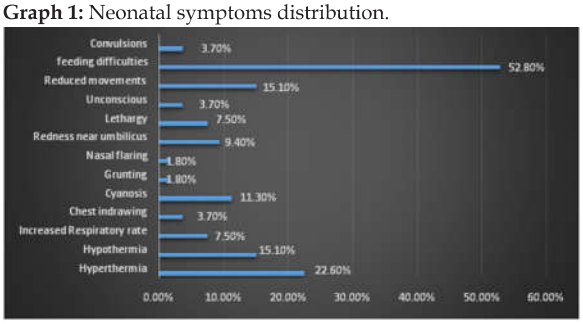
Sl. No.	Categorical variable		Frequency	Percentage
1	Sex	Male	59	55.6
		Female	47	44.3
2	Gestational age	Term	64	60.3
		Preterm	42	39.6
3	Onset of sepsis	EOS	82	77.3
		LOS	24	22.6
4	IUGR	Present	28	26.4
		Absent	78	73.5
5	HSS category	Category 1	32	30.1
		Category 2	48	45.3
		Category 3	26	24.5
6	Culture profile (88 cases)	Positive	27	30.6
		Negative	61	69.3

Table 3: Sex distribution according to age.

Age (days)	Male	Female	Total
<3	43	39	82
>3	16	8	24
Total	59	47	106

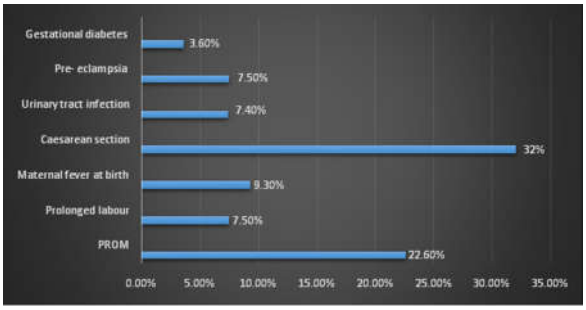
The feeding difficulties in the form of difficulty in suckling breast, difficulty to sustain breast feeding or reluctant to breast feeding was most common presentation (52.8% cases) followed by presentation with hyperthermia among 22.6% of cases. (Bar Graph 1)

Graph 1: Neonatal symptoms distribution.



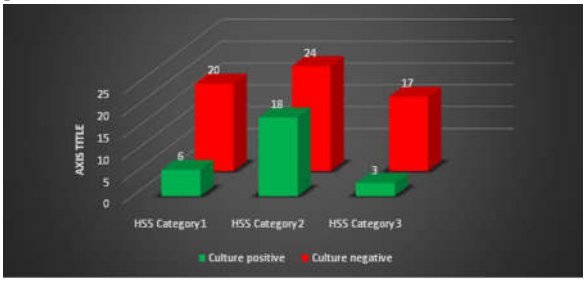
Maternal risk factors were recorded, caesarean section was noted most commonly (32% cases) followed by premature rupture of membrane, as depicted in Bar Graph 2.

Graph 2: Maternal risk factor distribution.

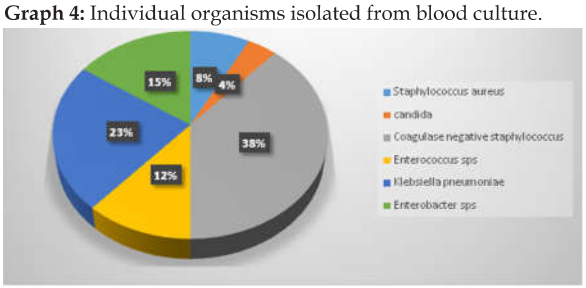


The blood culture correlation was possible in 88 cases among which 30.6% (n=27) cases were positive and 69.3% (n=61) cases were culture negative (Table 1). When correlated HSS category wise- 6, 18 and 3 culture positivity was seen for HSS category 1, 2 and 3 respectively. Similarly, 20, 24 and 17 cases were culture negative in category 1, 2 and 3 respectively. (Graph 3). The organisms that were isolated from the culture, coagulase negative staphylococcus was found to be most common in the present study, the distribution of which is shown in the Graph 4 as Pie chart.

Graph 3: Distribution of HSS categories in relation to culture profile.



Graph 4: Individual organisms isolated from blood culture.

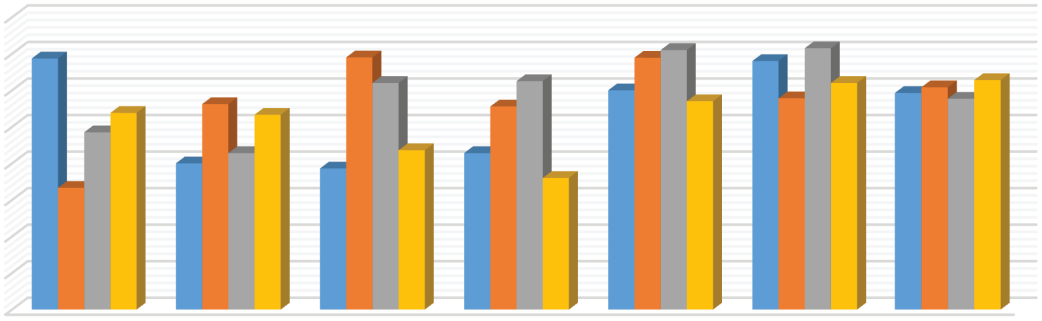


The performance of HSS scoring system based on the haematological parameter was analysed, it was found that TLC had highest specificity of 68.7% followed by Polymorph nuclear (PMN)

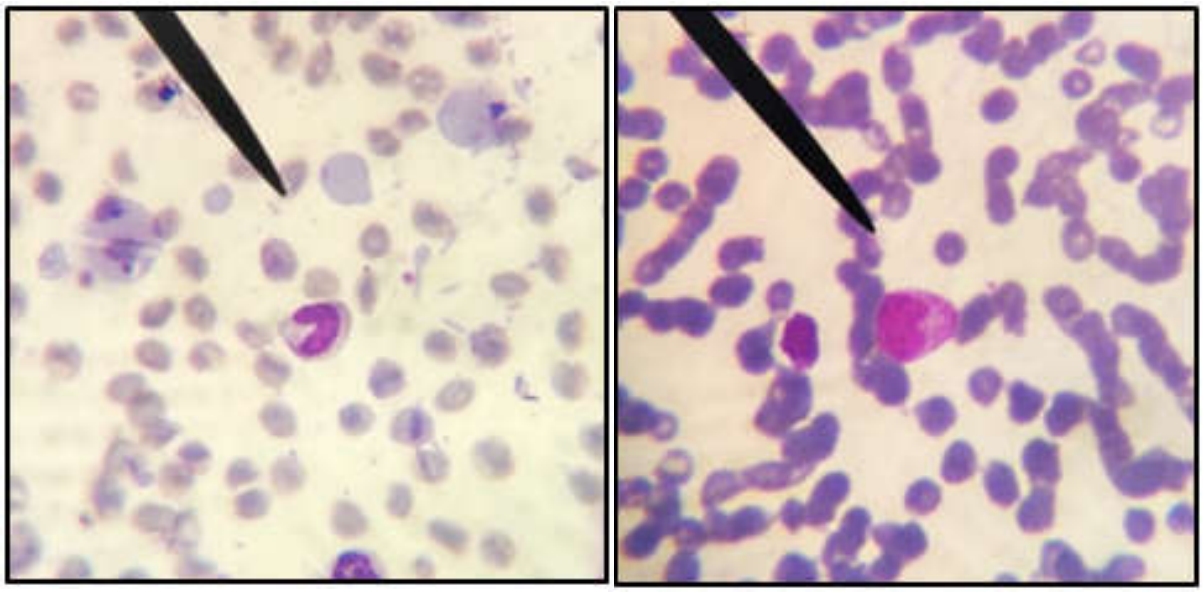
degenerative changes with 68% of specificity. Highest sensitivity recorded was of total Immature neutrophil count (INC) of 69.0% followed by 68.9% of I: M(Immature : Mature neutrophils) ratio parameter. I:M ratio showed highest positive predictive value (PPV) of 71.0% followed by 62.78% PPV of platelet count. Platelet count showed highest negative predictive value (NPV) of 62.78% and least NPV was measured by I:T ratio, as depicted in Graph 5 and immature forms of neutrophils is depicted in photograph 1.

As a part of sepsis screening test, C-reactive protein was done in all 106 cases of the present study. The quantitative distribution the CRP showed that 62.2%(n=66) cases measured CRP less that 6mg/dl while 7.5%(n=8) cases had CRP value between 6–8mg/dl and 30.2% (n=32) cases had >10mg/dl of CRP value.

Graph 5: Performance of hematological scoring system (in percentage).

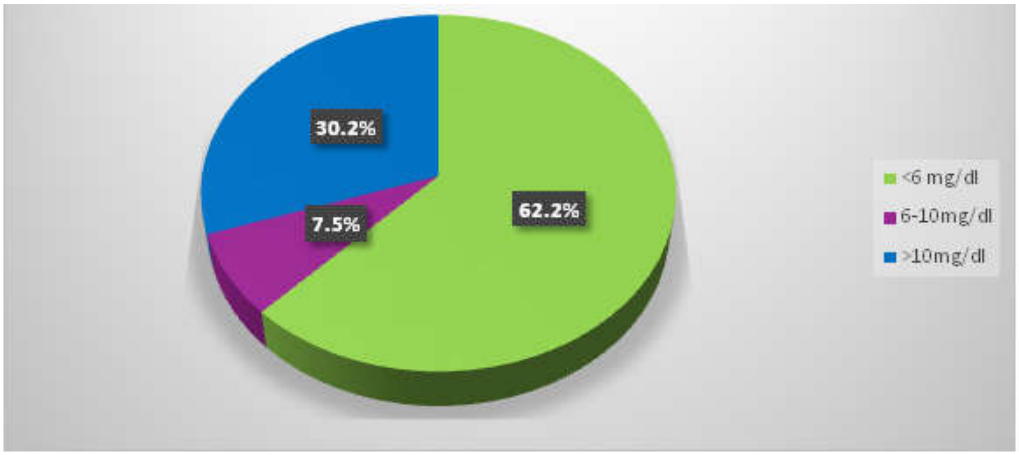


	TLC	Total PMN count	Total INC	I:T ratio	I:M ratio	PMN degenerative changes	Platelet count
specificity	68.7	40	38.6	42.8	60	68	59.26
sensitivity	33.3	56.2	69	55.56	68.9	57.8	60.81
PPV	48.5	42.8	62	62.5	71	71.5	57.69
NPV	53.8	53.3	43.6	36	57	62	62.78



Photograph 1: Microphotograph A and B: leishman stained peripheral smear showing band form and metamyelocyte

Graph 6: Distribution of nenatates according to C-reactive protein..



Discussion

HSS including blood parameters are reliable tools for screening and management of neonatal sepsis although blood culture is gold standard which although has less sensitivity and takes longer time for the culture reports.

Our study showed that majority (77.3%) of the cases were of less than <3 days and late onset sepsis cases with age more than 3 days accounted

Table 4: Comparison of the present study with recent studies.

Parameters		Values			
TLC		**Sensitivity**	**specificity**	**PPV**	**NPV**
	Priyanka T et al.[12]	23.6	71.2	35.8	–
	Bhalodia MJ et al.[22]	66.7	74.5	48	87
	Sharma MR et al.[23]	17.8	68	58.8	24.5
	Present study	33.3	68.7	48.5	53.8
Total PMN count	Priyanka T et al.[12]	52.8	91.1	80	74
	Bhalodia MJ et al.[22]	45.8	92.1	46	92
	Sharma MR et al.[23]	64.2	45.4	75	33.3
	Present study	56.2	40.0	43.0	53.3
Immature PMN count	Priyanka T et al.[12]	55.5	93	84.2	75.5
	Bhalodia MJ et al.[22]	–	–	–	–
	Sharma MR et al.[23]	67.8	40.9	74.5	33.3
	Present study	69.0	38.6	62.0	43.6
I:T ratio (>0.12)	Priyanka T et al.[12]	35.8	72.7	39.3	59.1
	Bhalodia MJ et al.[22]	91.6	92.1	92	93
	Sharma MR et al.[23]	57	50	74.4	31.4
	Present study	42.8	55.5	62.5	36
I:M ratio (>0.3)	Priyanka T et al.[12]	25.8	74.1	26.5	60.2
	Bhalodia MJ et al.[22]	93.7	94.2	93	94
	Sharma MR et al.[23]	26.7	68.2	68.2	26.7
	Present study	60.0	68.9	71	57
Degenerative changes	Priyanka T et al.[12]	45.6	86.2	69.3	70
	Bhalodia MJ et al.[22]	–	–	–	–
	Sharma MR et al.[23]	63.6	53.5	35	79
	Present study	52.6	52.3	48.7	56.1
Platelet count	Priyanka T et al.[12]	34.6	78.7	52.5	63.9
	Bhalodia MJ et al.[22]	56.3	55.9	56	58
	Sharma MR et al.[23]	59	80	54	83.3
	Present study	57.8	68.7	71.5	62.0

for 22.6% cases. Similar observations were seen in the study done by National Neonatal and Perinatal Database 13 and Sriram 14 with majority being early onset of sepsis.

Out of 106 cases, 59 cases were male and 47 were female with a ratio of 2.5:1 and a high prevalence in the age group of 24–48 hours. Hiral PS et al.[15], Garg et al.[16] Khair et al[8] and Bhat and Rao[17] observed male, female ratio as 1.7:1, 2.57:1, 1.39:1 and 1.08:1 respectively.

Neonatal sepsis presents with varied and non-specific symptoms, most common was refusal to feeding, fever and lethargy (present study- 52.8%), and similar findings were noted by Panwar et al.[18] (41%).

In the present study, the blood culture yield was 30.6%. This was similar to the 28.6 – 42.2% yield which was obtained by authors like Shrestha et al.[19] under the study conducted from July 2011 to January 2012, had 30.8% cases showing positive culture report. Sugandhi et al.[20] and Lal S et al.[21] reported 42.5% and 41.81% culture positivity respectively.

Comparison of the present study was done with few of the recent studies as shown in the Table 4:

Immature PMN count and I:M ratio are found to be very sensitive screening tool for neonatal sepsis as observed by the present study and studies concuded by Ghosh et al.[24] and Majumdar et al.[25]

Studies conducted by Basu S et al.[26] and Rodwell RL et al.[6] reported that thrombocytopenia is associated with poor prognosis in neonatal sepsis.

The traditional sepsis work up included various haematological parameters and CRP. Study done by Siddaiah et al.[27] concluded that neonates having raised CRP levels needed a long duration of antibiotic therapy than neonates having CRP levels within 6mg/dl.

Conclusion

Timely and aggressive treatment in the sepsis cases becomes very crucial for treating neonatologist as the symptoms at early sepsis phase can be subtle and deteriorate soon if treatment is not initiated timely. Measuring hematological parameters becomes mandatory in these cases and making hematological scoring system a routine in suspected cases can help as early indicators of neonatal sepsis as it is simple, cost effective, sensitive and quick with combination of parameter study further increases the sensitivity and specificity.

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