Utility of septic screen in early diagnosis of neonatal sepsis

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Abstract

Background and Objectives: (Signs of neonatal sepsis are subtle) and common to various illnesses making the diagnosis of sepsis difficult. Results of isolation of organism and culture are available only after 48 – 72 hours. Hence the need to find out different laboratory determinants helpful in detecting neonatal sepsis. A 'septic screen' of the following investigations was evaluated as a reliable indicator of neonatal sepsis – total leukocyte count, absolute neutrophil count, immature to total neutrophil ratio, micro erythrocyte sedimentation rate, C reactive protein.

Methods: This study was carried over a period of 18 months in a tertiary care hospital. A total of 250 neonates admitted with a clinical diagnosis of neonatal sepsis were included. Total leukocyte count was performed using an automated cell counter, differential count was done on peripheral smears stained with Wright stain, micro ESR was measured using capillary tubes with an internal diameter of 1.1 mm and length 75mm, CRP was estimated by commercially available latex agglutination kit. The cut off values used were Leukopenia < 5000 cells/mm^3, neutropenia < 1800/mm^3, immature neutrophils: total neutrophils count ratio >0.2, microESR >15mm, CRP positive (>1mg/dl)

Results: Blood culture was positive in 75 cases (30%) only and the commonest organism cultured was Acinetobacter. On comparing various tests with blood culture as gold standard they were found to be statistically significant ($p<0.01$)

Interpretation and Conclusions: The best three test combination was absolute neutrophil count + I/T ratio + ESR (AIE) having the best specificity and PPV.

Key Words: Neonatal sepsis, Septic screen

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Introduction

Neonatal sepsis remains a significant and frequent cause of morbidity and mortality in developing countries[1]. Blood culture is the definitive diagnosis of sepsis but requires **a well-equipped** laboratory and is also time consuming. Considering the outcome early diagnosis of this condition is desirable[2]. Combination of tests commonly recommended by various authors includes – total leukocyte count, absolute neutrophil count, immature: total neutrophil count ratio, C – reactive protein using **latex** agglutination test and micro ESR. Two or more positive tests have a good sensitivity and specificity.[3]

The purpose of the study was to use the following simple, economical, and rapid laboratory tests, (Septic Screen) which can be performed in the least equipped laboratory[4]:

- Total leukocyte count (TLC)
- Absolute neutrophil count (ANC)
- Immature : Total neutrophil count (I/T)
- Micro Erythrocyte sedimentation rate (m ESR)
- C- Reactive Protein (CRP)

Material and Methods

This study was done over a period of 18 months in LTMMC & LTMGH, Mumbai which is a tertiary care hospital with a neonatal intensive care unit facility. A total of 250 neonates admitted with a clinical diagnosis of neonatal sepsis were included. Ethical approval was taken from the institution ethics committee before initiation of the study.

Blood samples were collected under aseptic precautions for the following investigations – complete blood count, micro ESR, blood culture and serum CRP levels.

Total count was done using an automated three part cell counter. Differential count was performed by staining the peripheral smears using Wright stain and absolute neutrophil count was calculated. Micro ESR was measured using commercially available micro ESR kits using capillary tubes of 75mm length and internal diameter of 1.1 mm. Commercially available latex agglutination kits were used determine raised levels of CRP, agglutination present being interpreted as raised CRP levels.

Data was analyzed using the SPSS software, for Windows version 15.0 (Statistical Presentation Software SPSS Inc., 1999, New York) and Open Epi version 2.0 and categorical tables, Chi- square values, sensitivity, specificity, positive predictive value (PPV),

negative predictive value (NPV), relative risk of the three diagnostic methods derived and the results correlated. Test result was considered significant if *P* value is equal to or less that 0.05 (i.e. 5 %).

Results

A total of 250 neonates of both sexes with a clinical diagnosis of sepsis were included in this study. There were 155 (62%) males and 95 (38%) females. Of the 250 samples sent for blood culture only 75 (30%) were positive, while 175 (70%) were culture negative. Blood culture revealed that Gram negative septicemia is more frequent than sepsis caused by Gram positive organisms. Acinetobacter (24%) was the most common organism isolated followed by Klebsiella (21.3%) and Methicillin-Sensitive Staphylococcus aureus (21.3%). Enterobacter, Pseudomonas, E.coli were less commonly isolated.

The investigations included in the septic screen (total leukocyte count, absolute neutrophil count, I/T ratio, micro ESR, CRP) were compared to the gold standard i.e. blood culture and various statistical parameters were calculated and compared.

Table 1: Comparison of single septic screen tests with blood culture

Diagnostic Test		Gold standard: Blood Culture		Total	Chi Square value	P value	Relative risk / Diagnostic Odds
		Positive (75)	Negative (175)				
Single Tests							
TC	< 5000/mm3	32	15	47	39.97	< 0.01	7.94
	> 5000/ mm3	43	160	203			
ANC	< 1800/mm3	35	16	51	45.52	< 0.01	8.70
	< 1800/mm3	40	159	199			
I / T Ratio	> 0.2	59	71	130	30.53	< 0.01	5.40
	< 0.2	16	104	120			
Micro ESR	> 15 mm	42	8	50	86.79	< 0.01	26.57
	< 15 mm	33	167	200			
CRP	Positive (Agglutination + ve)	67	47	114	82.61	< 0.01	22.81
	Negative (Agglutination - ve)	8	128	136			

TC = Total Leukocyte Count, ANC = Absolute Neutrophil Count, I / T ratio = Immature: Total Neutrophil ratio, Micro ESR = Micro Erythrocyte Sedimentation Rate, CRP = C Reactive Protein

Table 2: Sensitivty, specificity, PPV & NPV of sepsis screen tests

Diagnostic Test	Sensitivity %	Specificity %	PPV %	NPV %
Single Test Positive				
TC (< 5000 /mm3)	43	91	68	79
ANC (< 1800 /mm3)	47	91	69	80
I / T Ratio (> 0.2)	79	59	45	87
Micro ESR (> 15 mm	56	95	84	84
CRP Positive (Agglutination + ve)	89	73	59	94

TC = Total Leukocyte Count, ANC = Absolute Neutrophil Count, I/T ratio = Immature: Total Neutrophil ratio, Micro ESR = Micro Erythrocyte Sedimentation Rate, CRP = C Reactive Protein PPV = Positive Predictive Value, NPV = Negative Predictive Value.

The percentage of culture positive cases was higher in those patients with leukopenia than with patients having a total count more than 5000 cells/mm^3 [Table 1]. The difference was statistically significant. Similar findings were observed with each of the other tests. So CRP is the most sensitive parameter for the diagnosis of neonatal sepsis and also has the highest Negative Predictive Value (NPV). Micro ESR is the most specific parameter for diagnosing neonatal sepsis with highest positive Predictive Value (PPV).[Table 2]

Table 3: Comparison of combination of 3 septic screen tests with blood culture

Diagnostic Test		Gold standard: Blood Culture		Total	Chi Square value	P value	Relative Risk / Diagnostic Odds
		Positive (75)	Negative (175)				
Combinations of 3 tests							
TAE	Positive	17	1	18	38.36	< 0.01	51
	Negative	58	174	232			
TAC	Positive	24	3	27	49.99	< 0.01	26.98
	Negative	51	172	223			
TAI	Positive	24	3	27	49.99	< 0.01	26.98
	Negative	51	172	223			
AIE	Positive	21	0	21	53.49	< 0.01	------
	Negative	54	175	229			
AIC	Positive	27	2	29	62.21	< 0.01	48.66
	Negative	48	173	221			
IEC	Positive	31	0	31	82.57	< 0.01	-----
	Negative	44	175	219			
IET	Positive	18	0	18	45.26	< 0.01	------
	Negative	57	175	232			
ECT	Positive	19	0	19	47.98	< 0.01	------
	Negative	56	175	231			
ECA	Positive	22	0	22	56.29	< 0.01	-----
	Negative	53	175	228			
TIC	Positive	23	2	25	50.85	< 0.01	38.26
	Negative	52	173	225			

T = Total Leukocyte Count, A = Absolute Neutrophil Count, E = micro ESR,
C = C Reactive Protein, I = Immature: Mature neutrophil ratio

Table 4: Sensitivty, specificity, PPV & NPV of 3 sepsis screen tests

Diagnostic Test	Sensitivity %	Specificity %	PPV %	NPV %
Three Tests Positive				
TAE	23	99	94	75
TAC	32	98	89	77
TAI	32	98	89	77
AIE	28	100	100	76
AIC	36	99	93	78
IEC	41	100	100	80
IET	24	100	100	75
ECT	25	100	100	76
ECA	29	100	100	77
TIC	31	99	92	77

T = Total Leukocyte Count, A = Absolute Neutrophil Count, E = micro ESR, C = C Reactive Protein, I = Immature: Mature neutrophil ratio, PPV = Positive Predictive Value, NPV = Negative Predictive Value

Table 3 shows that the combination of immature/mature neutrophil counts, micro ESR, and CRP has the maximum Chi square value. This combination (IEC) also has the highest sensitivity, highest specificity along with maximum positive predictive value (PPV) as well as negative predictive value (NPV) [Table 4].

Table 5: Comparison of combination of 4 and 5 septic screen tests with blood culture

Diagnostic Test		Gold Standard: Blood Culture		Total	Chi Square Value	*P* Value	Relative Risk / Diagnostic odds
		Positive (75)	Negative (175)				
Combinations of 4 tests							
TAIE	Positive	15	0	15	37.23	< 0.01	-------
	Negative	60	175	235			
TAIC	Positive	20	2	22	42.62	< 0.01	31.45
	Negative	55	173	228			
AIEC	Positive	20	0	20	50.72	< 0.01	-------
	Negative	55	175	230			
IECT	Positive	17	0	17	42.56	< 0.01	-------
	Negative	58	175	233			
TAEC	Positive	16	0	16	38.39	< 0.01	-------
	Negative	59	175	234			
Combinations of 5 tests							
TAIEC	Positive	14	0	14	34.6	< 0.01	-------
	Negative	61	175	236			

T = Total Leukocyte Count, A = Absolute Neutrophil Count, E = micro ESR, C = C Reactive Protein, I = Immature: Mature neutrophil ratio

Table 6:

Diagnostic Test	Sensitivity %	Specificity %	PPV %	NPV %
Four Test Positive				
TAIE	20	100	100	74
TAIC	27	99	91	76
AIEC	27	100	100	76
IECT	23	100	100	75
TAEC	21	100	100	75
Five Test Positive				
TAIEC	19	100	100	74

T = Total Leukocyte Count, A = Absolute Neutrophil Count, E = micro ESR, C = C Reactive Protein, I = Immature: Mature neutrophil ratio, PPV = Positive Predictive Value, NPV = Negative Predictive Value

All combinations of 4 tests and 5 tests of the septic screen, showed that the *p* value is statistically significant [Table 5]. These combinations showed specificity of 100% and PPV of 100%. The only exception was the combination of TAIC. The combination AIEC showed the highest sensitivity (27%) and specificity (100%) followed by TAIC (27%, 99%) [Table 6].

Table 7: Area under the curve for Total Leukocyte Count

Area under the curve – Test Results Variable : WBC / mm^3				
Area	Std. Error	Asymptomatic Significance	Asymptomatic 95% Confidence Interval	
			Upper Bound	Lower Bound
0.65	0.05	< 0.01	0.56	0.73

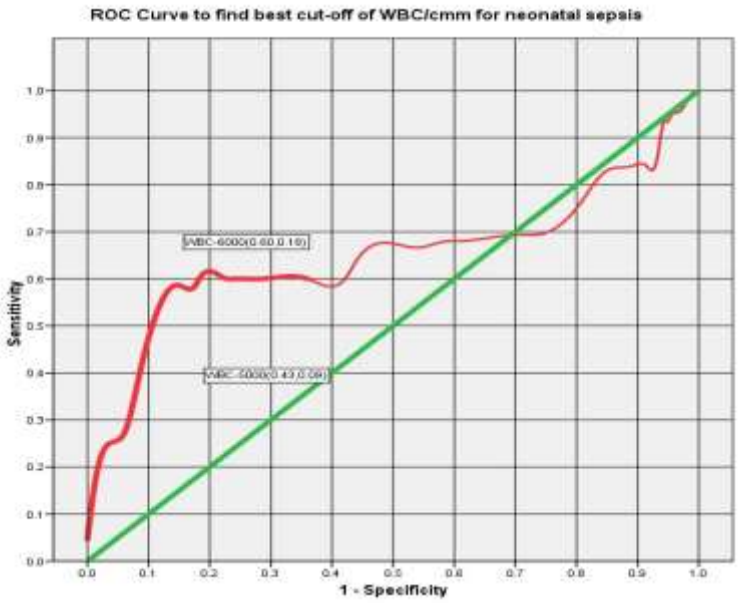

Fig 1

The ROC curve for total leukocyte count shows the value of < 6000 cells / mm^3 as the best cut off value significant for diagnosing neonatal sepsis.

Table 8: Area under Curve for Absolute Neutrophil Count

Area under the curve – Test Results Variable: Absolute Neutrophil Count/ mm^3				
Area	**Std. Error**	**Asymptomatic Significance**	**Asymptomatic 95% Confidence Interval**	
			Upper Bound	**Lower Bound**
0.65	0.05	< 0.01	0.56	0.74

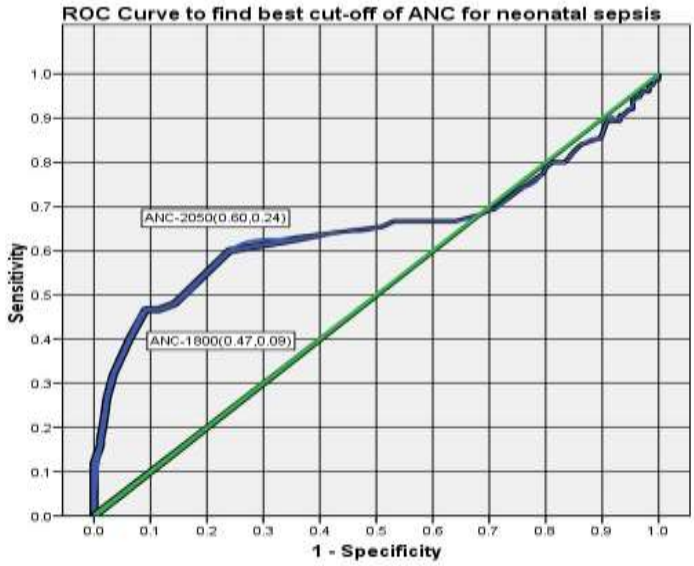

Fig. 2

The above ROC for absolute neutrophil count shows the best cut off value of < 2050 cells/mm^3 as significant value for diagnosis of sepsis.

Table 9: Area under Curve for Micro ESR

Area under the curve – Test Results Variable: micro ESR (mm)				
Area	**Std. Error**	**Asymptomatic Significance**	**Asymptomatic 95% Confidence Interval**	
			Upper Bound	**Lower Bound**
0.85	0.03	< 0.01	0.78	0.91

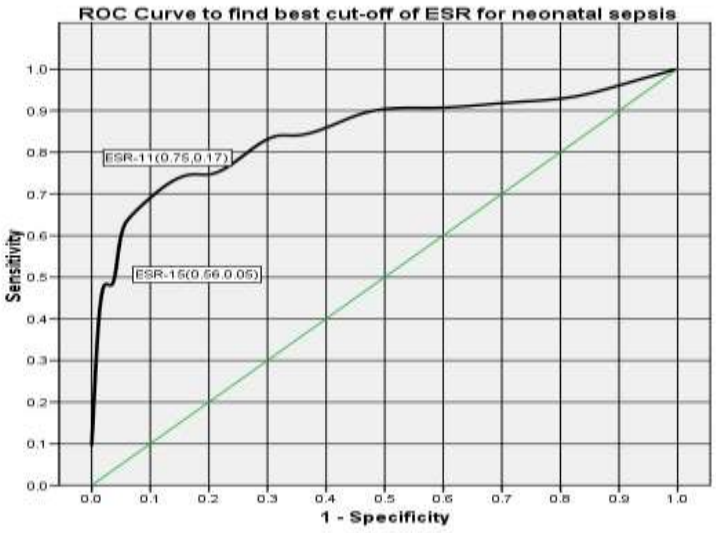

Fig. 3

The ROC curve for micro ESR shows the value of > 11 mm at the end of one hour as the best cut off value significant for diagnosis.

Discussion

In our study cases with early onset sepsis were more common (51%). The findings are consistent with other authors.[5]

We had an M: F ratio of 1.6:1 suggesting a slight male preponderance which was comparable to other studies.[2,6,7,8] As a single test screening parameter total leukocyte count, showed a better specificity (91%) in our study. This was consistent with the findings of Khair[6] et al and Supreetha et al.[14] [Table 10]

Table 10: Comparison with other studies with respect to individual tests, Total Count, Absolute Neutrophil Count, I /T Ratio

	Total Count				**ANC**				**I / T Ratio**			
	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Sensitivity (%)**	**Speci Ficity (%)**	**PPV (%)**	**NPV (%)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Our Study	43	91	68	79	47	91	69	80	79	59	45	87
Zaki[9] et al	48	77	67	62	55	74	67	64	76	87	85	79
Khair et al[6]	50	91	43	93	92	38	17	97	100	4	13	100
Shirazi[7] et al	35	77	----	------	35	74	------	------	------	------	------	------
Buch[10] et al	50.77	63.64	62.26	52.24	66.15	90.91	89.58	69.44	89.23	70.91	78.38	84.78
Sriram[8]	63.6	51.0	12.1	93.0	50	49.6	3.5	96.5	52.2	56.5	82.8	22.8
Waliulah et al[11]	------	-------	------	------	------	------	------	------	70	56	------	------
Basu[12] et al	54.62	50.64	45.77	59.40	55.46	53.85	47.83	61.31	56.30	53.85	48.20	61.76
Misra[13] et al	95.9	25	70	76.9	20	87.5	75	36.8	80	65	81.1	63.4
Supreeth	45	94	82	66	78	87	80	67	91	79	78	51

a et al[14]												
Mondal et al[15]	------	------	------	------	------	------	------	------	63	85	92	------

As a single screening test the absolute neutrophil count showed a high specificity of 91% which was comparable to the studies of Buch et al[10], Misra et al[13] and Supreetha et al.[14] [Table 10]

I/T ratio was found to be having a sensitivity of 79 % which was comparable to studies by Zaki et al[9], Misra et al.[13] [Table 10]

Our study and the study by Mondal et [15] al had a similar specificity for micro ESR as a single screening test. The sensitivity of CRP (89%) showed concordance with studies done by Zaki et al[9] and Misra et al.[14][Table 11]

Table 11: Comparison with other studies with respect to individual tests, micro ESR and CRP

	Micro ESR				**CRP**			
	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Our Study	56	95	84	84	89	73	59	94
Zaki et al[9]	------	-------	------	------	86	97	96	88
Buch et al[10]	63.08	72.73	73.21	62.50	68.46	73.64	71.83	71.43
Sriram[8]	73.1	56.2	32.8	87.7	52	61.5	91.4	14
Waliulah et al[11]	63.3	60	------	------	------	------	------	------
Basu et al[12]	64.71	80.77	71.96	75	79.83	83.97	79.17	84.52
Misra et al[13]	------	------	------	------	90.7	37.5	73.1	68.2
Supreetha et al[14]	------	------	------	------	82	70	54	91
Mondal et al[15]	63	94	92	------	84	65	69	------

Table 12: Comparison with other studies with respect to combination of 3 tests

	Our Study				**Buch et al[10]**			
	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Three Test Positive Combination								
TAI	32	98	89	77	76.92	78.18	80.64	74.14
AIE	28	100	100	76	78.46	90.91	91.01	78.12
AIC	36	99	93	78	87.69	83.64	86.36	85.19
IEC	41	100	100	80	86.15	76.36	81.16	82.36
ECA	29	100	100	77	80	83.33	88.14	78.69
TIC	31	99	92	77	83.08	70.90	77.14	78

Table 13: Comparison with other studies with respect to combination of 4 & 5 tests

	Our Study				**Buch et al[10]**			
	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Four Test Positive Combination								
AIEC	27	100	100	76	73.85	94.55	94.12	75.36
IECT	23	100	100	75	67.69	81.82	81.48	68.18
Five Test Positive Combination								
TAIEC	19	100	100	74	80	90.91	91.23	93.36

Utilizing a combination of three tests of the septic screen it was found that the combination of absolute neutrophil count + I/T ratio + ESR (AIE) had the best specificity and PPV followed by the next best

combination which was absolute neutrophil count + I/T ratio + CRP (AIC) [Table 12]. A similar trend was observed using a four test combination of absolute neutrophil count + I/T ratio + ESR + CRP (AIEC) followed by I/T ratio + ESR + CRP + Total count (IECT). This was observed in our study as well as the study by Buch et al.[11] [Table 13]

Summary & Conclusion

In this study screening for sepsis in 250 clinically diagnosed cases of neonatal sepsis was done. Bacterial culture though a gold standard has a low sensitivity and is relatively time consuming, the results being available in 72 hours. Hence the need for rapid screening tests of the septic screen. Five parameters - Total count, Absolute neutrophil count, immature to total neutrophil count, micro ESR, C - reactive protein were compared with the gold standard (blood culture) and an attempt was made to find a single best test or a combination of tests for easy and rapid diagnosis of neonatal sepsis. Micro ESR as a single screening tests had the best specificity as compared to other tests done singly. This was closely followed by total leukocyte count and absolute neutrophil count. Among single screening tests CRP had the best sensitivity. However as single screening tests these were not of much utility as evident from the PPV and NPV.

The best combination of tests observed was absolute neutrophil count + I/T ratio + ESR (AIE) had the best specificity and PPV followed by the next best combination which was absolute neutrophil count + I/T ratio + CRP (AIC). Based on the ROC plotted using our data the recommended cut of values significant for diagnosing neonatal sepsis are total leukocyte count < 6000 cells/mm^3, absolute neutrophil count < 2050 cells/mm3, micro ESR > 11 mm at end of one hour.

India is a country where tertiary centre health care is not available to all and where infant mortality still needs to be controlled. The tests used in our study are easily reproducible even at a basic level of healthcare. Further we have plotted the ROC curve and tried to find the best cutoff values based on our sample population. Further studies can be attempted to validate these values.

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The authors deny any conflicts of interest related to this study.

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