

Morphological Abnormalities of Leucocytes and Leucocytic Indices in Patient of Clinical Septicemia.

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Abstract

Background: Recognition of sepsis at an early stages is associated with favourable outcome. White blood cell parameters play an important role in diagnosis and prognosis of sepsis. The neutrophil cell population data is one of the meaningful exercise in the patients who are clinically suspected of sepsis.

Objectives: The objectives were to evaluate i) morphological alterations in neutrophils in state of septicaemia ii) to evaluate the changes in WBC count and absolute neutrophilic counts in septicemia and iii) to analyse neutrophil left shift parameters and immature cell indices in septicaemia.

Materials and method: Thirty patients who were clinically suspected of septicaemia by criteria of SOFA underwent neutrophilic parameters by automated cell counters for total leucocyte count, differential leucocyte count, absolute neutrophil count, immature to mature cell ratio and the evaluation of morphological alteration in neutrophils on peripheral blood smears.

Results: total leucocyte count and absolute neutrophil count was found raised in patients of septicaemia with 100 % sensitivity, SP, PPV and NPV. However NPV for band cells(40.63%), toxic granules(68.75%), Dohle bodies(9.37%), immature to mature cell ratio (78.12% and 21.87%) was found to be low.

Conclusion : the neutrophil population data is substantiates the diagnosis of septicaemia and therefore must be regarded high not for its diagnostic value but for its prognostic value.

Keywords: sepsis, neutrophil cell population data

INTRODUCTION

In spite of recent advances in antibiotic therapy and general critical care practices, including early goal-directed treatment for septic shock mortality of patients with severe sepsis/septic shock is still substantial. Early diagnosis of infection and sepsis before it progresses to organ dysfunction or circulatory failure has crucial impact on the clinical course and outcome of critically ill patients¹. White blood cell counts is at the center of diagnostic algorithm of sepsis.

The role of neutrophil cell population data, the mean neutrophil volume (MNV) and the standard deviation of the MNV, also known as neutrophil volume distribution width (NDW), in post-surgical bacterial infection have also been investigated². Timely diagnosing post-operative infection is critical for proper patient management. However, traditional parameters, such as WBC counts or body temperature, may not be adequate to detect the infection because non-infectious leukocytosis and fever are common phenomena in postsurgical period.^{3,4}

The systemic inflammatory response syndrome induced by bacterial infections and surgical trauma is a well-known entity, with resultant release of variety of inflammatory cytokines leading to fever and/or leukocytosis in spite of the absence of clinical signs of infection. In addition to the leukocyte numerical changes, there are other morphologic changes of reactive neutrophils, such as the presence of toxic granulation, toxic vacuolization and Dohle bodies in the cytoplasm and cytoplasmic vacuolation.^{5,6,7,8}

The findings of leucocytic alterations especially related to neutrophils and other granulocytes, it is evident that the condition of septicaemia can be recognized and predicted for its prognosis, if these alterations are properly noted. There are studies those reported not only the diagnostic importance but also predictive value of neutrophil parameter in patients of sepsis.^{8,9,10,11,12,13,14}. Therefore the present study has been carried out with following aims and objectives.

AIMS AND OBJECTIVES

The aim of the study was to note the different morphological abnormalities and counts of leukocytes in clinically suspected cases of septicaemia.

The objectives were to evaluate i) morphological alterations in neutrophils and granulocyte in state of septicaemia ii) to evaluate the changes in WBC count and absolute neutrophilic counts in condition of septicemia and iii) to analyse neutrophil left shift parameters and immature cell indices as a tool for diagnosis and prognosis in septicaemia.

MATERIALS AND METHODS

The study was carried out with following materials and method.

The study design - Prospective, Observational and Analytical

Source of Data- This study was performed in AVBRH Hospital attached to Jawaharlal Nehru Medical College, DMIMS (DU), in Department of Pathology Sawangi, Wardha, Maharashtra.

Case recruitment- The patient suspected of septicaemia with suggested infective focus at any site in the body. The patients of paediatric age group are excluded from the study. All the

patients recruited in study were of more than 18 years of age. The diagnosis of septicaemia was based on SOFA criteria.⁷

Patient details- the clinical details for the patients were recorded in proforma including important biochemical and radiological investigation apart of demographic details of the patients.

Sample size- Thirty patients were recruited in the study with suspected diagnosis of sepsis by criteria of SOFA.

Investigations – as the study was pertaining to haematological alterations in prediction and suggestion of septicaemia the following investigations were carried out by haematological cell coulter and morphological studies performed on Leishman stain peripheral smear. i) Total leucocyte count ii) Differential leucocyte count iii) Absolute neutrophil count iv) Granulocyte Immature ratio v) Band cell count vi) Percentage of distribution of toxic granules vii) Percentage of distribution of toxic vacuoles viii) Percentage of distribution of Dohle's bodies. The total leucocyte count and absolute count were calculated by haematological cell coulter. (Beckers coulter)

The statistical test performed were for sensitivity(Sn), specificity(Sp), positive predictive value(PPV) and negative predictive value(NPV)

Results

The following observations were made on the parameters chosen for the study in the clinically suspected cases of sepsis. The distribution of patients for their age and sex is with clinical diagnosis of sepsis is shown in table number 1.

Table 1: Age and sex distribution (n=32)

AGE RANGES	GENDER		TOTAL (%)
	MALE	FEMALE	
21-30	3	2	5 (15.6%)
31-40	5	1	6 (18.75%)
41-50	4	2	6 (18.75%)
51-60	0	4	4 (12.5%)
61-70	3	4	7 (21.87%)
71-80	0	3	3 (9.37%)
81-90	1	0	1 (3.125%)
Total	16	16	32 (100%)

Oldest patient was of 86 years and youngest patient was of 21 years in the study population.

All the 32 patients had clinical diagnosis of septicaemia with known underlying pathology except for 5 cases where the underlying pathology for septicaemia was unknown.

Maximum cases that developed septicaemia in due course of disease had a primary diagnosis of diabetes type II (4) followed by conditions of malignant neoplasia (3). Rare disease of scrub typhus too underwent septicaemia with clinical findings. The other diseases progressed to clinical septicaemia were cirrhosis of liver, chronic kidney disease with uremic encephalopathy with congestive cardiac failure and pulmonary tuberculosis.

The mean $\pm$ SD for total WBC count in comparison to total Absolute Neutrophilic Count with age fractionalisation is shown in table 2

Table 2: Total Leucocyte Count and Absolute Neutrophil Counts

PARAMETERS	Cut off -/ml	Less than 50 years age (n=17) -/ml	More than 50 years age (n=15) -/ml	TOTAL (%) (n=32) Mean $\pm$ SD
Total leucocyte count (mean $\pm$ SD)	11 X 10 ³	20287.5 $\pm$ 5584.25	24768.75 $\pm$ 11646.96	22528.125 $\pm$ 9268.72
Absolute neutrophilic count(mean $\pm$ SD)	8 x 10 ³	16034.8125 $\pm$ 5122.01	20138.5 $\pm$ 10278.16	18086.66 $\pm$ 8255.705

The mean $\pm$ SD value for total WBC count irrespective of age in adults above and below 50 years was 22528.125 $\pm$ 9268.72.

The mean $\pm$ SD value for ANC count was observed to be 18086.66 $\pm$ 8255.705 irrespective of age groups in adults.

Parameters pertaining to morphological alterations and ratio for the neutrophils in 32 cases are shown in table 3 with their mean and standard deviation.

Table 3: Morphological Indices and Ratio.

PARAMETERS		Less than 50 years age (n=17)	More than 50 years age (n=15)	TOTAL (%)	Mean $\pm$ SD
Toxic granule		11/17	11/15	22/32 (68.75%)	-
Toxic vacuolations (mean $\pm$ SD)		07/17	09/15	16/32 (50%)	-
Dohle body-		02/17	01/15	3/32 (9.38%)	-
Percentage of band cells		17/17	15/15	32/32 (100%)	5.34 $\pm$ 1.42
Immature to mature ratio cut- off	>0.25	12/17 (0.70)	13/15 (0.86)	25/32 (78.125%)	0.07 $\pm$ 0.02
	<0.25	05/17 (0.29)	02/15 (0.13)	07/32 (21.875%)	

The toxic granules were observed in 68.75 % of the patients. Toxic vacuolations were seen in the 7 of the 17 patients and 9 of 15 patients in age group of below and above 50 years respectively. Dohle bodies was observed in infrequent neutrophil in 2 cases. The band cells

were observed in peripheral smear of all cases. But its presence was variable. The mean value for the band cells 5.34 ± 1.42 .

The cut off of 0.25 for the immature to mature ratio was found to work well. The mean value for immature: mature ratio 0.07 ± 0.02 . Twenty five patients showed immature to mature ratio of more than 0.25 suggesting its diagnostic value.

The sensitivity, specificity, PPV and NPV for each parameter included for evaluation in diagnosis of septicaemia is shown in table 4.

Table 4: Parameters and value of correlation

Parameters	Cut-off values	Sensitivity	Specificity	PPV	NPV
TLC	11×10^3	100%	100%	100%	100%
ANC	$8 \times 10^3/\text{ml}$	100%	100%	100%	100%
Band cell %	4%	40.63%	100%	100%	40.63%
Toxic granules	*	68.75%	100%	100%	68.75%
Dohle bodies	*	9.37%	100%	100%	9.37%
Immature : mature ratio	<0.25	78.12%	100%	100%	78.12%
	>0.25	21.87%	100%	100%	21.87%

(* No cut off for this feature as it's a microscopic morphology)

The sensitivity of toxic vacuolation and Dohle body was lowest, so were the NPV. The highest specificity and PPV of 100% was observed with the features of TLC, ANC, band cells, toxic granules, Dohle bodies and immature to mature cell ratio associated with septicaemia. The immature: mature cell ratio with the cut off >0.25 was found to have the best impact at diagnostics of septicaemia.

The pattern for value of correlation were suggesting the high specificity for toxic granules though its sensitivity was less with septicaemia.

DISCUSSION

The mean age of patients in the study was 50.65 years and mean age of the patients below 50 years was 35 years and over it was 66.31 years, which was similar to a few studies reviewed for present work.²

The co-morbidities mentioned in the study of Annane et al were of immune deficiency, ID, carcinoma, hematological, chronic pulmonary disease, HIV⁴.

The present study had varied underlying inflammatory causes of sepsis as mentioned in the studies gathered for the review of present work includes necrotising enterocolitis, hepatitis, urosepsis, pulmonary tuberculosis, bronchopneumonia and typhus.

The co morbid condition that were associated with patient of sepsis included malignant neoplasia, cirrhosis of liver, diabetes mellitus, hypothyroidism, cardiogenic shock,

hemolytic anemia and idiopathic thrombocytopenia. These co-morbid conditions found in the patients included in the present study are much similar to the co-morbidities reported by Annane et al⁴ and Severson et al¹⁵ in cases of septicemia. The rise of in the total counts of WBC has been considered as hallmark in evidence of septicemia in these conditions.

The study of Park et al⁷ have reported the median count of TLC as 11610/ul (59 patients) in the patients of septicemia without septic shock and 30,820/ul (44 patients) with patients of septic shock.

The range that has been observed in the patients of septicemia was from 8870- 21505 per micro litre irrespective of severity of sepsis. This study has concluded that the total WBC counts in the diagnosis of septicemia has in significant P value of 0.655.

Seebach et al¹⁰ in their study of 320 patients found average of total leukocyte count was $12.4 \pm 7.9 \times 10^6$ /ml. The study has statistically concluded that the total leukocyte count in the diagnosis of septicemia has a low sensitivity but a high specificity. It has a role in the laboratory diagnostic process in the workup of septicemia, especially when the counts are above a foresaid levels.

Al-Gwaiz et al⁹ found that the total WBC counts vary in the septicemia with respect to each of the patient and severity of infections. Their study have found that $TLC > 9.6 \times 10^9$ /l had 36% sensitivity in the diagnosis of septicemia.

The present study has observed the mean total leucocyte count as 20287 ± 5584.25 for the patients of septicemia under 50 years of age and 24768.75 ± 11646.96 /ml for patients over the age of 50. The mean value when total population is included was found to be 22528 ± 9268.72 for total leucocyte count. These values are comparatively higher than the values observed by Seebach et al¹⁰ and Al- Gwaiz⁹.

This may be due to population character where the recruited population was of neonates.

Neutrophilic cell data population has been studied for diagnosis of septicemia by Xu et al⁸, Park et al⁷, Seebach et al¹⁰ and Buoro et al¹⁶ their studies have observed that the absolute neutrophilic count have varying sensitivity at prediction of septicemia.

Seebach et al¹⁰ observed the value of $>8.6 \pm 5.5 \times 10^6$ /ml of the diagnostic value in the patients below 50 years and $13.6 \pm 9.1 \times 10^6$ /ml. The observation of present study is different than the study of Seebach et al¹⁰ for absolute neutrophil count with its diagnostic significance with septicemia. The value of present study for the patients of septicemia below 50 years of age is $16.034 \pm 5122 \times 10^3$ while for the patients over 50 years was $18.086 \pm 8252.7 \times 10^3$ /ml.

The morphological assessment for toxic granules as an indicator for septicemia has been studied by Al-Gwaiz et al⁹ so also their study includes the observation for toxic vacuoles. The study has concluded that these three parameters when taken together will show lower sensitivity for the diagnosis of septicemia.

Their study have observed that the toxic granules had sensitivity of 48% Dohle bodies 32% and vacuoles had sensitivity of 22%. The study of Rathi et al¹¹ too have observed the lower percentage of positivity for the features of toxic granules, cytoplasmic vacuolations and

Dohle bodies in neonates. Twenty patients of sepsis showed toxic granules, 9 patients showed cytoplasmic vacuolations and 20 patients showed combination of toxic granules, cytoplasmic vacuolations and Dohle bodies out of 150 patients mostly belonging to department of Pediatrics. The comparisons of present studies data is in agreement with the observation of Al-Gwaiz et al⁹.

The study of Seebach et al¹⁰ reported 21.9 +/- 15.6% of bands in differential leucocyte count in the patients of septicaemia without significant difference whether the patients is below or above 50 years of age. The study of Al-Gwaiz et al⁹ reported >20% of bands in 32 patients of total of 105 cases. Band cells for its diagnostic importance in septicaemia has also been reported by Park et al⁷. However their absolute for differential value has not been reported in their publication. Study of Porizaka et al¹⁴ stress out on the importance of immature granulocytes as a sepsis predictor in the patients undergoing cardiac surgery but their absolute or differential values have not been reported.

Present study reported the band cells in 32 of 32 patients that is 100% with their average differential count of 5.34 %. The study of Seebach et al¹⁰ reported the high counts as previously described which is attributed to the high scores of sepsis for immature and band cells.

Immature to mature ratio have not been performed in many studies. The study of Seebach et al¹⁰ have reported the immature to mature ratio was found to be increased in the patients of the septicaemia either more than 0.3 and more than 0.25 in the studies of Rathi et al¹¹ and Seebach et al¹⁰ respectively. The other studies have given Immature: total ratio based on the findings of cell counters. The present study did not compare immature: total ratio. The present study observed the immature: mature ratio variability which is irrespective of the age groups.

Immature: mature cut off ratio of > 0.25% was observed in 25 of 32 cases (78.12%) and <0.25 was observed in 7 out of 32 cases (21.87%). This findings is similar to the findings of Seebach et al¹⁰.

Seebach et al¹⁰ reported sensitivity of 55% and specificity of 65% with the cut-off value of total leukocyte count for more than 11×10^3 . However Rathi et al¹¹ with the same cut-off found the sensitivity of 98.32% and specificity of 65.93% in neonates. Al-Gwaiz et al⁹ reported very low sensitivity of 36% at this cut-off of total leukocyte count. The present study had a dissimilar finding with that of Seebach et al¹⁰ and Al-Gwaiz et al⁹. However the findings of the present study of 100% sensitivity and 100% specificity of total leukocyte count with cut-off of 11×10^3 is 100% respectively which is close to the observation of Rathi et al¹¹ whose study has population of neonates.

Absolute neutrophil count with the cut-off of 8×10^3 was found to have sensitivity of 60% and specificity of 68% in the study of Seebach et al¹⁰. Rathi et al¹¹ reported sensitivity of 96.55% and specificity of 30.85% with the same cut-off in neonates.

Al-Gwaiz et al⁹ reported sensitivity of 48% at the above cut-off. The study of Seebach et al¹⁰ and Al-Gwaiz et al⁹ for sensitivity at the cut-off of more than 8×10^3 of absolute neutrophil count was very low and non-parallel with the present study.

Al-Gwaizet al⁹ reported the utility of morphological characters of the neutrophils for their diagnostic association with septicaemia. Rath et al¹¹ reported sensitivity of 88.80%, specificity of 87.02%, positive predictive value of 82.39 % and negative predictive value of 92.13% for combined morphological features of toxic granules, vacuoles and Dohle bodies in neonates. These observations are very close to the observations of the present study but the difference at sensitivity and negative predictive value. However Al-Gwaiz et al⁹ reported all the way a low sensitivity with these separate features of 48% sensitivity of toxic granules, 22% with toxic vacuoles and 32% with Dohle bodies. These observations of Al-Gwaizet al⁹ are contrary to the observations of present study.

The band cells with cut-off of 4% was reported to have sensitivity of 79% and specificity of 53% in the study of Seebach et al¹⁰. Rath et al¹¹ with the similar cut off reported sensitivity of 91.8% and specificity of 76.59% in neonates.

Al-Gwaizet al⁹ reported 32% sensitivity for band cells at the same cut-off. There appears to be a contradiction between the values reported for sensitivity and specificity at the cut-off of 4% of Band cells as present study observed sensitivity of 40.63% and specificity of 100%. Present study differs from report of Al-Gwaizet al⁹ as it observed 100% PPV and 40.6% NPV.

Seebach et al¹⁰ though reported band cells and immature cells but reported with the different cut off that is more than 15% for band cells and the ratio has been reported with immature to total cell which is not the selected objective cut-off of the present study. A few related studies on septicaemia were reported¹⁷⁻¹⁹. Related study on paediatric leukemia was reported by Jameel et. al.²⁰.

The present study with the cut off >0.25 found to have sensitivity of 28.87%, specificity of 100%, positive predictive value of 100% and negative predictive value of 21.87% thus the observations of immature: mature ratio is disagreement with a few other studies. This disagreement exist for sensitivity as well as negative predictive value. Thus presence of immature cells and their ratio comparisons with the mature cells appear to work low for its association with diagnosis of septicaemia.

CONCLUSION

It is concluded from observation that the total leukocyte count, absolute neutrophilic count and morphological alterations in the leukocytes appear statistically significant than isolated parameters such as percentage of band cells and immature: mature ratio. Therefore, the assessment of TLC, ANC, bandemia and morphological alterations in neutrophils are sensitive and specific at diagnosing the condition of septicaemia and predicting its course in early stage.

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