Original Article

Evaluation of Haematological Parameters as Prognostic Marker in Sepsis: An Observational Prospective Study

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ABSTRACT

Introduction: The haematological system is implicated early in pathogenesis of sepsis and is thought to be crucial to the illness' progression and recovery. Anaemia, thrombocytopenia, and leucocytosis are the most frequently detected anomalies in the cellular component of the haematological system however erythrocytosis, thrombocytopenia, and leukopenia may also be noted. The literature that is now accessible highlights the significance of undertaking additional study into the cellular component of the haematological system in order to pinpoint the precise function of specific indicators and their predictive value as sepsis markers.

Aims/ objective: To determine whether the parameters derived from cellular components of the haematological system can be helpful prognostic markers in critically ill septic patients

Materials and Method: Within 24 hours of admission into ICU, adult patients who were critically ill with a diagnosis of sepsis were included in the study. On the day of admission and on day 3, the following parameters were recorded—haemoglobin, haematocrit (PCV), red blood cell count, mean cell volume (MCV), mean cell haemoglobin (MCH), red blood cell distribution width, total leukocyte count (TLC), differential leukocyte count (DLC), platelet count and platelet distribution width. These parameters were compared between the patients who survived at 28 day of admission and the patients who died within 28 days.

Results: Non-survivors had lower RBC, leucocyte, lymphocyte, and platelet count on day 1 of admission and the difference was statistically significant ($p<0.05$). Thrombocytopenia was more pronounced in non-survivors as compared to other parameters. MCV was significantly greater in non-survivors. There was significant fall in neutrophil count on day 3 of admission as compared to day 1 ($p<0.05$). There was further decrease in platelet count on day 3 of admission.

Conclusion: The finding of our study suggested that thrombocytopenia, leukopenia and anaemia were significantly related to poor prognosis and mortality in sepsis. Haematological parameters should be assessed carefully in the patients of sepsis and the derangement in haematological profile should be considered as alarming sign.

Keywords: Sepsis, Haematological parameters, Mortality, Prognosis, Thrombocytopenia.

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INTRODUCTION

Sepsis is characterised as a potentially fatal organ malfunction brought on by an unbalanced host response to infection.[1] Sepsis diagnosis and prognostication are crucial since mortality increases by about 20% for every new organ failure.[2]

A two-point or more increase in the Sequential Organ Failure Assessment (SOFA) score is a sign of organ dysfunction in sepsis.1 Platelet count is utilised in the SOFA rating for the haematological system, and thrombocytopenia indicates a worse prognosis. Total leukocyte count is a component in the Simplified Acute Physiology II score, another predictive ICU scoring system.[3]

The haematological system is implicated early in pathogenesis of sepsis and is thought to be crucial to the illness' progression and recovery.[4] Anaemia, thrombocytopenia, and leucocytosis are the most frequently detected anomalies in the cellular component of the haematological system (however erythrocytosis, thrombocytopenia, and leukopenia may also be noted), but dysregulated haemostasis is frequently recognised in the fluid phase components.[5]

It is interesting to note that the protracted immunosuppressive condition of sepsis frequently replaces the initial proinflammatory state of sepsis. Apoptosis and a diminished sensitivity to inflammatory cytokines result in a decrease in the number of T lymphocytes (helper and cytotoxic).[6] Studies conducted in ICU patients who died from sepsis showed that lymphoid tissues like the spleen had the greatest overall loss of CD4+ and CD8+ T lymphocytes. Additionally, studies have shown

that in response to endotoxin, the production of vital cytokines including IL-6 and TNF decreases.[7, 8] Neutrophils in septic patients were shown to express less chemokine receptors, and their chemotaxis in response to IL-8 was reduced. [9]

The soluble blood components, including the coagulation proteins, antithrombins, and fibrinolytic system, have been the subject of much investigation to determine the aetiology and clinical progression of coagulopathy in sepsis. [10, 11] However, investigations on the cellular elements of the haematological system and their function as biomarkers for early identification and prognosis of sepsis have only lately been carried out. In their prospective study, Kim et al. discovered a strong correlation between the red cell distribution width (RDW) at 72 hours and the RDW at admission for predicting 28-90-day mortality in sepsis. [12] Immature platelet fraction (IPF) was discovered by Hubert et al. to have the highest diagnostic accuracy and to substantially correlate with sepsis severity score. [13] Seok et al. noted that death in sepsis may be predicted by the immature granulocyte fraction. [14]

The literature that is now accessible highlights the significance of undertaking additional study into the cellular component of the haematological system in order to pinpoint the precise function of specific indicators and their predictive value as sepsis markers. In order to determine whether the parameters derived from cellular components of the haematological system measured at admission to the ICU and at 3 days after admission can be helpful prognostic markers in critically ill septic patients, we conducted this prospective observational study.

MATERIALS AND METHODS

This was an observational and prospective study conducted in Department of Pathology and emergency ward of a tertiary care centre in south-west Bihar of India from July 2022 to December 2022. The study was done under the ethical guidelines of good clinical practice and declaration of Helsinki. Participant information sheet was provided to all study participants and then written informed consent was taken from them.

Sample Size

A total of 97 patients would be needed with an alpha error of 0.05 and power of 85% under the assumption that the incidence of mortality in sepsis is 50% and that early prediction is expected to reduce mortality by 15%. 11 An estimated 117 patients were needed if a 20% dropout rate with assumption of 20% drop-out rate.

Inclusion Criteria

Within 24 hours of admission into ICU, adult patients of age between 18 to 65 years of all genders who were critically ill with a diagnosis of sepsis were included in the study.

Exclusion Criteria

Patients who had a pre-existing haematological disorder or cancer, a history of sepsis or an ICU stay within the previous six months, any chronic disease that could affect the haematological system, or a history of blood transfusions within the previous month prior to admission or within the first three days of admission were all excluded from the study.

Based on the sepsis-3 definition, sepsis was identified at the time of admission.1 Demographic characteristics, associated disorders, SOFA and APACHE II scores were recorded at the time of admission. Additional parameters such as blood pressure, need of vasopressors, serum lactate level, base deficit, urine output, coagulation profile, serum electrolyte level, renal function test, liver function test, random blood sugar, need for mechanical ventilation, sign or symptom of ventilator associated pneumonia, need for nutrition support, and SpO_2 were observed and recorded daily starting from day 1 of admission to day-28 of admission or death or discharge.

On the day of admission and on day 3, the following parameters were recorded—haemoglobin, haematocrit (PCV), red blood cell count, mean cell volume (MCV), mean cell haemoglobin (MCH), red blood cell distribution width, total leukocyte count (TLC), differential leukocyte count (DLC), platelet count and platelet distribution width.

The primary outcome measure was mortality at 28 days, and secondary outcome measures included ICU stay time, days requiring mechanical ventilation, days requiring vasopressor support, and need for renal replacement therapy (RRT).

Statistical Analysis

All recorded variables were presented in tabular form using Microsoft Excel 365. Continuous variables like age, BMI, length of ICU stay, days on mechanical ventilation, number of days on vasopressor support, and haematological parameters were expressed as mean ± standard deviation (SD). Unpaired t-test was used to determine statistical significance of difference in continuous variables between survivors and non-survivors at day 28. Fisher's exact test was used to determine statistical significance of difference in categorical variables like sex and requirement of renal replacement therapy (RRT). P-value of less than 0.05 was taken as measure of statistical significance.

OBSERVATION AND RESULTS

A total of 117 patients were recruited in our study. At day-28, only *55 (47.01%) patients were survivors and 62 patients (52.99%) were non-survivors.*

Table 1: Comparison of baseline demographic and clinical characteristics between *survivors and non-survivors*

Variables	Survivors (n = 55)	Non-Survivors (n = 62)	P-Value
Age in years (mean ± SD)	51.38 ± 15.23	49.47 ± 15.91	0.51*
Sex			
Male	26	34	0.46**
Female	29	28	
BMI in kg/m^2 (mean ± SD)	23.18 ± 2.23	23.35 ± 2.41	0.69*
SOFA at the time of admission (mean ± SD)	6.11 ± 0.52	7.52 ± 0.63	**<0.0001***
*Unpaired t-test **Fisher's exact test			

The survivors and non-survivors didn't differ significantly with respect to age, sex and BMI (p>0.05). SOFA was greater in non-survivors with the difference being extremely statistically significant (p<0.0001).

Table 2: Comparison of outcome measures between *survivors and non-survivors*

Variables in mean ± SD	Survivors (n = 55)	Non-Survivors (n = 62)	P- Value (Unpaired t-test)
Length of ICU stay in days	6.23 ± 2.13	8.49 ± 1.97	<0.0001*
Days on mechanical ventilation	6.14 ± 2.24	8.17 ± 2.38	<0.0001*
Days on vasopressors	4.86 ± 1.54	7.21 ± 1.88	<0.0001*
Requirement of RRT			
Yes	*9*	*26*	*0.0043**
No	*46*	*36*	
**Unpaired t-test* ***Fisher's exact test*			

Non survivors had more days of ICU stays, more days on mechanical ventilation and more requirement of vasopressors and this difference was extremely statistically significant (p<0.0001). Requirement of renal replacement therapy was also significantly greater in non-survivors (p<0.05).

Table 3: Comparison of haematological parameters on day 1 of admission between *survivors and non-survivors*

Variables in mean ± SD	Survivors (n = 55)	Non-Survivors (n = 62)	P- Value (Unpaired t-test)
Haemoglobin (g/dl)	9.97 ± 1.98	9.79 ± 1.86	0.61
RBC count (10^6/ mm^3)	3.54 ± 0.27	3.21 ± 0.41	**<0.0001**
MCH (pg)	27.13 ± 3.08	27.96 ± 3.75	0.20
MCV (fl)	90.17 ± 8.21	94.34 ± 7.53	**0.005**
Haematocrit (%)	31.02 ± 7.14	31.95 ± 6.93	0.48
Total leucocyte count (10^3/ mm^3)	13.24 ± 2.18	12.05 ± 3.23	**0.02**
Neutrophil count (10^3/ mm^3)	9.75 ± 1.69	10.08 ± 1.76	0.30
Lymphocyte count (10^3/ mm^3)	1.43 ± 0.23	1.14 ± 0.13	**<0.0001**
Platelet (10^3/ mm^3)	161.73 ± 29.84	139.45 ± 24.19	**<0.0001**
Red cell distribution width (%)	16.38 ± 2.72	17.05 ± 2.88	0.20
Platelet distribution width (%)	16.47 ± 2.61	16.35 ± 2.57	0.80

Non-survivors had lower RBC, leucocyte, lymphocyte and platelet count on day 1 of admission and the difference was statistically significant (p<0.05). Thrombocytopenia was more pronounced in non-survivors as compared to other parameters. MCV was significantly greater in non-survivors.

Table 4: Comparison of haematological parameters on day 3 of admission between *survivors and non-survivors*

Variables in mean ± SD	Survivors (n = 55)	Non-Survivors (n = 62)	P- Value (Unpaired t-test)
Haemoglobin (g/dl)	9.49 ± 1.97	9.28 ± 1.88	0.56
RBC count (10^6/ mm^3)	3.39 ± 0.25	3.10 ± 0.37	**<0.0001**
MCH (pg)	26.62 ± 3.12	26.94 ± 3.14	0.58
MCV (fl)	92.25 ± 29.22	89.75 ± 28.25	0.63
Haematocrit (%)	28.97 ± 7.31	29.04 ± 7.11	0.96
Total leucocyte count (10^3/ mm^3)	12.53 ± 2.13	10.17 ± 3.09	**<0.0001**
Neutrophil count (10^3/ mm^3)	9.87 ± 1.73	8.78 ± 1.47	**0.0004**
Lymphocyte count (10^3/ mm^3)	1.47 ± 0.22	1.08 ± 0.11	**<0.0001**
Platelet (10^3/ mm^3)	150.18 ± 26.73	119.57 ± 18.11	**<0.0001**
Red cell distribution width (%)	16.49 ± 2.84	17.31 ± 2.92	0.13
Platelet distribution width (%)	16.09 ± 2.48	16.37 ± 2.61	0.55

There was significant fall in neutrophil count on day 3 of admission as compared to day 1 ($p<0.05$). There was further decrease in platelet count on day 3 of admission.

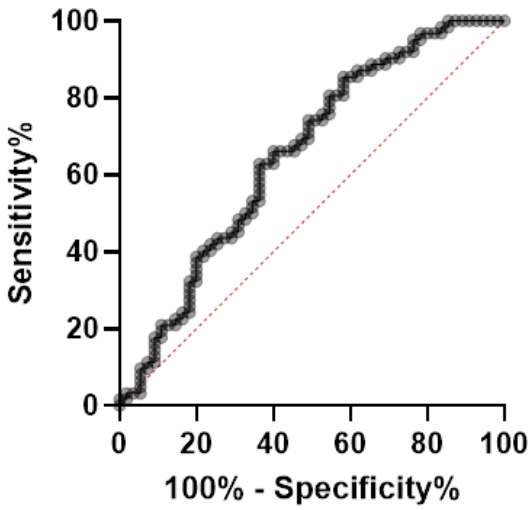

Figure 1: ROC curve of Platelet count as a prognostic marker in sepsis

(Area under ROC curve 0.6528, 95% CI: 0.5523-0.7533, P-value: 0.0044)

DISCUSSION

In this prospective observational study, we identified lower RBC, leucocyte, lymphocyte and platelet count as the most important prognostic factors for 28-day mortality in sepsis patients.

In the current study, higher MCV value was significantly related to the poor prognosis of sepsis. Muady et al.15 discovered that the initial haemoglobin upon presentation linked with in-hospital mortality in 815 patients with sepsis. However, the mortality at 28 days did not link with the haemoglobin level at presentation in our study. However, the length of mechanical ventilation, ICU hospitalization, and days requiring vasopressors were all substantially linked with low haemoglobin on day 1. Khamiees et al.16 found that individuals with anaemia were five times more likely to require reintubation following an initial successful extubation in patients with acute respiratory failure. In sepsis, anaemia can be caused by a number of factors, including inflammation, reduced production of erythropoietin, a reduced bone marrow response to erythropoietin, a reduced red cell survival rate, continual blood sampling, blood loss from surgery or trauma, newly diagnosed liver and kidney disease, disseminated intravascular coagulation (DIC), haemolysis, hypoadrenalism, haemodilution, and nutritional deficiencies.

In the present study, non-survivors had considerably greater MCV than survivors, and increased MCV was linked to longer stays in the ICU, the need for RRT, and the number of days on vasopressors. There was no evidence of such a mortality link. In Contrast, Meynaar et al. discovered that among 2,915 critically sick patients, MCV and MCH had no significant link with mortality, an extended ICU stay, or an extended period of mechanical ventilation. Elevated MCV may be a sign of elevated reticulocytotic following bleeding or haemolysis, vitamin B12 or folate insufficiency, or liver illness. Aged RBCs in normal persons shrink as a result of being exposed to oxygen radicals and shear stress.5, 17

High average RBC volume is likely caused by oxidative stress-related RBC membrane alterations that cause reduction in deformability and early RBC mortality in sepsis. However, more investigation is likely required to support this theory. 18 Additionally, survivors had greater red cell counts on days 1 and 3, and a reduced red cell count strongly predicted a longer stay in the intensive care unit

and more days requiring vasopressors. Studies on haemoglobin concentration predict that sepsis patients with low red cell counts will have poor prognoses. 4, 5

According to the SOFA score, thrombocytopenia can occur in 35 to 59% of sepsis patients and indicates a decline in the total haematological system's performance.4,5 In the early stages of sepsis, platelet production rises to keep up with the damage, releasing younger, larger platelets into the circulation. Later, however, bone marrow suppression sets in, resulting in thrombocytopenia. 19 The rise in IPF and large platelets in peripheral smears are indicators of this. In the current research, thrombocytopenia at admission predicted 28-day death, and non-survivors had decreased platelet counts on day 3 and at admission. In a similar way, platelet count at day 1 anticipated 28-day death in a prospective, multicenter trial of 1,486 patients with septic shock. 19 The probability of mortality progressively rose with the extent of thrombocytopenia.

In the early stages of sepsis, platelets may transform from their discoid shape to a sphere and create pseudopodia, which results in a greater surface area and a rise in PDW. 20 Elevated IPF and platelet deterioration also contribute to elevated PDW. In a retrospective cohort analysis, Guclu et al. discovered that PDW was higher in sepsis patients than in controls and higher in non-survivors than in survivors, and they came to the conclusion that PDW more than18% predicted mortality. 20

PDW on days 1 and 3 in the present study were within normal limits in either survivors or non-survivors and did not indicate mortality. The varying levels of thrombocytopenia, the variations in SOFA scores, or the types of patients could all be contributing factors.

Leukopenia, a bad prognostic indication, can develop from sepsis alone, which can depress the bone marrow. 21 In research by Bermejo-Martin et al., 195 individuals with sepsis had their levels of circulating neutrophil count (CNC) examined. 22 They discovered that the probability of death was about two times higher in patients with CNC less than 7,226 cells/dL. Reduced levels of circulating neutrophils in sepsis may be caused by greater adhesion to vascular endothelium. Neutrophils can be both damaging (by generating mediators causing organ damage) and beneficial (by defending against infections) in sepsis. In this study, there was no difference between survivors and non-survivors based on total leukocyte count or neutrophil count. The overall quantity of leukocytes and neutrophils did not seem to predict any of the negative outcomes.

In the present study, non-survivors had a considerably greater SOFA score (median score of 8) on first day of admission. Ferreira et al. reported that the early or highest score of greater than 11 or the mean score of greater than five was associated to death in more than 80% of patients in a prospective, observational study involving 352 critically sick patients. 23 While a rise in SOFA score throughout the first two days of an ICU stay indicated a mortality rate of at least fifty percent, both the mean and maximum SOFA scores also predicted negative outcome.

Strengths and Limitations

The current study thoroughly evaluated every cellular component of the haematological system and included a substantial number of patients with nearly minimal data loss during follow-up.

Our study has some restrictions. The study population was diverse, including a combination of medical and surgical cases from one centre. The first blood sample was taken at any time within the first 24 hours and the timing could not controlled. Prior to sampling, some individuals might have received antimicrobial agents and fluid resuscitation. It is yet unclear, though, how much of an impact such intervention will have on haematological variables.

CONCLUSION

The finding of our study suggested that thrombocytopenia, leukopenia and anaemia were significantly related to poor prognosis and mortality in sepsis. Haematological parameters should be assessed carefully in the patients of sepsis and the derangement in haematological profile should be considered as alarming sign. There is need of further research to refine and strengthen the evidences and to evaluate the effect of antibiotics and fluid resuscitation on these haematological parameters.

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