International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 13, No. 9, September 2024 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_13.9.2024.67

ORIGINAL RESEARCH

A study on hematological parameters predictive of mortality in Covid-19

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Received Date: 26 July, 2024 Acceptance Date: 29 August, 2024

ABSTRACT

Introduction: Among the many challenges faced by Covid-19, identification of a comprehensive, economical and early biomarkers of critical illness gain much attention. Such a parameter impacts best use of man and material. This study evaluates the predictive potential of haematological indices for outcome of hospitalization. **Material and Methods**: Laboratory data of tests done at admission of 308 Covid-19patients was analyzed retrospectively. The patients were divided in two groups, survivors (n=270) and non-survivors (n=38). Routine haematological parameters such as neutrophil, lymphocyte, platelet counts and calculated composite indices such as Systemic immune inflammation index (SII); Prognostic nutritional index (PNI); Neutrophil:Lymphocyte ratio (NLR); Platelet:Lymphocyte ratio etc were correlated with outcome. **Results**: We found statistically higher mean for total leucocyte count, neutrophil count, SII, NLR among deceased than discharged patients. Mean for lymphocyte count, serum albumin and PNI were statistically lower among non-survivors. **Conclusion**: This information on circulatory bio-markers serves a marker for early identification of high-risk patient requiring intensive care.

Keywords: Covid-19, Prognostic markers, Hematology, Mortality.

INTRODUCTION

As Covid-19 is no longer a global health emergency, the strategies for testing, treatment, and control are changing. WHO has noticed trends of increased cases during holidays and gatherings, followed by the emergence of new variants. WHO has now called on all governments to maintain surveillance and sequencing. The increase in ICU admissions and thousands of deaths noticed in recent months in Europe and the Americas is putatively preventable.[1] The availability of updated bedside tools at admission to identify patients who are at risk of poor outcomes shall aid in providing timely care and effective utilization of hospital resources in resource-intense settings. This pressing need for risk stratification has necessitated identifying economical and comprehensive bio-markers of value in prognostic prediction.[2,3,4] In this regard, we conducted a retrospective analysis of haematological parameters and composite indices in hospitalized patients with COVID-19. Our study aimed to investigate whether haematological indices measured and calculated at the earliest hours of admission, would facilitate predicting the outcome of hospitalization.

MATERIALS & METHODS

The present study is a hospital-based retrospective observational cohort study conducted at a coastal tertiary care centre. All consecutive admitted cases with positive result RT-PCR for Covid-19 on a nasopharyngeal swab were included in this study. At admission, patients were assessed for severity and admitted either in ward or ICU. Patients with one of the following features: respiratory distress (respiratory rate > 30 / min, oxygen saturation (O^2Sat) ≤ 80 %t at rest); respiratory failure receiving mechanical ventilation, shock, and/or organ failure other than lung necessitating intensive care were identified as critically ill and admitted in ICU. After inclusion, we assimilated following data from medical records: demographic details [age, sex, admission date,], clinical characteristics [O^2 at the time of admission, co-morbidities, duration of hospital stay, events during hospitalisation, outcome], laboratory data after admission [routine blood counts, serum albumin]. Patients were excluded for incomplete data. The primary outcome for all patients admitted was discharge, referred or in-hospital death. Charlson

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Comorbidity Index (CCI) score was used to evaluate the presence of comorbidity. Each comorbid condition present was assigned a weight of 1 to 6 points. The CCI score was categorized as none (CCI=0); low (CCI=1) or high (CCI more than 2). Serum albumin was classified into hypoalbuminemia (albumin< 3.5 g/dl) and normal albumin (albumin >3.5 g/dl).[5]

The following composite indices were calculated:

1. Neutrophil:Lymphocyte ratio (NLR) was calculated by dividing absolute neutrophil count by absolute lymphocyte count.[6,7]
2. Platelet:Lymphocyte ratio (PLR) as platelet count/absolute lymphocyte counts and Platelet:Neutrophil ratio (PNR) as platelet count/absolute neutrophil counts.[6]
3. Systemic immune inflammation index (SII) was calculated using the formula: SII= Platelet (x 10^9/L)X Neutrophil (x10^9/L)/Lymphocyte count(x10^9/L). [5, 8, 9]
4. Prognostic nutritional index (PNI) =10×albumin (g/dL) + 5 ×lymphocyte count (10^9/L).[9,10,11]

Requirement of written consent was waived in view of the retrospective design of this study. The study was approved by the Institutional Ethics Committee.

SPSS software was used for data analysis. Continuous data was expressed as mean, SD and examined by Student-t test. The p value of <0.05 was considered significant. Subgroup analyses was according to admission (ward or ICU); CCI SCORE (0,1,>2); outcome of hospitalization. Categorical data was summarized as counts and percentage, compared using chi-square test.

RESULTS

A total of 350 consecutive Covid-19 patients were admitted at our institute from April 2020 to December 2021 were surveyed. After excluding those with incomplete data (n=38), those referred to higher centre (n = 4), the rest 308 patients were included for analysis. While 166 (54%) among them were categorized as non-critical and admitted in general ward, 142 (46%) critical patients required ICU care. Thirty eight patients died during the hospital stay. A comparison of hematological indices measured at the time of admission between non-survivors and survivors patients was done (Table 1). The mean age for general ward patients was 47 years (4-85 years), 126 of whom were males. Mean age of ICU patients was 56 years (17-92 years), 101 of whom were males. The mean for total leucocyte counts, absolute neutrophil count, SII, NLR and PLR was higher in non-survivors patients as compared to discharged patients, while Serum albumin, PNI and PNR was decreased in demised patients. The magnitude of anemia, neutrophilia, leucocytosis, leucopenia, lymphocytopenia and thrombocytopenia among survivors were 30%, 25%, 23%, 06%, 15% and 13% respectively (Table 2). The most common haematological abnormality among non-survivors patients was neutrophilia (58%). The magnitude of lymphocytopenia and thrombocytopenia among non-survivors patients was 31% and 21% respectively. Serum albumin measures were available in 169 patients only. Hypoalbuminemia was recorded in 50% of survivors and 89% of non-survivors patients at admission. A chi-square test of independence showed that there was significant association between CCI score and outcome, p <0.00001 (Table 3). While 20% of patients with CCI score >2 demised, a lone case with zero CCI score had fatal outcome.

Table 1: Comparison of haematological parameters and indices among survivors and non-survivors with Covid-19

Characteristic	Discharge (n = 270)	Death (n = 38)	P value
Ward admission, n (%)	162 (97%)	4 (3%)	NA
ICU admission, n (%)	108 (76%)	34 (24%)	NA
Haemoglobin, g/dl, mean (SD)	13.1 (2.17)	13.2 (2.27)	.424871
Leucocytes, 106/L, mean (SD)	8027 (3658)	11013 (5619)	< .00001
Neutrophils, 106/L, mean (SD)	5762.9 (3532)	9297.95 (4907)	< .00001
Lymphocytes, 106/L, mean (SD)	1562.8 (822.9)	1132.5 (1289)	.00286
Platelets, 109/L, mean (SD)	238 (90)	218 (103)	.099573
Serum albumin, g/L, mean (SD)	3.45 (0.52)	2.95 (0.49)	.000052
SII, mean (SD)	1244.76 (1387.46)	2512.55 (1880.47)	< .00001
NLR, mean (SD)	5.57 (6.74)	11.33 (6.52)	< .00001
PLR, mean (SD)	0.2 (0.16)	0.28 (0.16)	.001725
PNR, mean (SD)	0.06 (0.04)	0.03 (0.02)	< .00001
PNI, mean (SD)	102.09 (47.51)	67.66 (29.66)	.000333

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Table 2: Proportion of haematological abnormalities among survivors and non-survivors with Covid-19.

	WARD ADMISSIONS		ICU ADMISSIONS	
OUTCOME	**DISCHARGE**	**DEATH**	**DISCHARGE**	**DEATH**
Characteristics				
Anemia	48/162	1/4	36/108	11/34
Leucocytosis (>9800)	32/162	2/4	31/108	17/34
Leucopenia	12/162	none	4/108	1/34
Neutrophilia (>7300)	30/162	02/4	37/108	20/34
Lymphocytopenia (<700)	23/162	none	19/108	12/34
Thrombocytopenia	19/162	2/4	16/108	6/34
Thrombocytosis	8/162	none	2/108	2/34
Hypoalbuminemia (<3.5)	24/67	03/03	51/83	14/16

Table 3: Correlation of CCI scores with outcome of hospitalisation.

	DEATH	DISCHARGE	TOTAL
CCI = 0	01	101	102
CCI = 1	05	41	46
CCI >2	32	128	160
TOTAL	38	270	308

DISCUSSION

Covid-19 exhibits clinical heterogeneity with symptoms varying from non-specific symptoms to severe respiratory distress. [2,3,11,12,13,14] Although pneumonia and ARDS are typical respiratory manifestations, Covid-19 is now recognized as multi-system disease.[15] Around 15% - 32% of patients require critical care at ICU, which has placed a huge burden on existing healthcare facilities. [15, 16] Early risk stratification aids selection of appropriate therapies and timely treatment.[15] As pathogenesis of Covid-19 involves complex interplay of immuno-inflammatory dysregulation and derangements of coagulation cascades, laboratory bio markers can provide additional information to identify patients at risk of becoming critically ill. [11,13,15]

A complete hemogram is a universally available, simple, rapid and inexpensive laboratory diagnostic test performed on all admitted patients.[2,3,6] Blood tests in such patients are likely to aid in stratifying.[3] The circulating parameters discussed herein can be easily calculated from cell count results. We have evaluated the predictive value of such parameters in Covid-19.

Non-survivors patients had higher absolute neutrophil counts (M = 9297, SD = 4907) than survivors patients, $p < .00001$ in the present study. Higher absolute neutrophil count is commonly observed in severe Covid-19 requiring ICU admission.[15] Further, an increased number of immature neutrophils with progenitor like phenotype and neutrophil extracellular traps (NETs) observed in Covid-19 correlate with viral load and severity of disease.[13, 17, 18, 19] As neutrophils secrete pro-inflammatory mediators, cytokine storm characterized by overproduction of such mediators is associated with critical illness and adverse outcomes.[4]

Lymphocytopenia is the most common manifestation of viral infection.[2,11] The mechanisms attributed to causing lymphocytopenia include reduced lymphocyte proliferation induced by lactic acidosis, T cell response to disseminated viremia and high viral loads, cytokine storm induced chemo-attractants for lymphocytes, lymphocyte apoptosis induced by interleukins and lysis of lymphocytes carrying ACE2 surface receptors.[12,13,15] Low absolute lymphocyte counts < 0.8 x 109/l on admission were significantly associated with higher mortality as shown in meta analysis done by Kiss et al.[4,5] Lymphopenia is not specific for COVID-19and is a common finding among the elderly. Hence, it is combined with other parameters such as neutrophil count to generate Neutrophil Lymphocyte Ratio.[2]

Higher NLR is demonstrated to be an independent risk factor of mortality in diseases such as infections, myocardial infarction, intracerebral hemorrhage, polymyositis and solid tumors.[6,5,20,21, 22, 23] We evaluated its early predictive value in Covid-19. The 38 non-survivors patients (Mean = 11.3, SD = 6.52) when compared to survivors patients had significantly higher NLR values, $p < .00001$.

There was no significant difference of platelet counts between non-survivors and survivors patients, despite non-survivors patients demonstrating lower platelet counts (Mean = 218, SD = 103). Inflammation and platelet consumption are suggested to cause thrombocytopenia.[15] Inflammatory response in viral infection involves uncontrolled platelet activation, which is postulated to cause marked thrombocytopenia.[6,15] Virus induced endothelial damage and inflammation sets in hypercoagulability which results in thrombocytopenia.[13, 18, 19] Thrombocytopenia is shown to correlate with increased risk of mortality and severe outcomes.[15,13,18,19]

Systemic Immune Inflammation Index (SII) is a composite indicator integrating neutrophil, lymphocyte and platelet counts.[5] SII has been shown

DOI: 10.69605/ijlbpr_13.9.2024.67

to be a prognostic predictor in varied diseases such as coronary artery diseases, stroke, malignancies and fractures.[5, 8] SII is an objective marker of systemic inflammation, and easy to calculate from hemogram results.[5] Systemic inflammation is related to poor survival in hospitalized elderly with cardio-vascular disease. Subgroup analysis revealed that SII remained an independent risk factor for severe disease our observation.

Elevated Platelet:Lymphocyte ratio (PLR) is shown to be a predictor of mortality among the elderly with hip fractures.[5] Survivors patients reported lower values of PLR than non-survivors patients, $p < .00001$.

The pathogenesis of COVID-19 involves immunothrombosis which is characterised by a vicious cycle of NETs from neutrophil recruiting platelets, followed by activated platelets interacting with neutrophils to stimulate formation of NETs containing tissue factor.[18] A lower mean PNR was observed among non-survivors in the present study.

Turcato et al. in a retrospective observational study showed that serum albumin of <3.5 g/dl measured on admission among SARS COV2 patients at the emergency department is an independent risk factor for severe disease and mortality.[24] Hypoalbuminemia is a marker of functional status connecting inflammation, nutrition and general status.[25, 26] Hypoalbuminemia is multifactorial and is attributed to increased capillary permeability, oxygenation deficit, decreased protein synthesis, increased turnover and increased volume of distribution.[11, 15, 13, 24, 27, 28, 29] Serum albumin inhibits NETosis. As NET is an important mediator of tissue damage in Covid-19, correlation of hypoalbuminemia with higher risk of ARDS or death is evident.[27] While other inflammatory markers such as CRP and Leucocytosis may not be altered in the first few hours of the infectious process, microcirculatory dysfunction induced hypoalbuminemia may be present on arrival at an emergency. Therefore, it is postulated that altered albumin levels at admission correlate better with poor outcome.[24, 27] The independent t test revealed significantly lower mean values of Serum albumin among non-survivors patients in the present study. This demonstrates the importance of this immune-nutritional index in predicting Covid-19 severity.

Prognostic Nutritional Index (PNI) was first employed by Buzby and colleagues to estimate operative risk in gastrointestinal surgery. PNI is a surrogate marker of immune and nutritional status, both of which are associated with susceptibility and severity of infections.[11] PNI is demonstrated as an independent marker for poor prognosis in cancer patients.[10] Albumin is related to nutritional status and inflammation. Lymphocytes are regulators of immune responses in viral infections.[11] Evidently, both hypoalbuminemia and lymphocytopenia are seen in severe Covid-19 when compared with non-severe disease.[2,11,19,30] Our study also highlighted association of lower PNI with poor outcome.

There was no statistically significant difference for Hemoglobin and Platelets counts between survivors and non-survivors patients. A single indicator may be affected by many confounding factors. Thus, composite markers are more reliable biomarkers of poor prognosis.[10]

Our study had a few limitations. As this is a single centre study, the sample size is smaller; studies with larger sample sizes are required to confirm the findings. Due to retrospective design of this study, data regarding some variables such as body mass index, time since first symptom to hospital admission, other laboratory data and confounders were not obtained.

CONCLUSION

The ability to predict the outcome would facilitate decision-making, scenario planning and resource allocation.[3,4,29] Our study has identified SII, PNI, CCI as potential predictors of the poor outcome in Covid-19 admissions. We found NLR to have a high sensitivity in predicting mortality among admitted people. These simple, feasible, easily assessable, repeatable, economical and easily calculable composite haematological indexes have promising value in the management of Covid-19 infection. Upon incorporating within routine clinical practice to predict poor outcome, this can aid early identification of high-risk patients requiring intensive care and consequently appropriate resource allocation.

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