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Original Article

Association of Neutrophilia with Disease Severity in Patients with COVID-19

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ABSTRACT

COVID-19 has become a global pandemic with limited data on prediction of disease severity and management of critically-ill patients. **Objective:** To assess associations between routine Haematologica parameters especially neutrophil counts and severity in COVID-19 patients. **Methods:** The study was a cross-sectional study involving 133 non-severe and 120 severe category patients. This study was conducted at Chughtai Institute of Pathology from 1st June till 31st August, 2020. The association of severity with parameters was determined using Chi-square and Fisher´s Exact test. **Results:** Absolute Neutrophil Count (ANC) and NLR were significantly higher in Severe Group category. Neutrophilia and raised NLR were observed in 81.7% and 93% of the severe group respectively. Lymphopenia was observed in only 36.7% of Severe Group. Comorbidities such as, hypertension (82,1%), diabetes (85.5%), IHD (100%) and COPD (83.9%) had significantly high frequency of increased NLR. Also, clinical symptoms like fever (77.9%), cough (80.9%), shortness of breath (94.3%) and abdominal symptoms (88.2%) also had same significant association. **Conclusions:** It was observed that high NLR ≥ 3 was associated with severe disease along with high ANC. However, lymphopenia as expected, was not observed in significant population. Instead, neutrophilia was a more consistent finding in the concerned group.

INTRODUCTION

Corona virus is a family of viruses whose members are known to cause diseases ranging from flu-like-illnesses to severe respiratory syndromes. Two notable viruses from this family, severe acute respiratory syndrome (SARS) and Middle East Respiratory Syndrome (MERS) have caused epidemics in the recent past [1, 2]. On 17th December, 2019 a new member of this family, SARS-CoV-2 emerged in Wuhan, China causing an unfamiliar pneumonia like illness called as Corona Virus Disease (COVID-19) [3]. It rapidly became an epidemic in Wuhan spreading via direct personal contact and droplet infection [4, 5]. On 11th March 2020 World Health Organization (WHO) declared SARS-CoV-2 as a pandemic and international emergency. As of October 13th, 2020, 38, 049, 049 cases worldwide have been reported with 4% of mortality [6]. It has been observed that SARS-CoV-2, attaches to the apical pulmonary epithelial membrane via ACE2 receptors and gain entry in to the alveolar membrane and cause its destruction. These pulmonary epithelial cells were shown to produce IL-6 and IL-8. IL-8 is known to act as a chemo-attractant for neutrophils and T lymphocytes. Infiltration of lungs by these cells induced lung injury, immune response, cardiac and intestinal changes [7]. In minor fraction of patients infected with Covid-19 massive inflammatory response occurs occasionally called as "Cytokine storm" contributing to weak immune response and imbalance [8]. This massive cytokine release leads to neutrophil recruitment causing neutrophilia and also causes peripheral lymphocyte counts to fall [9]. Clinically COVID-19 manifests as fever, cough, sore throat, nausea, vomiting and diarrhoea [10]. Severe

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cases lead rapidly to acute respiratory distress syndrome, coagulopathy, metabolic acidosis and shock. Patients with mild to moderate disease can be managed by isolating them and giving symptomatic treatment. Those with critical illness require Intensive Care Unit (ICU) management and invasive therapies. Early severity identification in this disease would help in saving medical resources as well as reduce death rate [11]. Preliminary studies on COVID-19 haematological parameters have shown that there is increased Total Leukocyte Count (TLC), Neutrophil Count and Neutrophil-to-Lymphocyte Ratio (NLR) in symptomatic patients [12]. Among these NLR has gained importance as early as well as independent identification and prognostic marker for severity in COVID-19 patients [8, 11]. Lymphopenia has also been associated with poor outcome in this disease [13]. But other Complete Blood Count (CBC) parameters are not being used for severity identification. Thus, we wondered are these CBC parameters are associated with the severity of this disease. Was high NLR caused by absolute lymphopenia or due to neutrophilia in the peripheral blood? And last of all are comorbidities and clinical symptoms associated with high NLR? As CBC is a reliable and inexpensive tool to predict prognosis as compared other routinely used tests such as CRP, LDH, D-dimers etc. These questions are still unanswered so this study was undertaken to determine the association between these hematologic parameters and severity of disease. By collecting data from 233 confirmed cases, we attempted to determine the association between various haematological parameters and disease severity by using Pearson Chi-Square and Fisher´s Exact test.

METHODS

A cross-sectional study was conducted by consecutive sampling of patients admitted in Mayo Hospital COVID-19 ward from 1st May, 2020 till 30th June, 2020. All the patients testing positive by Reverse Transcriptase-Polymerase Chain Reaction assay (RT-PCR) were included in the study [14]. The sample size was calculated using OpenEpi, Version 3, open-source calculator taking COVID 19 prevalence as reference parameter. Complete epidemiological and clinical data of 113 non-severe and 120 severe cases was obtained from Mayo Hospital COVID-19 ward. These cases were categorized into "Non-severe Group" and "Severe Group" (including critically-ill) according to WHO interim guidelines and international pulmonologist´s consensus on COVID-19 [5, 15]. Non-severe Group patients (including asymptomatic patients) met the following conditions: (1) Travel or contact history, (2) Fever or other symptoms suggestive of this infection, and (3) Typical X-Ray findings. Severe Group patients (including critically-ill) additionally met at least one of the following conditions: (1) Respiratory rate ≥ 30 times /min, Resting oxygen saturation < 93%, (3) PaO2/FiO2 < 300mmHg, (4) Shock and (5) multi-organ dysfunction. Ethical approval was taken by the review board of Mayo Hospital and Chughtai Institute of Pathology. The laboratory assessments of the samples (on admission CBC) were done at Chughtai Institute of Pathology, Lahore. 2 mL blood samples were taken in EDTA vials and were brought to Chughtai Institute of Pathology from Mayo Hospital Lahore stored at 4°C. They were instantly run on Sysmex-XN9000 Automated haematology analyser (after proper quality control and calibration). The laboratory reference values for TLC, ANC and ALC were 4-11, 2-7 and 0.8-4 x 10^3/µL. Neutrophil to lymphocyte ratio of 1-3 was taken as normal and >3 as high. Continuous variables were summarized as the appropriate means and standard deviations. Categorical variables were expressed as mean and standard deviation in each group. Chi-square and Fisher´s exact tests were applied to categorical variables. P < 0.05 was recognized as statistically significant. All these statistical calculations were performed using the SPSS version 24.0.

RESULTS

The mean age of the patients was 46.58 ± 16.30 years. Higher number of 119(51.1%) female and lesser male 114(48.9%). Comorbidities at the time of presentation were noted as 110(47.2%) having and 123(52.8%) were not having any comorbidity. 67(28.8%) were hypertensive and 16(71.2%) cases were normal. Few cases 55(23.6%) were having diabetes and 178(76.4%) were non diabetic. 31(13.3%) were with chronic obstructive pulmonary disease and 202(86.7%) were not. Ischemic heart disease was found in 20(8.6%). On clinical evaluation, fever was noted in 149(63.9%), Cough in 115 (49.4%), shortness of breath in 110 (47.25%) and abdominal symptoms in 7.3% of total study population. These symptoms were more pronounced in the Severe Group. A significant difference was noted for age, gender and other clinical profiles among the patients of Severe Group as compared to Non- Severe Group. as shown in Table 1.

Table 1: Comparison of the Clinico-Demographical Findings in the Population in Both Strata

Clinico-demographics		All Cases of Covid (233)		p-value
		Non-Severe cases n= (113)	Severe Group(n=120)	
Age(years)		36.69±12.27	55.90±14.02	0.000*
Gender	Male	45(39.5%)	69(60.5%)	0.007*
	Female	68(57.1%)	51(42.9%)	
Comorbidities Hypertension	Yes	12(17.9%)	55(82.1%)	0.000*
	No	101(60.8%)	65(39.2%)	
Diabetes Mellitus	Yes	8(14.5%)	47(85.5%)	0.000*
	No	105(59%)	73(41%)	
Chronic Obstructive	Yes	5(16.1%)	26(83.9%)	0.000*

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Pulmonary Disease	No	108(53.5%)	94(46.5%)	
IHD	Yes	0(0%)	20(100%)	0.000*
	No	113(53.1%)	100(46.9%)	
Symptoms Fever	Yes	33(22.1%)	116(77.9%)	0.000*
	No	80(95.2%)	4(4.8%)	
Abdominal symptoms	Yes	2(11.8%)	15(88.2%)	0.002*
	No	111(51.4%)	105(48.6%)	
Shortness of breath	Yes	7(5.7%)	116(94.3%)	0.000*
	No	106(96.4%)	4(3.6%)	
Cough	Yes	22(19.1%)	93(80.9%)	0.000*
	No	91(77.1%)	27(22.9%)	
O2 Required	Yes	4(3.2%)	120(96.8%)	0.000*
	No	109(100%)	0(0%)	
X-Ray infiltrates	Yes	26(17.9%)	119(82.1%)	0.000*
	No	87(98.9%)	1(1.1%)	
Severity of disease	1	78(100%)	0(0%)	0.000*
	2	35(100%)	0(0%)	
	3	0(0%)	80(100%)	
	4	0(0%)	40(100%)	

*p-value is <0.05 and significant

Similarly, it was observed that TLC level and ANC level were raised in the cases with severe disease, with a significant difference (p-value<0.05). ALC and N/L ration was low and high respectively as compared to non-severe cases (p-value<0.05). No significant difference was noted for the platelet count among the both groups of the COVID patients. (P-value>0.05) as shown in Table 2 and Figure 1.

Table 2: Comparison of the Biochemical Profile in the COVID-19, Severe Group versus Non- Severe Group

Blood profile	Group	Mean ± SD	p-value
TLC level	Non-Severe	8.21±2.47	0.000
	Severe	13.05±5.45	
ANC level	Non-Severe	4.72±1.83	0.000
	Severe	11.24±5.32	
ALC level	Non-Severe	2.69±0.85	0.000
	Severe	1.14±0.68	
N/L Ratio	Non-Severe	1.93±1.64	0.000
	Severe	15.49±26.27	
D-NLR level	Non-Severe	1.45±0.95	0.000
	Severe	7.81±5.52	
Hb level	Non-Severe	12.86±1.83	0.004
	Severe	12.03±2.45	
PLT count	Non-Severe	287.75±91.84	0.745
	Severe	283.05±124.67	

*p-value is <0.05 and significant

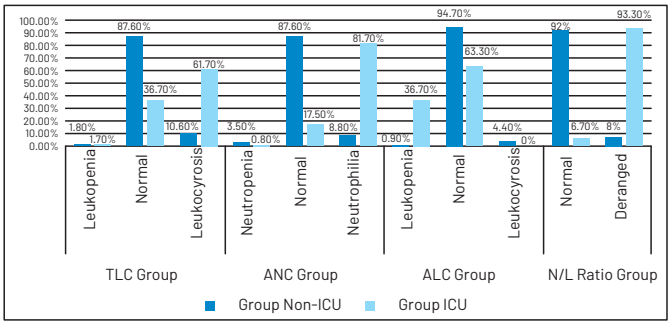

Figure 1: Prevalence of Abnormality in the Hematological Profile among the Severe/ICU versus Non-severe/non-ICU cases

DISCUSSION

COVID-19 infection spreading rapidly via human contact has caused a worldwide pandemic since December 2019. A recent report showed that, it has an incubation period of 3-7 days and around 26% of patients required ICU care with a mortality rate of less than 4% [16]. Mostly patients developed severe disease by 7-10 day. The number of cases worldwide is increasing day by day especially in USA and India as of October 5, 2020. Early detection of critical illness and risk identification will allow save medical resources, especially of intensive care and will further reduce the mortality rate. Recent studies have shown that mostly old age patients and those with risk factors developed severe or critical disease with COVID-19 [17]. This research also showed that mostly patients with severe or critical disease were over 45-50 years of age. Most of them had concomitant risk factors most common of which were hypertension and diabetes. Lymphopenia [13] and thrombocytopenia [18] have been associated with poor outcome in this disease. The exact mechanism of lymphopenia is unclear. However, recent researches predict it may be either due to inflammatory cytokine storm (TNF-α and IL-6) or the virus may directly infect T lymphocytes and their damage directly related to patient's deterioration [19]. In this study only 36.7% of critically ill patients showed lymphopenia. Rest of the cases had normal lymphocyte count. Contrary to this, according another study 85% of severely ill patients have lymphopenia [20]. No significant association was found with decreased platelet counts. NLR was increased in 93% of severe cases as shown in Table 2. It was consistent with latest studies suggesting high NLR predicting severity of disease [8, 11]. The results of neutrophil count, lymphocyte count and NLR suggest that instead of absolute lymphopenia, high NLR occurs due to neutrophilia in the peripheral blood. Absolute neutrophil count was increased above normal in 81.7% of severely ill patients. TLC was raised in 61.7% population of this group. Hence, suggesting high peripheral neutrophil counts are more related to poor prognosis than lymphopenia. Likewise, Chevrier *et al.*,

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found out that patients with severe disease had increased neutrophils which strongly correlated with disease poor outcome [21]. The above-mentioned results can be explained by following reasons: Neutrophils are major component of white cell population that activates and migrates from venous blood to immune systems. These neutrophils by interacting with distinct cell populations release large amounts of reactive oxygen species, numerous pro-inflammatory cytokines, Tumour Necrosis Factor (TNF) angiogenic /fibrogenic factors and formation of Neutrophil Extracellular Traps (NETs) causing extensive tissue damage and thrombotic complications as explained by recent studies [9, 22, 23]. There was also a limitation in this study that lymphocyte counts were only taken at time of admission. The CBC parameters were not followed after admission as patients received dexamethasone injections to relieve their respiratory symptoms. Recent studies suggested that dexamethasone reduced mortality in ICU patients with COVID-19 [24]. Dexamethasone is a corticosteroid, which is postulated to cause lymphopenia in peripheral blood after a few hours of injection as well as peripheral recruitment of neutrophils [25]. The follow up of leukocyte counts after dexamethasone administration would have caused BIAS in the study. Due to this reason our conclusion may differ from conclusion of other studies. In the study we also observed strong association of NLR with comorbidities and clinical symptoms of patients with COVID-19. Majority of the patients having these risk factors and showing symptoms had increased NLR. A range of 1-3 was established as normal. As shown by recent studies NLR > 3 was associated with severe disease and poor outcome [8, 11]. In this research NLR > 3 also showed significantly high association with comorbidities and clinical symptoms such as fever, cough and shortness of breath. These patients eventually required ICU admission and some form of mechanical ventilation. In contrast cases having no prior risk factors and showing either no or only mild symptoms had NLR < 3. These patients were either discharged by day-3 or required only supplemental low flow oxygen in some cases. Thus, explaining that old age, comorbidities, severe symptoms and high NLR are all strongly associated with critical disease and their presence in patients can predict poor outcome of patients infected with COVID-19. However, 7% of study subjects in severe group were young and below the age of 30 years with no concomitant risk factors. These cases also had high NLR and showed poor outcome. Same was observed in a study by Jimeno *et al.*, where NLR was high in non survivors [26].

CONCLUSIONS

Old age, comorbidities and severe symptoms are associated with poor disease outcome. Haematological parameters such as high neutrophil count and NLR are associated with severe disease and critical illness. However, lymphopenia was not present in significant population. Instead, high neutrophil counts showed stronger relation with severe disease and poor prognosis in these patients. NLR and neutrophil count in combination can predict better outcome of these patients. In future studies should be conducted to observe serial lymphocyte counts in patients with COVID-19 with and without the use of dexamethasone or any other corticosteroid. This would help us assess the true levels and trends of lymphocyte and neutrophil counts, effects of dexamethasone on them and their relationship with the outcome of these patients.

Authors Contribution

Conceptualization: NI
Methodology: NI, AMM, AI
Formal Analysis: NAM
Writing-review and editing: AMM, NAM, ASC

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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