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PRESENCE OF HIGH NEUTROPHIL-LYMPHOCYTE RATIO(NLR) IN COVID PATIENTS: -

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Abstract

Objective: The present study was done to evaluate prognostic role of select immunological biomarkers in COVID-19 positive patients for early identification and grading of symptomatic patients who might need critical care support with adequate and optimal triage for resource division and definitive management protocol.

Materials and Methods: The present retrospective observational study, on 60 symptomatic patients, was carried out in KPC Medical College and Hospital, Jadavpur comparatively assessing laboratory investigations.

Results: During the course of study, 60 symptomatic patients were assessed. All the patients were of Indian origin and had a recenent historyof contact with a COVID 19 positive patoent. Male patients constituted of 60 % of the population. Lymphopenia (< 20% of total leucocyte[TLC]) was observed in 23 patients (**38%**) . Neutrophilia (> 75% of total leucocyte[TLC])was observed in 21 patients (**35%**).

NLR(neutrophil to lymphocyte ratio) was increased in 29 patients out of the 60 patients studied**(49%)**.

Conclusion: Lymphopenia is an effective and reliable finding in COVID-19 patients. Raised NLR is significantly higher in COVID 19 patients

Keywords:TLC= Total Leucocyte Count ; NLR= Neutrophil to Lymphocyte Ratio

Introduction

The World Health Organization (WHO) has recently declared coronavirus disease 2019 (Covid-19) a public health emergency of international concern(1). The causative organism for this outbreak is a novel beta-coronavirus identified as Severe acute respiratory syndrome coronavirus 2 (SARS CoV 2).(2) As of May 10 2020, a total of 39,25,815 laboratory confirmed cases have been documented globally.(3)

The pathogenesis of SARS-CoV-2 has not been well understood, but extensive lung damage in COVID-19 patients appears to be associated with high viral load, neutrophil infiltration in the lungs along with elevated levels of serum proinflammatory markers with an abrupt decrease of peripheral T lymphocyte.(4) Therefore, clinical deterioration and tissue damage during SARS-CoV-2 infection may result from direct virus-induced cytopathic effects along with overt immune hyper-responsiveness. SARS-CoV-2 infection has been found to reduce peripheral T lymphocyte and impair immune response during acute infection.(5,6) In articles from Huang C et al and Yang X(7,8), 85% of critically ill patients with COVID-19 showed lymphopenia, a finding confirmed by Wang D et al, who, in their study, reported that ICU patients suffering from this infection had a median lymphocyte count of 800 cells/mm(9).Also, Positive correlations have been found(10) between red cell distribution width (RDW) and biomarkers of inflammation namely, sICAM, IL-8, IL-6, SAA, fibrinogen, D-dimer, CRP, CD4/CD8 ratio and others.

Biomarkers representing the immune status of the patient that can identify the ones who may become clinically ill could help in optimizing the resource allocation significantly reducing the eventuality of overtreatment or undertreatment. The present study was designed to identify presence of abnormalities in complete blood count, particularly absolute lymphocyte count and RDW, in patients with COVID-19 and to establish an association with appearance of symptoms and severity of the disease.

Objective: The present study was done to understand prognostic significance of specific immune mechanistic biomarkers in COVID-19 positive patients for early identification and categorization of symptomatic patients who may need critical care support resulting in adequate and optimal resource allocation and definitive management protocol.

Material and Methods

Study Design

The present observational study was conducted on laboratory RT-PCR (real-time polymerase chain reaction) confirmed cases of COVID 19 admitted in COVID 19 wards and ICU of KPC Medical College and Hospital, Jadavpur. Patients with complete clinical data, were randomly selected between 1 April 2020 and 30 April 2020. The secrecy and confidentiality of participants of the study was strictly maintained.

Statistical Analyses

Continuous variables were depicted as appropriate medians and interquartile ranges. Categorical variables were summarized as counts and percentages in each category. Fisher's exact tests were used for categorical variables. $P<0.05$ was recognized as statistically significant. All these statistical calculations were performed using the SPSS 17.0 software.

Data Collection

Epidemiological, clinical, radiological and laboratory data were obtained and COVID-19 positive cases were diagnosed on basis of interim guidelines of World Health Organization (WHO). The symptomatic patients satisfied at least one of the following conditions namely, (1) Fever or any respiratory complaint (2) RR≥30 times/min (3) Oxygen saturation (Resting state) ≤93%, or (4) PaO2 / FiO2 ≤300mmHg. Patients with COVID-19 were confirmed by a positive result on real-time reverse transcriptase-polymerase chain reaction assay for nasal and pharyngeal swab specimens. Only laboratory-confirmed cases, based on diagnostic criterion recommended by National Institute of Virology, Pune were included in the analysis. Laboratory assessments consisted of complete blood count and blood chemical analysis, coagulation testing, renal and liver function tests.

Inclusion Criteria:

Those who satisfied the criteria for WHO guideline for Symptomatic case -at least one of the following conditions namely, (1) Fever or any respiratory complaint (2) RR≥30 times/min (3) Oxygen saturation (Resting state) ≤93%, or (4) PaO2 / FiO2 ≤300mmHg. Patients with COVID-19 were confirmed by a positive result on real-time reverse transcriptase-polymerase chain reaction assay for nasal and pharyngeal swab specimens

Exclusion Criteria:

1)Those who failed to satisfy the above criteria.

2)Those who did not give consent for the study.

3)Pre-diagnosed cases of Fever.

4)Tested negative for COVID-19 RTPCR.

Results

A study of hematological parameters (Total leucocyte count, Absolute lymphocyte count,NLR(neutrophil to lymphocyte ratio)) comparison was done in 60 symptomatic patients of COVID 19 disease. All patients were of Indian ethnicity with a history of contact with COVID 19 positive case.

On admission, leukopenia was observed in 2 patients (3.3%) belonging to symptomatic patient population .Laboratory reference value for leucocyte was 4000 – 10,000 cells/ul and for lymphocyte was 1000 – 4000 cells/ul. Lymphopenia was observed in 23 patients (38%) **FIG 1.**An ALC value approaching scoring scale of severe lymphopenia of <0.6 × 109/L could possibly be considered as one of the indicators for early and timely admission for supportive care in the ICU.

Neutrophilia were observed in 21 COVID-19 positive patients **FIG 2**. The laboratory reference values of neutrophil count were 1500 – 8000 cells/ul.

NLR(neutrophil to lymphocyte ratio) was increased in 29 patients out of the 60 patients studied**(**49**%)**. **FIG 3**

Fig 1 shows Lymphopenia in our Study Population

Fig 2 shows Neutrophilia in our Study Population

Fig 3 depicts Raised NLR in our Study Population

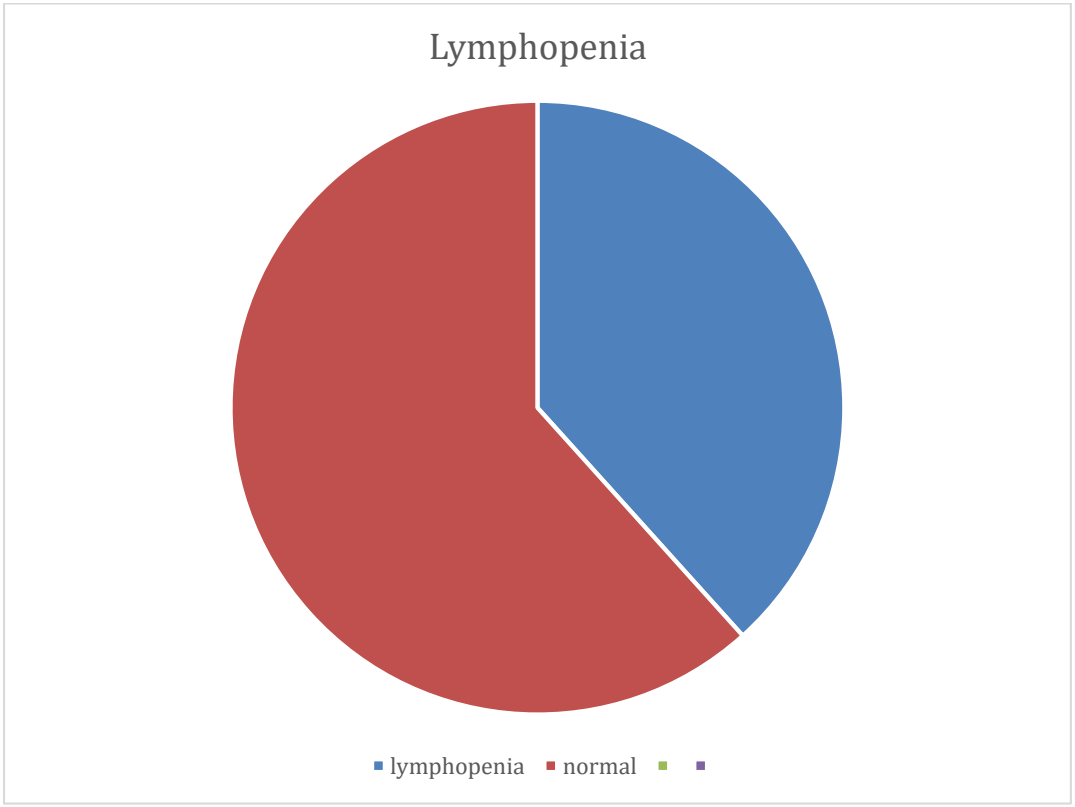

Fig 1: Lymphopenia in our Study Population

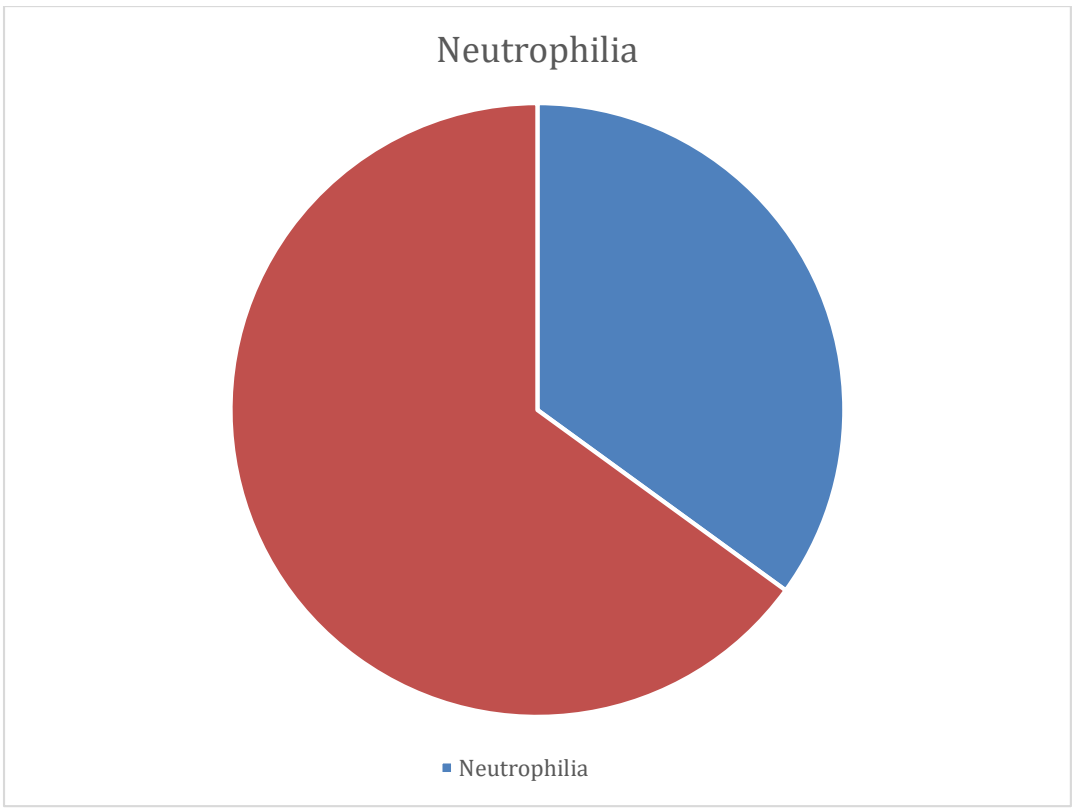

Fig 2: Neutrophilia in our Study Population

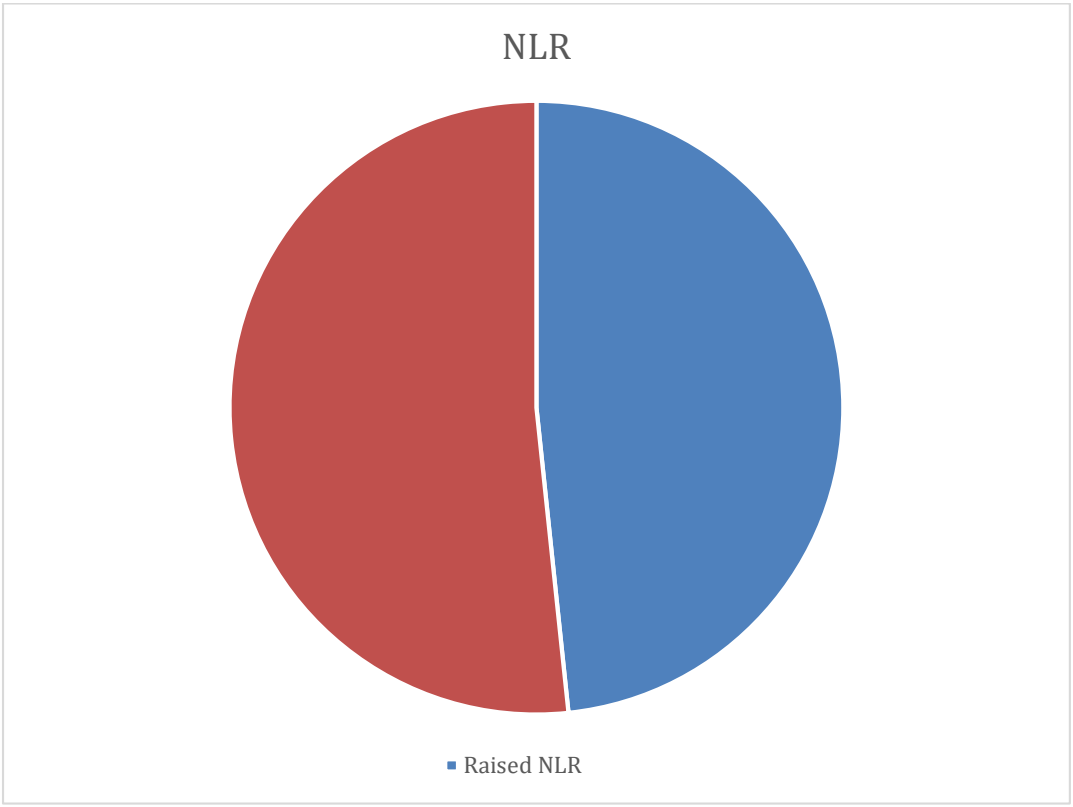

Fig 3: Raised NLR in our Study Population

Discussion

Since December 2019, COVID-19 was first reported in Wuhan and has rapidly spread across the world. Like MERS-CoV, SARS-CoV-2 is a member of the β coronaviruses family, with predominantly human to human transmission and incubation period of 3 days with a low fatality rate.(12)Coronavirus spread among humans leads to a spectrum of respiratory diseases ranging from flu-like symptoms or pneumonia to acute respiratory distress syndrome (ARDS).(12) Infection by these coronavirus can cause sustained immune responses of cytokines and chemokines known as cytokine storm, resulting in a high incidence of immune disorders and mortality in select individuals.(13)

In the present study, lymphopenia, a decrease in ALC, was observed in 23% of symptomatic COVID-19 positive patients indicating a dysfunctional inappropriate immune response of ageing individuals during the course and progression of SARS-CoV-2 infection.

Lymphopenia has been reported in various types of viral diseases such as SARS, MERS and RSV. In the study of Cui et al on SARS, incidence of lymphopenia observed was 84%, CD4 T cells decreased in 100% of patients, CD8 T cells decreased in 87% of sample population.(14) In the study conducted by Assiri et al on MERS, lymphopenia occurred in 34% of patients.(15) Huang et al and Yang et al have documented lymphopenia to occur in 85% of critically ill patients with COVID 19.(7,8)The presence of lymphopenia in severe COVID-19 was confirmed by Wang et al, who reported a median lymphocyte count of 800 cells/mm in ICU patients, with non survivors exhibiting persistent lymphopenia. The presence of hyper-cytokinemia in COVID-19 patients with lymphopenia may point to a poor control of the pathogen, as evident

in critically ill patients due to (SARS-CoV)which emerged in 2003 .(16)These features of lymphopenia and hypercytokinemia have been observed to be associated with incidental increased severity, mortality from the infection.(17)

Lymphocytes play a vital role in maintenance of immune system function. As with immune diseases and other infectious disease, virus infection can lead to dysregulation along levels and strata of lymphocyte subsets.(18) Adaptive immune responses develop precise and powerful immunity against viruses. The adaptive immune response to viral infections is exerted through the effector function of cytotoxic T lymphocyte (CTL) response(19) which specifically recognize and kill virus infected cells and/or release inhibitory antiviral soluble factors.Hence, a prominent increase in CTL count was to be expected in patients with SARS-CoV-2 infection. However, unlike the conventional immune responses against viruses, SARS-CoV-2 infection is accompanied with T cell lymphopenia, with a sharp fall in both CD4 and CD8 T cell subsets.(20,21)Another report, indicated a reduction in number of CD4+ and CD8+ T cells in the peripheral blood of SARS-CoV-2-infected patients, similar to patients with SARS-CoV, and was associated with hyperactivated T cells shown by high proportions of CD4-restricted HLA-DR (3.47%) and CD38 (CD8 39.4%) positive populations.(22)

The possible explanation of lymphopenia in COVID-19 need further investigation. The potential underlying mechanisms could possibly be, (1) The virus might directly infect lymphocytes, resulting in lymphocyte death as lymphocytes express coronavirus receptor ACE2 and may be a direct target of viruses,(23) (2) Infiltration of T cells and subsequent sequestration in the lower respiratory tract as well as immune cell death,(24) (3) The virus might directly destroy lymphocyte harboring lymphatic organs. Acute lymphocyte decline might be related to lymphocytic dysfunction, and the direct damage of novel coronavirus virus to organs such as thymus and spleen cannot be ruled out. This hypothesis needs confirmation by pathological dissection in future,(25)(4) Inflammatory cytokines such as TNF alpha, IL-6 continued to be disordered, perhaps leading to lymphocyte apoptosis,(26) (5) Inhibition of lymphocytes by molecules produced by metabolic disorders, such as lactic acidosis. In severe COVID-19 patients -elevated blood lactic acid levels have been found, which might suppress the proliferation of lymphocytes,(27) (6) Viral infections would inevitably lead to the activation of hypothalamic-pituitary-adrenal axis under stress, resulting in up-regulation of endogenous corticosteroids, which might be involve in immunopathogenic mechanisms of lymphopenia of COVID–19.(28)

Conclusion

Lymphopenia is a useful and reliable indicator of onset of symptoms and severity of disease in COVID-19 patients. Lymphocyte count can easily be obtained at admission to the emergency room. In areas with sustained circulation of the new Coronavirus, evaluation of lymphocyte counts could help to identify and prioritize those individuals who require or will shortly require critical care.

Limitations

The limitation of the present study was inadequacy of serial profiling of blood counts and bio-chemistries of patients who were minimally symptomatic. It is also reckoned that correlating onset of symptoms (days of illness) with hematological parameters is important and would require a case note review that would be taken in further studies. Further studies need to be conducted comparing patient onset of symptoms and their correlation clinically with laboratory findings inclusive of lymphocyte subset alteration in COVID 19.

Declarations

Funding: This research was self-supported.

Competing interests: The authors declare no competing interests.

Ethics approval and consent to participate: Written consent was obtained from all patients and the study was approved by the Ethical Committee of KPC Medical College and Hospital, Jadavpur.

Consent for publication: Not applicable.

Availability of data and material: Not applicable.

Code availability: Not applicable.

Authors' contributions: Conceptualization ,Formal analysis, Methodology, Supervision, Validation, Writing – Dr.Kaushik Munsi ,Dr Shikhar Bansal, Dr. Sayonee Das, Dr. Ramiz Islam ,Dr. Parvez Shahide Biswas , Dr. Ahsan Ahmed

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