National Board of Examination - Journal of Medical Sciences
Volume 1, Issue 6, Pages 357–364, June 2023
DOI 10.61770/NBEJMS.2023.v01.i06.005

ORIGINAL ARTICLE

NLR, CRP, LDH as severity markers in Coronavirus Positive patients

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Accepted: 08-May-2023 / Published Online: 01-June-2023

Abstract:
Background: Coronavirus is a deadly respiratory virus and was discovered way back in 1965 but made a recent comeback in November- December 2019. In March 2020, coronavirus infection was declared a pandemic, since then there has been widespread research taking place on this virus. For confirmatory diagnosis of coronavirus infection, Rapid Antigen detection test, TRUNAAT, RTPCR are used.
Aim: Correlation of NLR, CRP, LDH as a marker of severity, morbidity, and mortality of coronavirus positive patients.
Discussion: Coronavirus has undergone multiple small changes in its RNA genome known as mutation leading to production of variants. A lot of variants have been produced and detected since then because of its high replicability and mutation rate. The more dangerous variants have been labelled as Variants of Concern and less severe variants have also been detected which are known as Variants of Interest. Although, a viral infection is usually associated with lymphocytosis in most cases, but coronavirus infection has been reported to be associated with lymphopenia.
Conclusion: There is a significant correlation of Neutrophil to Lymphocyte Ratio, S.CRP, S.LDH with the severity of coronavirus disease. They can be used individually as well as in combination as markers to establish severity of the coronavirus disease and helps in deciding prognosis of the patient.

Keywords- NLR, coronavirus, severity marker, CRP, LDH

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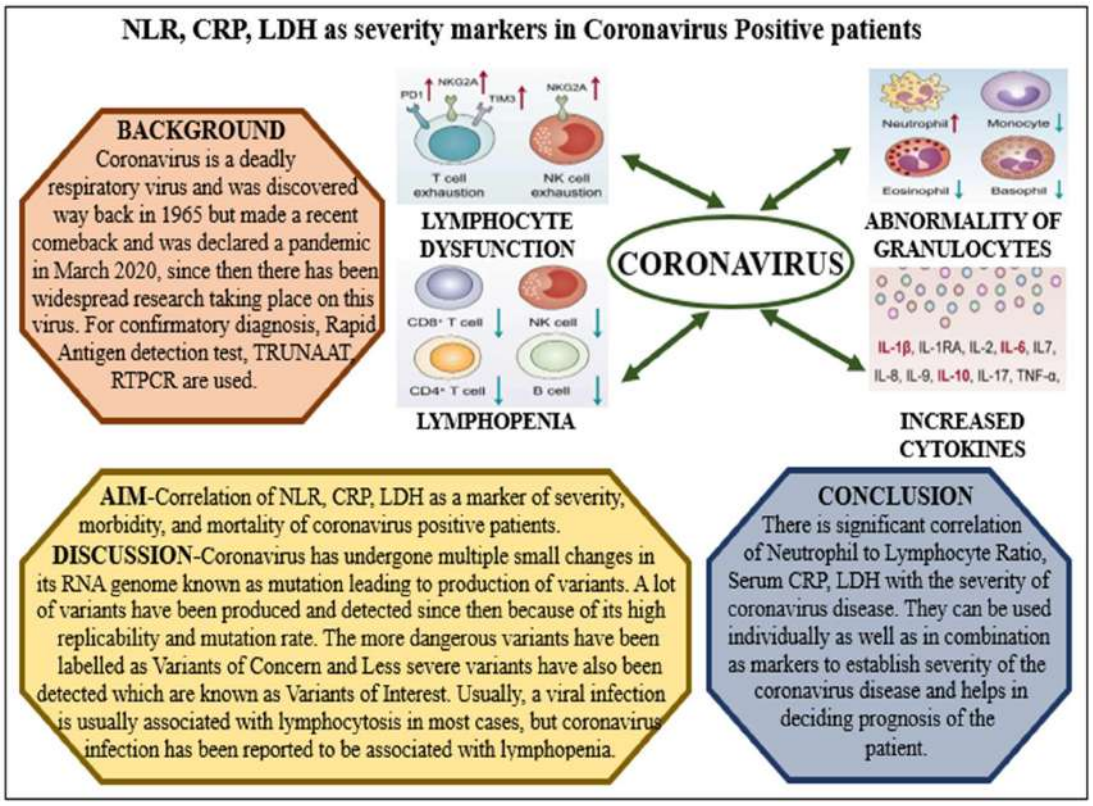
Abbreviations

LDH	:	Lactate Dehydrogenase
CRP	:	C-Reactive Protein
NLR	:	Neutrophil to Lymphocyte Ratio
RNA	:	RiboNucleic Acid
TRUNAAT	:	Taqman Cartridge Based Nucleic Acid Amplification Test
CoV	:	Coronavirus
SARS	:	Severe Acute Respiratory Syndrome
RTPCR	:	Reverse Transcriptase Polymerase Chain Reaction
ACE	:	Angiotensin Converting Enzyme
HRCT	:	High Resolution Computed Tomography
CTSS	:	Computed Tomography Severity Score

Graphical Abstract



Introduction

Coronavirus is a deadly respiratory virus and was discovered way back in 1965 but made a recent comeback in November-December 2019 which belonged to same lineage (A lineage is a group of closely related viruses with a common ancestor) [1]. In March 2020, coronavirus infection was declared a pandemic, since then there has been widespread research taking place on this virus. Coronavirus has undergone multiple small changes in its RNA genome known as mutation leading to production of variants. A lot of variants have been produced and detected since then because of its high replicability and mutation rate.

For confirmatory diagnosis of coronavirus infection, few antigens and nucleic acid-based tests are advised that are Rapid Antigen detection test, TRUNAAT,

RTPCR. Out of these, TRUNAAT and RTPCR work based on detection of nucleic acid of the virus by amplifying it several times via PCR [2].

Pathophysiology

Sars-Cov2 is transmitted via aerosol droplets from an infected individual. Once transmitted, it goes to upper respiratory tract and get bound to ciliated epithelial cells with the help of spike protein 1. Spike protein 2 helps in fusion with host cell membrane. After fusion, it transmits nuclear material (+ssRNA) to host cell which in turn produce a negative strand of ssRNA and then undergoes viral replication. After newer RNA copies are made from negative strand via RNA polymerase activity, it also produces nucleocapsid and other viral packaging proteins [3]. New viral particles are released via exocytosis ready to infect new respiratory host cells and cause induction of apoptosis in the lymphocytes by indirect mechanisms such as soluble Fas ligand (sFasL), vascular cell adhesion molecule-1 (VCAM-1) [4].

In almost 80% of cases, the infection is contained in upper respiratory tract but in rest 20% cases it may invade lower respiratory tract. It has a special affinity towards ACE-2 receptors which are found on type 2 pulmonary alveolar epithelial cells. After the invasion, it leads the host cells to produce a series of inflammatory cytokines like IL-6, TNF-α etc. The cytokines are associated with increased production of neutrophils in the bone marrow and cause recruitment of CD4 and CD8 cells resulting in systemic inflammation [5]. Although, a viral infection is usually associated with lymphocytosis but coronavirus infection has been reported to be associated with lymphopenia [6]. CD8 mediated cytotoxicity and sequestration of inflammatory cells in the lungs leads to diffuse alveolar damage which finally causes the dreaded Acute Respiratory Distress Syndrome as shown in Figure 1 [7].

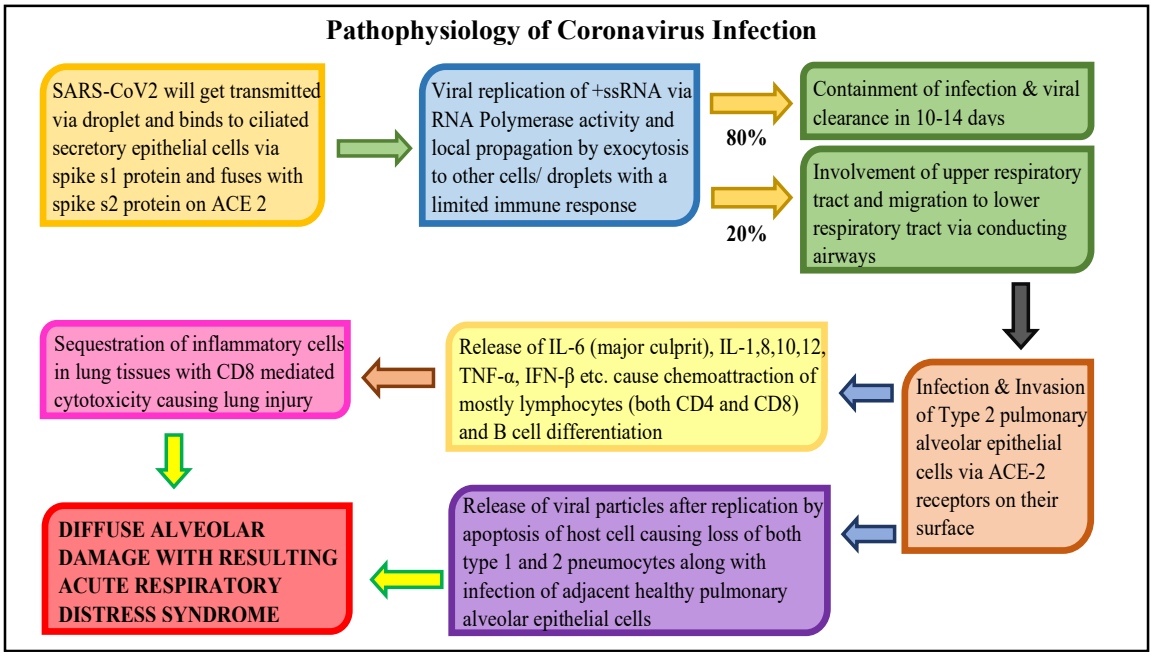


Figure 1. Pathophysiology of coronavirus infection and mechanism of ARDS

Variants of Concern

The more dangerous CoV2 variants are categorised as Variants of Concern as shown in Figure 2. It is based on ability to cause more severe disease like increased hospitalizations or deaths, evidence of increase in transmissibility, reduced effectiveness of treatments or vaccines, significant reduction in antibody mediated neutralization generated during previous infection, or diagnostic detection failures [8].

Variants of Interest

Less severe variants are categorised as Variants of Interest as shown in Figure 3. These are labelled based on reduced efficacy of treatments, reduced neutralization by antibodies generated against previous infection/vaccination, evidence of mutations causing altered receptor binding, predicted increase in transmissibility or potential diagnostic impact [9].

Figure 2. Variants of Concern

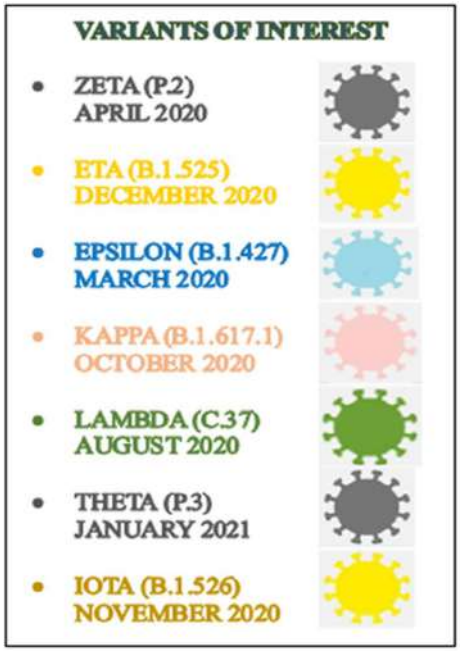


Figure 3. Variants of Interest

Clinical Features

Mild coronavirus illness

1. Mild symptoms such as fever, sore throat, nasal discharge, headache, malaise, cough, muscular pain, nausea, loose stools, loss of olfactory/gustatory sense.
2. SpO_2 >94% on room air,
3. Respiratory rate < 24 per minute,
4. No abnormal radiological imaging.
5. Also, no evidence of end organ damage or other severe comorbidities [10].

Moderate coronavirus illness

1. Shortness of breath,
2. SpO_2 = 90-94% on room air,
3. Respiratory rate between 24-30/min,
4. PaO_2/FiO_2 > 300 mmHg.
5. Infiltrates < 50% in HRCT (CTSS ≤12/25)[10]

Severe coronavirus illness

1. Shortness of breath,

2. SpO_2 < 89% on room air,
3. Respiratory rate > 30 times/min,
4. PaO_2/FiO_2 < 300mm Hg
5. Infiltrates > 50% in HRCT (CTSS ≥13/25) [11]

Critical Illness

The last but the most lethal presentation of coronavirus infection is critical illness which is defined as when the patients is suffering from (ARDS) acute respiratory distress syndrome, cardiac dysfunction, septic shock, elevation of inflammatory cytokine levels thus provoking a cytokine storm, and/or exacerbation of underlying co-morbidities that are already existing in the patient [12].

CT Severity Score

CTSS also known as CT Severity Score is a scale to quantify the lung involvement in coronavirus positive patients. Both lungs contain 5 lobes in total (3 in right lung and 2 in left lung) which are scored according to extent of involvement in each lobe between 0 to 5. The total score of all 5 lobes is the resultant score with a minimum score of 0 denoting no lung involvement and 25 denoting complete lung involvement. It has been widely used for quantifying the severity of covid illness and in predicting the prognosis also [13].

Markers of severity for Coronavirus disease

Neutrophil to Lymphocyte Ratio (NLR)

Neutrophil-to-lymphocyte ratio (NLR) is calculated by dividing the absolute/ percentage neutrophil count with absolute/ percentage lymphocyte count. Based on general trend in normal population, neutrophils are usually more in number than lymphocytes, so the normal ratio comes out to be greater than one [14].

Lactate Dehydrogenase (LDH)

LDH is a universal intracellular enzyme which is found as 5 different isozymes:
Type 1 in cardiac muscle cells,
Type 2 in reticuloendothelial cells and is also highest isoenzyme component,
Type 3 rich in lung tissue,
Type 4 found in kidneys & pancreas and
Type 5 in hepatocytes and striated muscle cells [15].

Since LDH's type 3 isoenzyme is present in pneumocytes, patients with coronavirus infection usually involving lungs produces large amount of LDH and increase the serum LDH levels, although whether it is only type 3 component is not determined. Additionally, LDH levels are elevated in thrombotic microangiopathy which is also seen in coronavirus infection, so other isoenzyme components of LDH can also be contributing factors in total serum LDH levels [16].

Normal Reference Range:
Serum LDH: < 248 IU/L.

C-Reactive protein (CRP)

CRP is a proteinaceous compound and is also an acute phase reactant produced in liver in response to inflammation. It has a regulatory mechanism controlled by cytokines IL1 and IL6 at transcriptional level. In recent studies, it is seen that the acute respiratory distress syndrome in Coronavirus infected patients is due to hyper-inflammation supported by cytokine storm which specially includes IL-6 and IL-1, hence making CRP a reliable marker for assessment of severity of coronavirus infection [17].
Normal Reference Range:
Serum CRP: 0-5 mg/L.

Methods

For NLR Calculation

Method: 2 ml fresh EDTA sample was taken using all aseptic precautions and was processed in fully automated 5-part Haematology Analyzer:

Result: The reports of samples from the analyser were used to calculate NLR by dividing absolute/percentage value of neutrophils and lymphocytes. The same were confirmed by peripheral blood film checked by the Pathologist under the microscope.

For CRP and LDH calculation

Sample: 2 ml fresh sample was collected using all aseptic precautions and serum was then separated by centrifuging the sample at 2500-3000 rpm. Sample was processed on fully Automated Biochemistry Analyzer.

Method for CRP: We had taken Immuno-turbidimetric test for quantitative determination of serum CRP levels. When serum was mixed with R1 buffer and R2 latex suspension, CRP reacts specifically with anti-human CRP antibodies coated with latex particles to yield insoluble aggregates. The absorbance of the aggregates was proportional to CRP concentration in serum.[18]

Method for LDH: LDH catalyses the oxidation of lactate to pyruvate coupled with reduction of NAD+ to NADH. The increase of NADH was measured at 340 nm which was directly proportional to enzyme activity in serum.[19]

Results

The study was conducted amongst 90 patients enrolled in the study who presented to GMSH, Sector 16, and Chandigarh as in patients or in OPD of medicine department. Out of 90 patients, 47 patients were found to be RTPCR positive whereas 43 patients were tested RAT positive. All the patients included in this study were adults and of age more than 18 years. The mean age of the study group was found to be 45.02 years. While comparing the male to female number in all 90 coronavirus positive patients, 51 (56.6%) patients were male and 39 (43.4%) were females with the ratio coming to 1.3:1. Analysis of data found that 49 (54%) patients belonged to mild category, 23 (26%) belonged to moderate category and 18 (20%) belonged to severe category.

Severity of the disease vs NLR

NLR was found to be positively correlated with severity index of the disease. Mean ± SD of NLR in mild, moderate, and severe cases were 2.62 ± 1.68, 4.86 ± 3.25 and 9.32 ± 5.48 respectively. NLR was significantly high in severe cases as compared to mild/moderate cases (p-value <0.005). Similarly, NLR was significantly high in moderate cases as compared to mild cases (p-value = 0.004).

Severity of the disease vs S.CRP

CRP and severity of the disease was positively correlated. Mean ± SD of CRP in mild, moderate, and severe cases were 4.6 ± 4.4, 56.9 ± 30.5 and 180.4 ± 61.5 respectively. CRP was significantly high in severe cases as compared to mild/moderate cases (p-value <0.005). Similarly, CRP was significantly high in moderate cases as compared to mild cases (p-value <0.005).

Severity of the disease vs S.LDH

LDH and severity of the disease was positively correlated. Mean ± SD of LDH

in mild, moderate, and severe cases were 217.2 ± 26, 289.2 ± 76.8 and 481.1 ± 165 respectively. LDH was very significantly high in severe cases as compared to mild/moderate cases (*p*-value <0.005). Mild cases had their LDH value within normal range.

Conclusion

In our study, there was significant degree of correlation of NLR, S.CRP, S.LDH with the severity of coronavirus disease. They can be individually used as markers to establish the severity and helps in deciding prognosis of the patient.

Future Scope

There is still ongoing emergence of new variants of coronavirus like Arcturus variant (formerly XBB 1.16) which are difficult to detect because of similarity with influenza virus. Hence, more effective clinical criteria's and diagnostic tools are required for early and specific detection. Vaccine development is an important area to work upon for eradication of coronavirus pandemic. Various DNA, RNA vaccines are under the research and will benefit the humanity once approved. Therapeutic drugs which are effective in severe covid are yet to be approved and are in different research phases. Long term covid and post covid adverse effects are yet to be fully understood and needs a lot of research tools.

Overall, the world has come a long way since the start of pandemic but there many more challenges in the way to come.

Conflicts of interest

The authors declares that they do not have conflict of interest.

Funding

No funding was received for conducting this study

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