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The integrating baseline inflammatory biomarker to enhance the forcasting of COVID-19 severity

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ABSTRACT

Early intervention can be facilitated by combining inflammatory indicators such as IL-6 with ferritin, Lactate Dehydrogenase, Procalcitonin, Neutrophils, neutrophil/lymphocyte ratio (NLR), Lactate Dehydrogenase/ Lymphocyte Ratio and CRP on admission levels, since this can enhance estimating the Coronavirus disease 2019 patients' severity.There have been several studies undertaken on the condition,including biomarkers for diagnosis, prevention strategies and prospective treatment. In this study, a comprehensive analysis of data from non-severe and severe confirmed Patients with COVID-19 revealed that males were more significantly affected by the severe disease, and diab. Furthermore, the most prevalent presenting symptoms during the hospital admission were cough, dyspnea, and fever. According to the current study's findings, individuals with severe COVID-19 consumed considerably elevated grades of inflammation and coagulation indicators (ferritin, CRP, neutrophils, and D dimer) than those with mild COVID-19. This study was directed in patients who confirmed COVID-19. The practical study also examined the possible suggestions of targeting these inflammatory biomarkers to improve treatment effectiveness and minimize negative side effects. This study was conducted to evaluate if combining baseline inflammatory biomarkers could improve COVID-19 severity prediction. This study targeted to see whether integrating baseline inflammatory indicators and the NLR and LDH/L ratio could enhance the forecast of COVID-19 severity and are reliable predictors of outcomes. ***Conclusion:*** The purpose of this study is to see whether integrating baseline inflammatory indicators could enhance the forecast of COVID-19 severity. Discovery a reliable and useful biomarker or score that can assist physicians in finding patients who are at high risk of passing away is crucial, particularly for individuals who have severe COVID-19.

Key Words: COVID-19; Biomarkers; Combination; Interleukin-6; C-reactive protein (CRP); Ferritin; Severity

1. INTRODUCTION

1. Origin and structureof SARS-COV2

Severe Acute Respiratory Syndrome Coronavirus 2 is a new β-coronavirus after the previously identified SARS-CoV and middle east respiratory syndrome-Coronavirus (MERS-CoV) which cause fatal

respiratory tract infections and pulmonary failure, coronavirus 2 is a new type of coronavirus **[1]**. This positive sense RNA virus is enveloped, non-segmented, and belongs to the orthocoronavirinae subfamily of sarbecoviruses, which is widely dispersed in humans and other mammals. Its diameter ranges from 65 to 125 nm**[2]**.The majority of modifications barely affect the virus's characteristics. Numerous notable variations, including Alpha, Beta, Delta, and Omicron, have been observed since the start of the COVID-19 pandemic **[3]**. The current variety of concern is called Omicron, which spreads more readily than the original Covid-19 virus **[4]**. Acute Severe Respiratory Syndrome Coronavirus 2 has a length of roughly 29.9 kb **[5].** SARS-CoV-2 has a single positive strand of RNA that makes up its genome and is ready for translation, which will lead to the creation of its proteins. Open Reading Frames, or ORFs, are a minimum of fifty distinct locations where translation can start **[6].** The two major transcriptional units, ORF1a and ORF1ab, which encode two polyproteins (PP1a and PP1ab, respectively), are encoded by the first two thirds of the RNA sequence. ORFs for at least 16 nonstructural proteins (Nsp1-16) are present in the bigger unit, PP1ab. Non-structural proteins play a variety of roles in biological processes **[6]**. The last third of the RNA codes for accessory proteins as well as those that specify the structure of SARS-CoV-2. At least nine ORFs for accessory proteins are found in the 3' end of the genome and are distributed among the genes that encode structural proteins.

2. Epidemiology of Covid-19

The World Health Organization (WHO) announced on March 11, 2020 that the coronavirus disease for 2019 has changed from an epidemic to a pandemic sickness due to the rapid spread of SARS-CoV-2 globally **[7]**.

In December 2019, this virus was discovered for the first time in the respiratory systems of pneumonia patients in Wuhan, Hubei, China **[8]**. From that point on, the illness spread quickly and the number of cases rose dramatically. The disease spread to every continent in a few of months after the first case was recorded outside of mainland China on January 11th **[9]**.The death rate rises with age, according to numerous research. For individuals above 50, the death rate begins to rise **[10]**.Infection with SARS-CoV-2 increases the risk of severe sickness in older adults and younger adults with significant illnesses such diabetes, heart disease, and lung disease, according to the Centers for Disease Control and Prevention (CDC) and numerous other research **[11, 12]**.

3. Clinical Characteristics o of SARS-CoV-2 Infection

3.1. Incubation Period:

Typically, SARS-CoV-2 incubates for 14 days after infection; most individuals manifest symptoms four to five days after exposure. This time frame is determined by the patient's age and immune system condition. **[13]**.

3.2. Initial Presentations:

About 80% of positive symptomatic instances of the infection recover without the need for treatment, and some infected people never show any symptoms at all **[14]**.

There is a broad spectrum of symptoms associated with confirmed COVID-19 instances, ranging from moderate to more severe cases as follows: **Mild cases**: Symptomatic with no HRCT evidence of COVID-19 pneumonia OR asymptomatic with aberrant laboratory results or high (HRCT) findings of COVID-19 pneumonia. **Moderate cases**: Symptomatic with aberrant lab test results, HRCT evidence of COVID-19 pneumonia, and clinical indications of non-severe pneumonia. **Severe cases**: COVID-19 pneumonia detected by HRCT, together with clinical indicators of severe pneumonia. **Critical cases**: The occurrence of sepsis, other organ failure, shock, and respiratory failure necessitating artificial ventilation **[15]**. As a serious symptom, severe respiratory pneumonia is most common **[16]**.

3.3. Acute Course and Complications:

Although the primary target of SARS-CoV-2 is the lung, due to the extensive distribution of ACE2 receptors in these organs, it is crucial to periodically check for any harm to the central nervous system, liver, kidney, gastrointestinal tract, heart, and eyes **[17]**.

4. Diagnosis of Covid-19

4.1. Laboratory Findings:

4.1.1. Hematologic Markers:

Evident alterations in hematologic biomarkers in severe and critical COVID-19 have been documented, including persistent lymphocytopenia and a notable reduction in monocytes, basophils, and eosinophils but a notable rise in leukocytes and neutrophils. For the early detection and identification of severe disease, these fundamental biomarkers can be useful **[18]**.

a. Lymphopenia: A typical observation in patients infected with COVID-19 is lymphopenia, which is thought to be a sign of a compromised immunological response to the virus**[18]**.Lymphopenia (as an absolute lymphocyte count of less than 1.0 × 109/L) showed up in 83% of patients. Several reasons could account for the notable decline in lymphocyte count, such as: (1) direct SARS-CoV-2 infection in these cells, leading to their lysis (2) Potentially brought on by the systemic inflammatory process, lymphocyte apoptosis may result in a significant generation of cytokines (3) lymphoid organ atrophy (4) Lactic acidosis preventing the growth of lymphocytes **[19]**.

b. Leukocytosis: In a small percentage of COVID-19-infected patients, leukocytosis is observed; it may indicate either bacterial infection or superinfection, regardless of whether it is neutrophilia, lymphocytosis, or both**[18]**. The cytokine storm and hyperinflammatory state, which are expressed as neutrophilia, have a significant pathogenetic role in COVID-19 and related illnesses like SARS **[20]**.

c. Monocytes, Basophils and Eosinophils: In extreme situations, the percentages of monocytes, eosinophils, and basophils decline significantly **[21]**. The continuous fall in eosinophils is indicative of a poor prognosis, since the proportion of eosinophils in fatal inpatients continues to decline much before corresponding clinical signs arise **[22]**.

d. Thrombocytopenia: In COVID-19 patients, thrombocytopenia is a significant marker of severe illness. It shows the consumption of platelets as a result of thrombus development. Nonetheless, a higher platelet count indicates that inflammation has promoted the formation of megakaryocytes and boosted platelet synthesis **[23]**. In addition, the formation of IgG antibodies against the platelet factor 4-Heparin complex causes platelet activation and removal, which in turn induces a prothrombotic state (HIT). When hypercoagulability and a platelet count reduction of greater than 50% occur following heparin exposure, HIT is considered **[24,70]**.

4.1.2.Biochemical Markers:

Because COVID-19 individuals have concomitant organ damage, particularly liver, kidney, and heart disease, several biochemical markers are aberrant. The changes were most noticeable for CRP and lactate dehydrogenase (LDH), then for creatinine, alanine aminotransferase (ALT), aspartate transaminase (AST), and creatine kinase (CK) **[25]**.

a. C-Reactive Protein: In many different inflammatory diseases, there is a rise in CRP, an acute phase reactant produced by the liver. Those with COVID-19 infection had higher rates of it (75%–93%). If patients are experiencing an increasing level of infection, it can be tracked using other biomarkers such the absolute lymphocyte count **[18]**.

b. Lactate Dehydrogenase: In the ICU context, elevated LDH is typical for COVID-19 patients and is indicative of a poor prognosis **[20]**. The majority of COVID-19 patients had high levels of this enzyme, which may be explained by the fact that the lower respiratory tract is the principal site of the SARS-CoV-2 infection and that LDH is a significant indicator of lung damage **[1]**.

c. Alanine Aminotransferase: Since ALT is more prevalent in COVID-19 patients with severe illness, it may be helpful to keep an eye on patients who are admitted to the intensive care unit **[26]**.

d. Bilirubin: Research has demonstrated that elevated total bilirubin levels can differentiate between COVID-19 patients who require ICU care and those who have less severe illness **[26]**.

e. Troponins: The defining feature of myocardial infarction and acute coronary syndrome diagnosis is elevated serum levels of the cardiac-specific troponins, troponin I and troponin C. In COVID-19 individuals, it is now recognized that underlying cardiovascular disease is a strong predictor of severe disease **[26]**.

f. Procalcitonin (PCT): Prohormone in nature, it is an early precursor to the hormone calcitonin, which is essential for maintaining calcium homeostasis. Elevated levels are most commonly observed in cases of septic shock and organ malfunction that need medical attention when a patient is in sepsis. PCT is often significantly elevated in individuals with severe COVID-19 infection **[26]**. Furthermore, based on PCT values greater than 0.1 ng/ml and if immunodeficiency was clinically relevant or reasonably suspected, a number of patients were cautiously precluded **[27]**.

g. Serum Creatinine: Patients with severe COVID-19 disease are more likely to have elevated creatinine than those with milder symptoms, and patients who have both elevated blood urea nitrogen and elevated creatinine had a higher incidence of unfavorable outcomes **[26]**.

h. Albumins: Low serum albumin levels have been linked to unfavorable outcomes in COVID-19 patients **[18, 26]**.

4.1.3. Coagulation Markers:

Hemostasis test alterations have also been documented during the COVID-19 treatment, including longer durations for prothrombin and activated partial thromboplastin and higher D-dimer values **[28]**.

D-dimer, which is produced when fibrinolytic fibrin is broken down, is very helpful in the identification of thrombotic disorders since high levels of it suggest that the body is hypercoagulable and that secondary fibrinolysis is occurring. **[29].**

A patient's illness may be more significant if their D-dimer value is higher. Some people may even have additional serious complications. Potential explanations for a COVID-19 patient's elevated D-dimer levels are: (1) Creation of an inflammatory storm by the production of pro-inflammatory cytokines **[30]**. Endothelial cell dysfunction, aberrant coagulation system activation, systemic small vessel vasculitis clinical symptoms, and widespread microthrombosis could result from this **[31]**. (2) Inflammation can result in thrombosis or increased oxygen demand, and different COVID-19 patients have varying degrees of hypoxia. In aberrant hemodynamics, the absolute oxygen demand rises. (3) Rise in levels of plasminogen activator inhibitor 1 (PAI-1) and excessive inhibited fibrinolysis, which will eventually activate the coagulation cascade, as well as inhibit fibrinolysis and promote thrombosis, are two more ways that severe infection or acute inflammation brought on by sepsis can affect blood coagulation **[32]**.

4.1.4.Miscellaneous Markers:

a. Interleukin 6 and Cytokine Storm: A crucial factor in acute inflammation, IL-6 is a multifactorial cytokine. It is generated by Toll-like receptors stimulating macrophages and monocytes, which in turn activate different cell types. The two primary factors that activate IL-6 expression are TNFα and IL-1β **[33]**.

Patients with severe Covid-19 usually demonstrate an elevated level of IL-6, which is typically linked to the spontaneously secreted other cytokines in a cytokine storm that is characterized by a decrease in pulmonary tissue oxygenation and a rise in alveolar-capillary gas exchange **[33]**.

b. Ferritin:

Patients with severe COVID-19 infections have a significantly higher serum ferritin level. The immunological and inflammatory responses are linked to ferritin's function, which includes iron binding and storage **[34]**. Hemochromatosis, prolonged transfusion, and bacterial or viral infections are among the causes of elevated ferritin **[35]**. The release of iron from the reticuloendothelial system, the reduction of ferritin transport capacity in the liver and spleen, and the enhanced production and release of intracellular ferritin are all associated with elevated serum ferritin levels in cases of bacterial and/or viral infection. The elevated ferritin may be used as a predictor of a bad prognosis in COVID-19 cases if there is a serious subsequent bacterial infection **[36]**.

4.1.5.Molecular Markers:

The most popular and trustworthy test for COVID-19 diagnosis is reverse transcriptase polymerase chain reaction (RT-PCR), which is the gold standard for SARS-CoV-2 detection. Nasopharyngeal swabs and other upper respiratory tract specimens, such as a throat swab or more, are used in the RT-PCR test **[37]**. Viral RNA (ORF1ab gene) in the nasopharyngeal swab, as determined by the cycle threshold (Ct), becomes detectable as early as the first day of symptoms in the majority of persons with symptomatic COVID-19 infection, and peaks during the first week of symptom onset. Higher viral RNA loads are indicated by lower Ct values, which are the number of replication cycles needed to generate a fluorescent signal. By week three, this enthusiasm is beginning to fade and eventually disappears. When most moderate cases provide a negative result after three weeks, PCR positivity may continue beyond that time. Nevertheless, the Ct values achieved in acutely sick hospitalized patients are lower than the Ct values of mild cases. **[38]**. Even so, a "positive" PCR result does not always mean that a living virus is present; it just indicates that viral RNA was detected **[39]**. The primary causes of false-negative results were inadequate sampling methodology, particularly with regard to nasopharyngeal swabs, and improper timing of sample collection in relation to the onset of sickness **[40]**. Reagent contamination and technical faults can occasionally lead to false positive results **[40]**.

4.1.6.Serological Markers:

Measuring the host immunological response to SARS-CoV-2 infection is another indirect method of detecting COVID-19 infection. For patients with mild to severe sickness who may present later than the first two weeks of illness start, a serological diagnosis is particularly crucial. It is a crucial tool for comprehending the scope of COVID-19 and for identifying the persons **[41]**.

Total antibodies are the earliest and most sensitive serological marker. greater levels occur in the second and third week of sickness, while IgM and IgG ELISA have been observed to be positive even as early as the fourth day after symptom start **[42]**. Despite that, even with IgG continues for more than seven weeks, IgM starts to decrease, reaching lower levels by week five, and nearly vanishes by week seven **[43]**.

4.2.Imaging:

Imaging results are often lacking at presentation and should not be relied alone for diagnosis of COVID-19 ,nevertheless the following abnormalities have been observed in chest X-ray and CT scan, such as bilateral, peripheral, patchy opacities (ground glass appearance) and consolidation **[44]**.

5. Treatment of SARS-CoV-2 Infection and COVID-19 Disease

SARS-CoV-2 sickness is treated with a combination of strategies that target distinct stages of the viral replication cycle and alter the host's immunological response to the infection **[45]**.

5.1.Antiviral Treatment:

a. Chloroquine and Hydroxychloroquine: are lysosomotropic drugs that operate as antimalarial and immunomodulatory agents. They aggregate inside lysosomes and other intracellular compartments, lowering the acidity of these vesicles and preventing viral fusion and reproduction. decreasing the chance of cytokine storm **[46]**.

b. Azithromycin: similarly has an alkalinizing action at least equal to that of hydroxychloroquine, but it is a weak base that builds up in endosomes. Periodically, azithromycin is utilized for its immunomodulatory qualities in addition to its antibacterial ones **[47]**.

c. Remdesivir and Other Nucleoside Analogues: They merge with the viral RNA to cause RNA synthesis disruption and non-obligate RNA chain termination **[48]**.

d. Umifenovir (Arbidol): works as a broad-spectrum antiviral by preventing membrane fusion, which prevents the spread of viruses **[49]**.

e. Camostat Mesylate: is an antagonist of the protease TMPRSS2, which SARS-CoV-2 uses to prime spike proteins during host cell entrance **[50]**.

f. Lopinavir–Ritonavir (LPV/r): The purpose of LPV/r, a protease inhibitor, is to treat COVID-19 patients because proteases play a crucial role in the maturation and reproduction of many viruses in the host cell and because proteases also impede the host innate immune system **[51]**.

5.2. Immunotherapy of COVID-19

5.2.1. Interferon-Based Immunotherapy:

An essential part of the immune system's defense against viruses is played by interferons **[52]**. The synthesis of IFN-1 can be inhibited by SARS-CoV-2, or severe acute respiratory syndrome **[52]**. Due to IFN's low IFN-producing threshold, which triggers early IFN induction and ultimately inhibits SARS-CoV-2 infection, the reduced mortality rate associated with the virus in children has been explained. However, the increased threshold for IFN production in elderly individuals is at least partially responsible for their greater death rate, as it causes a delay in IFN production and an insufficient immunological response **[53]**. Antiviral medications by themselves were found to be less successful than early combined therapy of SARS-CoV-2 with IFN and antiviral medicines in the clinical context **[54]**.

5.2.2. Antibody-Based Treatments:

In COVID-19 patients, antibody-based therapies are a type of passive immunotherapy that can boost immunity and prevent SARS-CoV-2 infection **[55]**.

a. **Convalescent plasma therapy (CP):** Using convalescent plasma from recovered SARS-CoV-2 patients, people with COVID-19 can develop passive immunity. There are several different blood-derived components that make up the varied composition of CP. In addition to water and over a thousand different proteins, plasma also includes a variety of organic and inorganic salts. Albumin, immunoglobulins, complement, coagulation, and antithrombotic components were present in the latter **[56]**.

It can inhibit the progression of the infection and reverse the inflammatory process in COVID-19 patients

b. **Monoclonal antibody:** (mAbs) are a collection of antibodies made by a particular B cell population that are directed against a certain antigen's epitope **[57]**. They bind to a non-overlapping epitope on the surface spike protein RBD of SARS-CoV-2 with great affinity, preventing the virus from attaching itself to the human ACE2 receptor and preventing the virus from spreading **[58]**.

c. **Intravenous immunoglobin (IVIG):** produces polyclonal IgGs from the serum of hundreds of donors, resulting in a biological product **[59]**.

5.2.3. Management of the Cytokine Storm:

When there is significant hyperinflammation, controlling the cytokine storm can help with respiratory failure. There are two ways to go about doing this. To stop inflammatory pathways, the first step is to block interleukin. The use of immunosuppressive cytotoxic medications, such as corticosteroids and dexamethasone, in critically ill patients experiencing cytokine storm is the second **[60]**.

5.2.4. Cell Therapy:

An immunotherapeutic strategy that has received a lot of interest is cell therapy, which is used to treat cancer and viral respiratory infections **[61]**.

a. Mesenchymal stem cell therapy: a kind of stem cells that, by blocking the release of proinflammatory cytokines, have strong immunomodulatory effects **[62]**.

b. Chimeric antigen receptor NK cell therapy: Gene-modified receptors, such as (CAR), are extensively used to treat various malignancies **[63]**.

5.2.5. Immune Checkpoint Blockade:

May offset the two primary obstacles lowering the lymphocytes' ideal antiviral immune response in COVID-19 **[64]**. Cases of:

a. **PD-1 and Tim-3 inhibition**
b. **NKG2A inhibition**
c. **Janus-Kinase (JAK) inhibition**
d. **Granulocyte-macrophage colony-Stimulating factor (GM-CSF) inhibition**
e. **C-C chemokine receptor type 5 (CCR5) inhibition**

5.2.6. Active Immunotherapy:

Active immunotherapy employing antiviral vaccinations has been explored from the early stages of the pandemic due to the difficulties associated with passive immunotherapy treatment, including its high cost and resource constraints **[65]**.

6. SARS-CoV-2 Vaccination

By exposing the immune system to the virus-specific antigen, inactivated viral antigen, virus-specific APCs, adenoviral vectors, viral DNA/RNA vaccine, or attenuated virus, viral vaccines are bioproducts that have the ability to produce active immunity. The majority of vaccine-based research has focused on the virus's spike (S) glycoprotein **[62]**.

• **RNA Vaccines:** Viral RNA/mRNA is a component of RNA vaccines, which are administered to the host body via lipid nanoparticles as a vector. Following transduction, the target cells express the viral antigen due to the influence of mRNA **[66]**.

• **DNA Vaccine:** consist of a vector that transfers the plasmid into the host cell and an antigen-expressing plasmid. A pathogen-specific antigen is encoded by the genetically altered plasmid **[66]**.

• **Inactivated/Attenuated Viral Vaccines:** belong to the most traditional approaches for creating vaccines. For a variety of viral illnesses, such as measles and influenza, this type of vaccination has been effectively developed. Injecting the host with virus particles that have been inactivated or attenuated is included **[67]**.

• **Spike Protein Vaccine:** Within the SARS-CoV-2 structure, the primary immunogenic protein is the spike (S) protein. Strong immune responses against the virus can be elicited by the (S) protein vaccines **[66]**.

• **Artificial APC Vaccine (aAPCs):** supply the pathogen-associated antigen to the adaptive immune cells, which aids in the antiviral immune response. The adaptive immune response to the pathogen is triggered by the antigen's presentation. aAPCs are lentivirally altered APCs that express a protease or structural protein unique to a particular pathogen **[68]**. Preclinical and clinical investigations have taken into consideration the aAPCs vaccine design for the anti-SARS-CoV-2 vaccine **[69]**.

7. CONCLUSION

The role of laboratory evaluation of inflammation markers in the classification and grading of COVID-19 patients have a duty to be explored. In the current study, among all the studied inflammatory biomarkers, IL-6, LDH, PCT, Ferritin and CRP were autonomous variables for estimating the COVID-19 patients' severity. The analysis also explores the combining IL-6 and CRP on admission levels, or other inflammatory indicators like ferritin, can improve COVID-19 severity prediction and allow for early intervention.The investigation's outcomes displayed that, among Egyptian patients with COVID-19, the NLR and LDH/L ratio are reliable predictors of outcomes. They might be applied as a quick, dependable tool for COVID-19 patient care that is done quickly and efficiently.

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