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# The role of neopterin as macrophage activation and C-Reative protein in disease prognosis of coronavirus disease-2019 patients

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***Abstract*---** Coronavirus disease 2019 (COVID 19) has the potential to affect several organs owing to viral-induced hyper inflammation. Hyper inflammation is linked to the severity of coronavirus disease 2019. In severe COVID-19 patients, high levels of circulating cytokines have been recorded. High levels of circulating cytokines were reported in severe COVID-19 patients. Based on current knowledge, the "cytokine storm" appears as one of the most dangerous and potentially life-threatening events related to COVID-19 sustaining its major clinical consequences. Macrophage activation syndrome and its possible contribution to COVID-19, and cytokine targeted attempts in determine severity COVID-19 cases. Serum levels of the immune activation marker neopterin has shown to be of prognostic value in patients with SARS. Release of this cytokines is followed by rapid development of lung tissue damage resulting in acute respiratory distress syndrome, sepsis, and organ failure. In this study, it was aimed the role of macrophage activation in immune response of covid 19 & progressive of disease to determine the prognostic Neopterin levels as prediction of severe disease in patients with COVID-19. Severe, moderate and mild cases of COVID-19 were compared in terms of clinical and laboratory findings at hospital admission. Measure the level of neopterin protein from macrophage activated in the covid 19 patients with COVID-19 produced macrophage to active T cell.

***Keywords*---**neopterin, macrophage activation, C-Reative, prognosis, coronavirus.

## Introduction

Coronaviruses are a genus of viruses that may infect a variety of animals and cause moderate-to-severe respiratory illnesses in humans [1]. Two highly pathogenic coronaviruses with zoonotic origin, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), respectively, emerged in humans and caused fatal respiratory illness in 2002 and 2012, bringing emerging coronaviruses into the twenty-first century as a new public health concern [2]. A new coronavirus known as SARS-CoV-2 developed in the Chinese city of Wuhan at the end of 2019 and triggered an atypical viral pneumonia outbreak[3, 4]. This unique coronavirus illness, also known as COVID-19 (coronavirus disease 2019), has spread rapidly over the world due to its high transmissibility [5, 6]. High levels of circulating cytokines were reported in severe COVID-19 patients[3, 4]. Based on current knowledge, the "cytokine storm" appears as one of the most dangerous and potentially life-threatening event related to COVID-19 sustaining its major clinical consequences. The immune mediated events related to the response to SARS-CoV-2 infection, and the role of the chemokine/chemokine receptor system, will be further and more extensively characterized with the final goal to identify targeted therapeutic strategies [7].

When macrophages are triggered by interferon gamma (INF-), they produce neopterin (NPT) (6-(D-erythro-1′, 2′, 3′-trihydroxypropyl)- pterin) is a well-established immune activation marker with elevated concentrations seen in many inflammatory states including infections, autoimmune disorders, and cancer a member of the pteridine family. An NPT is a macrophage stimulation indicator, a sign of cellular immunological activity, and a type 1 immune response of the T helper 1 (Th1) type [8] . High blood levels of neopterin (NPT) were connected to illness severity and a poor clinical course [9] and linked to diverse infectious diseases and immunological disorders, including autoimmune diseases [10] and viral infections, like rubella, cytomegalovirus, hepatitis C, and dengue fever [11]. As a result, NPT levels reflect underlying cell-mediated immunological activity because various forms of viral infections can induce macrophages to create and release NPT by activating the synthesis and release of INF- from infected cells [12]. Most COVID-19 patients will develop mild to moderate symptoms, while some infected people may face a hyper-inflammation induced by massive cytokines/chemokines production [7].

The virus first infects the respiratory mucosa, then spreads to other cells, generating a cascade of immune responses and resulting in the development of a systemic known as cytokine release syndrome (CRS) that is characterized by an exacerbated pro-inflammatory cytokines release, usually leading to a systemic inflammation with high inflammation parameters and multiple organ failure [13]. CRS can be caused directly by viral damage or indirectly by immune system overactivation, which causes immune cells like neutrophils and macrophages to infiltrate the tissue. In theory, this is a defensive reaction to prevent viral transmission, but it really has the opposite effect [14]. Changes dramatically in severe COVID-19 patients identified initially by C-reactive protein (CRP) as an essential marker who is a liver protein that acts as an early indicator of infection and inflammation [15]. CRP levels can trigger the immune system's traditional

complement cascade and alter the activity of phagocytic cells during infectious or inflammatory disease states, indicating that CRP plays a role in the opsonization of infectious pathogens and dead or dying cells [16]. In COVID-19, the exact effect of CRP remains unclear, but it was reported that their level can be used for early diagnosis of pneumonia [17] and assessment of severe pulmonary infectious diseases it is considered predict disease severity and guide management of COVID-19 patients to different extents [18].

**Subject and Methods**

This study a cross-sectional consists of three groups, each of which consists of Eighty specimens of blood samples that have been collected from hospitalized patients infected by the coronavirus COVID-19 in Al- Zahraa teaching hospital in the governorate of Wasit, Iraq from October 2021 to January 2022. who was diagnosed by a consultant physician based on RT-PCR and CT-scan and thirty healthy non-infected subjects. Patients were subdivided according to their infected disease severity into severe , moderate and mild groups. The range of age for patients is between 20 and 80 years. These age dividing to the four categories ( 20 -35 ),(35- 49) , ( 50- 64) , (65-80). Male consisted 63% of them and 76% aged ≥35 years, distribution of cases and control. Peripheral blood samples were collected from each patient and controls,and the separated serum was used for measurement CRP concentration by ARCHITECT c4000 Abbott. Neopterin concentration were measured by using Enzyme-Linked Immunosorbent Assay technique (ELISA).Data of normal distribution measured parameters were presented as mean (±SD) values and p.Value.

**Results**

Investigated blood Neopterin, across patients with mild , Moderate and severe COVID-19 to see if they were linked to disease severity. NPT level has a significant correlation with the severity of COVID-19 (P <0.001) (Table 1) , the area under the ROC curve (AUC) is 0.969( Figure 1)

Table 1
Mean differences of neopterin in cases groups compared to control

| Markers | Groups | N | Mean±SD* (range) | Kruskal-Wallis H |
|---|---|---|---|---|
| Neopterin (ng/L) | Severe | 30 | 24.8±13.6 (9.5-67.5) | H=95.98 Sig. <0.001 |
| | Moderate | 25 | 7.7±0.79 (6.0-9.1) | |
| | Mild | 25 | 4.3±1.85 (1.1-7.9) | |
| | Control | 30 | 1.9±0.82 (0.1-3.3) | |

Investigated blood neopterin concentrations across patients with mild , Moderate and severe COVID-19 to see if they were linked to disease severity. found a more than three -fold higher mean concentration of neopterin in severely ill patients. the Kruskal-Wallis H test indicated that neopterin mean rank levels were statistically significantly different in the studied groups (P<0.001). The control

group presented with lower mean of neopterin 1.9 (mean rank 18.02), and all cases with different severity have the higher mean level of neopterin (≥4.3) with remarkably increased with severity, The post hoc pairwise comparison revealed that the mean rank of neopterin level in control group was statistically significantly lower than in all other groups (P<0.05), the mild group also have neopterin mean rank (41.86) lower than moderate and severe, in addition, there was significant neopterin mean rank between moderate cases and severe cases.

NPT level has a significant correlation with the severity of COVID-19 and can be considered a macrophage activation in order to limit proinflammatory responses and account for the efficient resolution of inflammation to release Neopterin as a consequence of T-cell-dependent interactions [19] also; Macrophages have remarkable plasticity that allows them to efficiently respond to environmental signals and change their phenotype, with M1 macrophages representing one extreme and M2 macrophages representing the other. M1 macrophages are the classically activated macrophages, while the M2 are designated for alternatively activated macrophages and all other types of macrophages. During organs defect alternatively activated macrophages can contrast the production of pro-inflammatory cytokines by classically activated macrophages and can contribute to wound and tissue, allowing the tissue homeostasis reestablishment [20]. Later on, a distinct population, named 'regulatory macrophages,' is able to dampen pro-inflammatory immune responses. Since renal insufficiency may affect serum neopterin levels [21] and the observed difference in neopterin concentrations between mild and severe SARS-CoV-2 is in agreement with previous results from SARS patients[9] and also with other study which including Neopterin is an independent prognostic factor for COVID-19 severity [22].

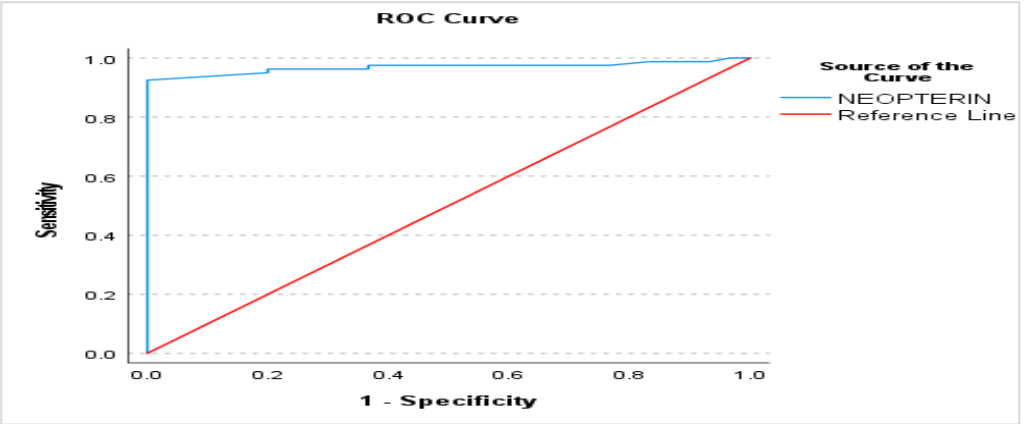


Figure 1. ROC curve to determine neopterin accuracy in detection of cases in compare to control

ROC test (Receiver Operating Characteristic test) was used to study the sensitivity and specificity of critical value of the studied parameters with healthy control . Based on the ROC analysis between mild/moderate and severe patients, the results revealed perfect diagnostic positivity rate of SARS-CoV-2 by investigation of neopterin protein level with high accuracy of the test, the area under the ROC curve (AUC) is 0.969 (P <0.001), figure [1].

Table 2
Mean differences of CRP in cases groups compared to control

| Markers | Groups | N | Mean±SD* (range) | Kruskal-Wallis H |
|---|---|---|---|---|
| CRP (mg/dL) | Severe | 30 | 5.9±3.49 (1.7-18.2) | H=95.51 Sig. <0.001 |
| | Moderate | 25 | 2.16±0.88 (0.9-4.3) | |
| | Mild | 25 | 1.37±0.49 (0.0-0.7) | |
| | Control | 30 | 0.30 SD ± 0.14 | |

This table show higher mean concentration of CRP in three cases groups , severely ill patients (mean value 5.9 mg\dL (SD 3.4)) compared to patients with moderate symptoms (2.1 mg\dL (SD 0.88)) and mild symptoms (1.3 mg\dL (SD 0.45)) and healthy patients as control (0.3 mg\dL (SD 0.2)) significant increase in CRP (p<0.0001) and mean levels of the Age groups were insignificantly comparison with the gender group p.value 0.43), we show that healthy elderly individuals show low serum levels of CRP and pro-inflammatory cytokines, but higher when compared to the younger population when infected .

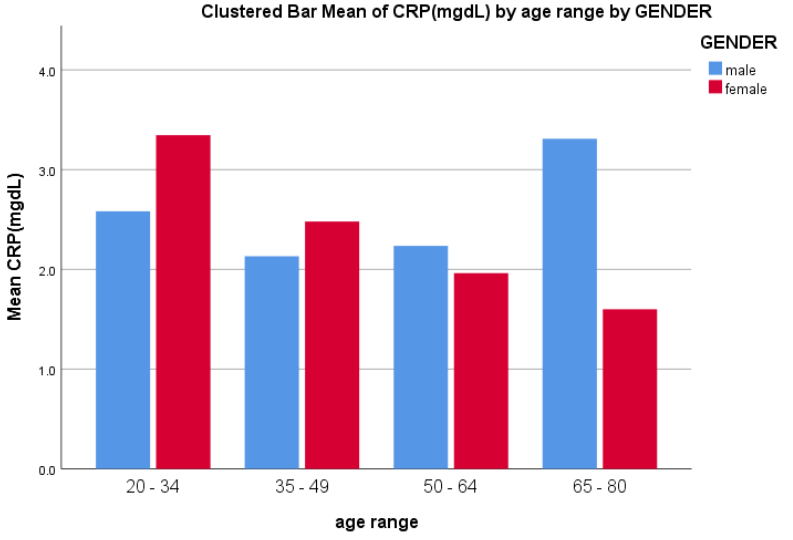


Figure 2. Mean CRP levels comparison of age groups and gender

The results of the present study also showed that serum CRP mean levels of the Age groups were in significantly comparison with the gender group p.value 0.43). Our study also agreement with [23] that observed CRP levels are linked to the severity of inflammation, and their concentration is unaffected by age, gender, or physical condition. However, the host is immune response and tissue-focused inflammation in the lung and other organ likely plays an

important role in COVID-19 , which suggests it will be important to monitor hospitalized COVID-19 patients for evidence of cytokine storm. Thus critically-ill COVID-19 patients often demonstrate features suggestive of cytokine storm, including, characteristic lab changes, and ARDS.

**Conclusion**

- Macrophage activation syndrome and its possible contribution to COVID-19, and cytokine targeted attempts in severe COVID-19 cases.
- Neopterin levels can be used as an early prognostic biomarker for COVID-19 on admission. Neopterin can identify patients with increased risk for the worsened outcome and appear to relate to a productive infection and thus to an increased infectious situation, whereas a low or normal neopterin is indicative for silent infection without or with less active virus production therefore may be a useful marker for more accurate estimation of extent of disease and hence prognosis will be further and more extensively characterized with the final goal to identify targeted therapeutic strategies.
- CRP levels are linked to the severity of inflammation, and their concentration is unaffected by age, gender, or physical condition.

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