Reduction of Inflammatory C3 and C4 Complement Proteins in Severe COVID-19 Patients

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Research

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Abstract

Background: The complement system, consisting of more than 20 soluble proteins, has a key role in innate immunity and inflammation that eliminates pathogens and viral infections. Therefore, we investigated the titer of C3, C4, and total IgG in the serum of the non-severe and severe COVID-19 patients.

Methods: For this purpose, peripheral blood samples were collected from 30 non-sever, 30 severe COVID-19 patients, and 30 healthy individuals with similar age and sex as the control group. The amount of total IgG, C3, and C4 were analyzed in the serum samples. Also, white blood cells, platelets (PLTs), and lymphocytes were counted by the auto-analyzer.

Results: White blood cells had no difference between patients and control groups. The results showed a significant decrease in lymphocyte and PLTs in COVID-19 patients compare to control. Complement proteins including C3 and C4 were increased in non-severe COVID-19 patients than the other groups. Total IgG showed a notable decrease in severe patients. In conclusion, the level of C3 and C4 complement proteins were increased in non-severe-COVID-19 patients; however, in the severe COVID-19 patients their concentrations were decreased.

Conclusion: However, inflammatory C3 and C4 complement factors increase in non-severe COVID-19, it decreased in the severe patients that may be because of more consumption by the formation of the immune complex. These results can shed light on the inflammatory role of C3 and C4 proteins in various phases of the disease and could provide a basis for further exploration of the pathophysiological significance and can suggest them for specific interventions.

Background

The novel coronavirus 2019 (COVID-19) belongs to the family of Coronaviruses [1]. The global outbreak of COVID-19 started in Wuhan city, China, and now is known as a great threat to the public health systems [2]. Until now only a few Asian countries have controlled the disease, but the second wave of the infections is expected [2]. According to the pandemic of coronavirus that rises daily all over the world, any researcher efforts to overcome the virus. Therefore, there is an urgent need to better understand the immune response to COVID-19 until we can find the best treatment and management of the disease [3].

The most important reaction to control and resolve the viral infection is the effectual host immune response including innate and adaptive immunity; however, the severity and outcome of the COVID-19 might be associated with the excessive immune response [4–6]. In this respect, the complement system has a key role in the innate immune system and contributes to the systemic inflammation against different pathogens. The complement system uses various mechanisms such as enhancing humoral immunity, regulating antibody effector mechanisms, and modulating T-cell function by linking the innate and adaptive immune responses. The complement system is activated in three general pathways including classical, alternative, and lectin with different proteins like C3 and C4 molecules [7].

The C3 protein has a central role in the complement cascade and its investigation could be an appropriate criterion that showing activation of three pathways of complement [8]. The C4 protein has a key role in the immune response via classic and lectin complement pathways. Complement neutralization exerted with C4 by enveloped viruses had shown in the past studies [9]. However, a recent study suggested that the presence of an unknown C4-dependent mechanism blocks the infection of non-enveloped viruses [10]. Furthermore, previous studies showed that gene polymorphisms of mannose-binding lectin (MBL), which plays an important role in the lectin pathway, was significantly associated with susceptibility to SARS-CoV infection [11, 12]. Here, we measured the complement proteins (C3 and C4) and total IgG antibody concentration in severe and non-severe COVID-19 patients.

Materials And Methods

Patients

COVID-19 patients were categorized into 30 patients with severe symptoms (severe COVID-19 patients) that were admitted in ICU and 30 patients with mild symptoms (non-severe COVID-19 patients) and 30 healthy individuals as the control of similar age and sex. For confirmation, all of the patients were initially recognized based on the clinical manifestation and ultimately by quantitative RT-PCR (qRT-PCR) analysis of throat swab samples. We excluded the patients who received immunosuppressive therapies in the past and those who died of the disease. All of the samples were collected according to the laboratory testing of humans suspected of novel coronavirus infection guidelines.

Blood cells measurement

Whole blood samples were collected from patients and healthy individuals, then blood specimens were analyzed for counting white blood cells (WBCs), platelets (PLTs), and lymphocytes by hematology auto-analyzer (Sysmex, KX-21N).

Nephelometry

Measuring C3, C4, and total IgG were performed by nephelometry assay according to the manufacture's instruction (The Binding Site Group Ltd., UK). Briefly, serum samples were diluted with buffer, and anti-serum was added to the samples and mixed well. The absorption was immediately recorded by a MININEPH PLUS device and C3, C4, and IgG concentrations were measured using a standard curve.

Statistical analysis

Statistical analyses were performed in SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). Data presented as mean ± standard deviation. One-way ANOVA with Tukey post hoc test was used for comparison of means between the groups. A P-value of < 0.05 was considered statistically significant.

Results

WBCs and PLTs Evaluation

White blood cells count showed no changes between the studied groups ($P < 0.05$) (Fig 1A); however, PLTs were decreased significantly in severe COVID-19 patients compared with healthy individuals (P=002) and non-severe COVID-19 patients (P=0.031) (Fig 1B). Also, the results showed a significant (P=0.024) increase of polymorphonuclear leukocytes (PMN) in severe COVID-19 patients than the other groups (Fig 1C). Furthermore, lymphocytes were decreased significantly in severe and non-severe COVID-19 patients (Fig 1D).

Complement C3 and C4 proteins and total IgG evaluation

Examination of total IgG in the serum samples by nephelometry was showed a significant decrease in severe COVID-19 patients (P=0.037). Complement proteins including C3 and C4 detection in the serum samples confirmed increased concentration level of C3 (P=0.017) and C4 (P=0.012) proteins in non-severe COVID-19 patients than the healthy individuals. Also, the results showed a significant decrease in C3 and C4 proteins in severe COVID-19 patients than non-severe COVID-19 patients (P=0.014).

Discussion

The most important responses to viral infections are the innate and acquired immune systems. Innate immune responses include the use of cells such as macrophages and neutrophils, activation of the complement system, and production of antimicrobial peptides. The complement system uses various mechanisms to remove pathogens [4]. In this study, we evaluated blood cell count, C3, and C4 complement factors level, and total IgG in patients with severe and non-severe COVID-19 patients.

Examination of white blood cell counts showed that there was no difference in WBC levels among the studied groups, whereas patients with severe and non-severe COVID-19 had lymphopenia. In parallel, a study of 108 patients with COVID-19 reported that the WBC count was not different in 97% of patients compared to healthy individuals, while 67% of patients had lymphopenia, and lymphopenia was confirmed in COVID-19 infection in the past studies [5, 13].

In this study, the number of platelets counted in patients with severe COVID-19 was reduced compared to the other two groups and the patients had thrombocytopenia. Previous studies have shown thrombocytopenia in HIV-infected patients [14]. A study of 1,476 patients with COVID-19 in China found that 238 of the patients (16.1%) had died and 306 (20.7%) had thrombocytopenia. From these studies, it can be concluded that thrombocytopenia is common in patients with COVID-19 and is associated with an increased risk of mortality [5, 15].

Also, our results showed neutrophil count in patients with severe COVID-19 increased compared to the other groups. Neutrophils can be increased due to the inflammatory cytokines and chemokines including interleukin (IL)-8 and IL-6 produced from the innate immune system [6, 16].

IgG total titers decreased in patients with severe disease compared to non-severe patients and healthy individuals. A study by the Zhe Du group of 60 patients recovering from COVID-19 showed that antibody detection could serve as an indicator of COVID-19 progression. Also, past studies showed antibodies can be reduced in COVID-19 patients. Patients' antibody levels and clinical manifestations indicate that antibody titer detection could be an indicator of the severity of disease [17]. Also in another study on 38 patients in the acute phase of SARS-CoV-2 infection, IgM and IgG titer 31 of them have been reported to be negative [18], which could be the reason for the decrease in total antibody in people with severe COVID-19.

In this study, the level of C3 and C4 factors in the group with moderate disease form increased significantly compared to the control group as well as patients with severe disease form. There was also a significant reduction in complex C3 and C4 proteins in the group of patients with severe COVID-19 disease compared with non-severe patients. Li and colleagues conducted a study on patients with SLE and RA and examined serum levels of C3 and C4 factors. The results showed that with increased disease activity, the levels of C3 and C4 complexes in patients with SLE gradually decreased [19].

Measurement of serum C3 and C4 factors are useful in diagnosing and monitoring immune complex diseases such as SLE and some blood-related infectious diseases. Complement is the same as acute-phase proteins and it may be normal to take complement some inflammatory and infectious disorders. C3 and C4 are measured simultaneously as this indicates the activation of both the classical and alternative complement pathways. Therefore, due to the activation of C3 alone, the concentration of this component in some infectious diseases (septicemia, endocarditis) is reduced, and also the C3 and C4 titers are often reduced in both immune complex diseases. For example, C4 alone is reduced as a diagnostic marker in angioedema, immune complex diseases, especially vasculitis and cryoglobulinemia, and cold agglutinin [20].

In conclusion, C3 and C4 complement factors increase in non-severe COVID-19; however, in the severe COVID-19 patients by the formation of the immune complex, the complement proteins will be consumed then the concentration of complement was reported decreased. These results can shed light on the role of these proteins in various phases of the disease and could provide a basis for further exploration of the pathophysiological significance and can suggest them for specific interventions.

Declarations

Ethics approval and consent to participate

All of the participants were informed about the objectives of the research and completed the consent forms. This study was approved by the Human Ethics Committee of Arak University of Medical Sciences, Arak, Iran (No IR.ARAKMU.REC.1399.079).

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors report no conflicts of interest.

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Authors' contributions

Conceptualization: [Ali Ganji], [Ali Ghazavi]; Methodology: [Nafiesh Keshavarzian], [Somayeh Rabiemajd], Formal analysis and investigation: [Ghasem Mosayebi], [Ali Ganji]; Writing - original draft preparation: [Ali Ghazavi], [Nafiesh Keshavarzian]; Writing - review and editing: [Ali Ganji]; Supervision: [Ali Ganji]

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Figures

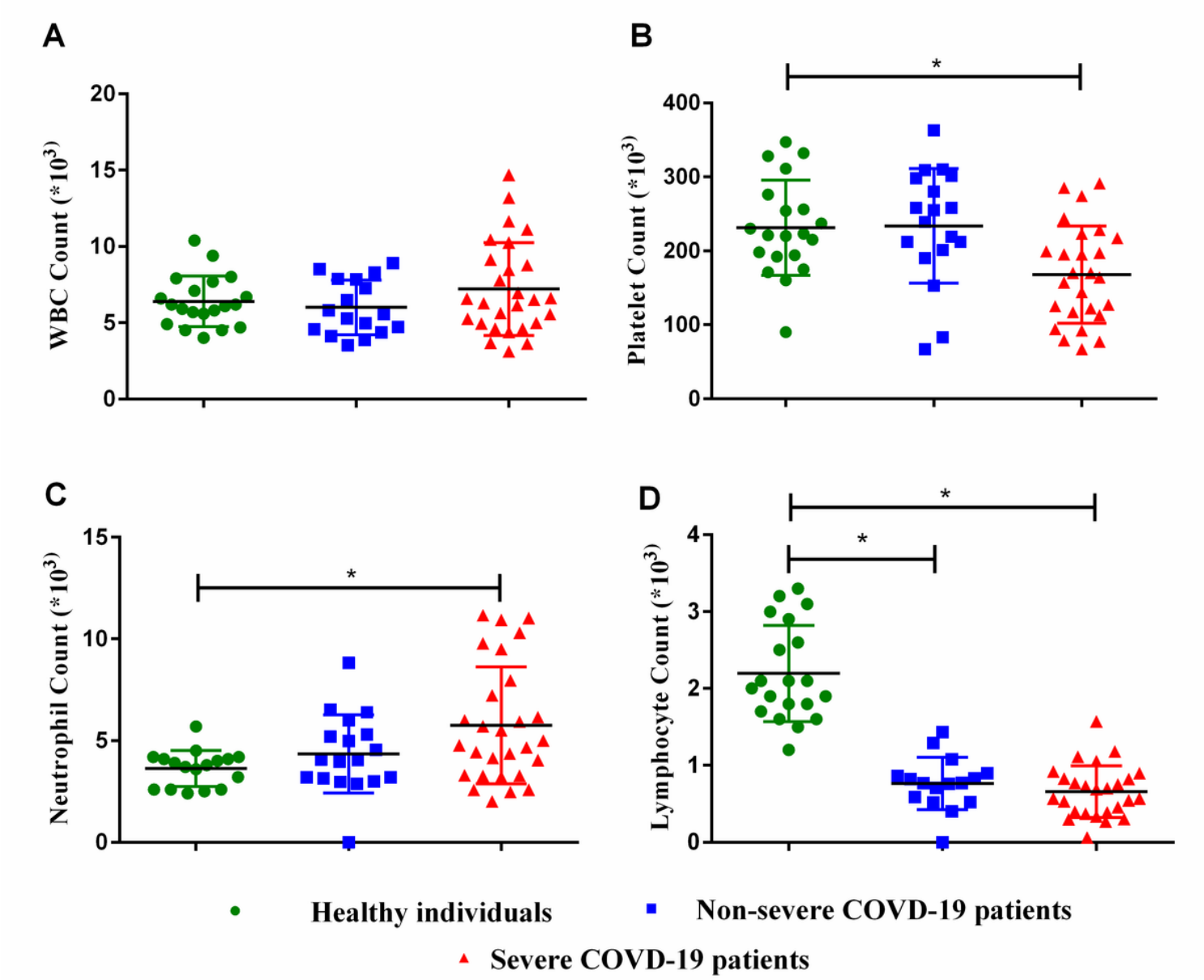
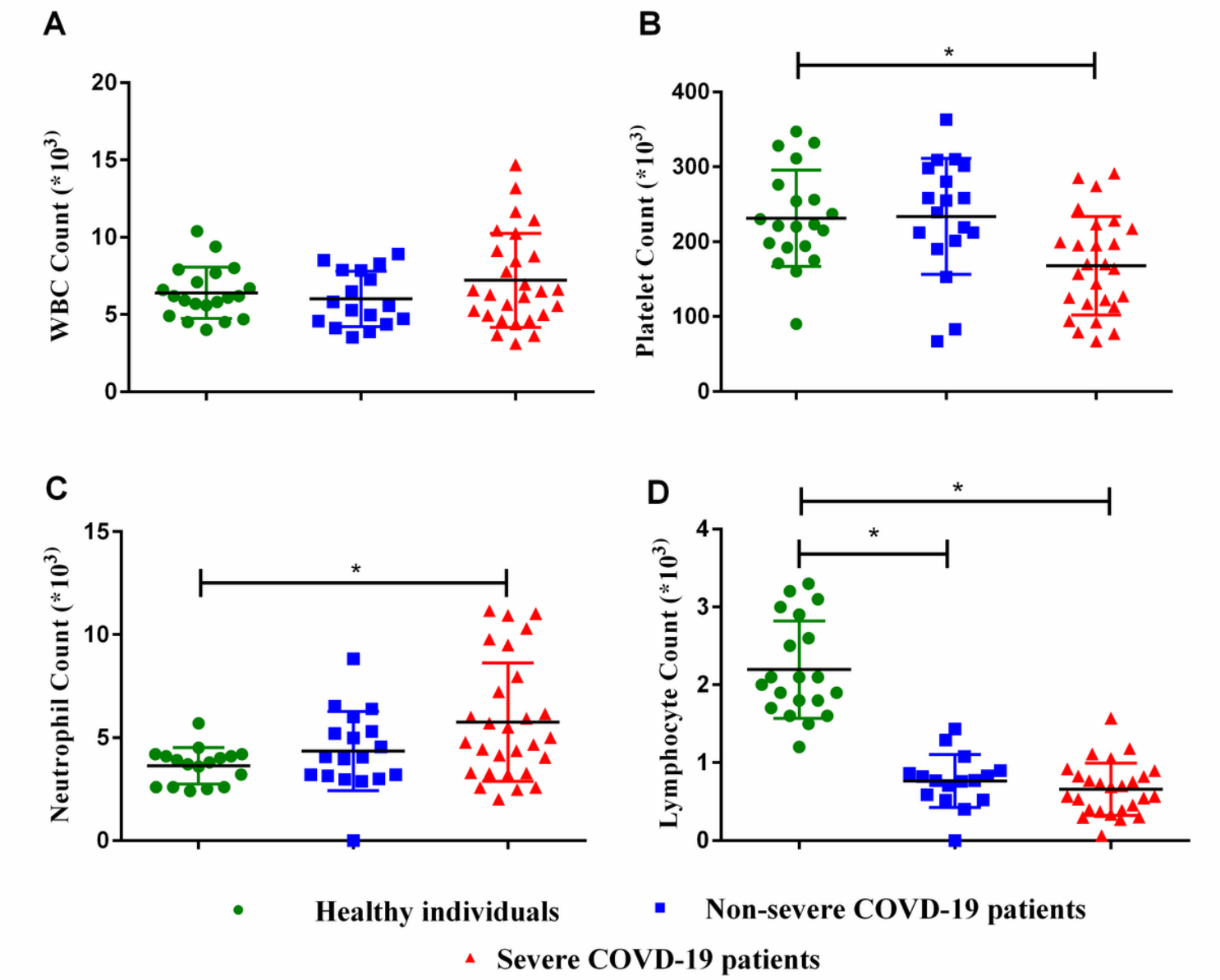


Figure 1

Blood cells analyzing in severe and non-severe COVID-19 patients. (A) WBC count, (B) Platelet count, (C) Neutrophil count, (D) Lymphocyte count. *P < 0.05.



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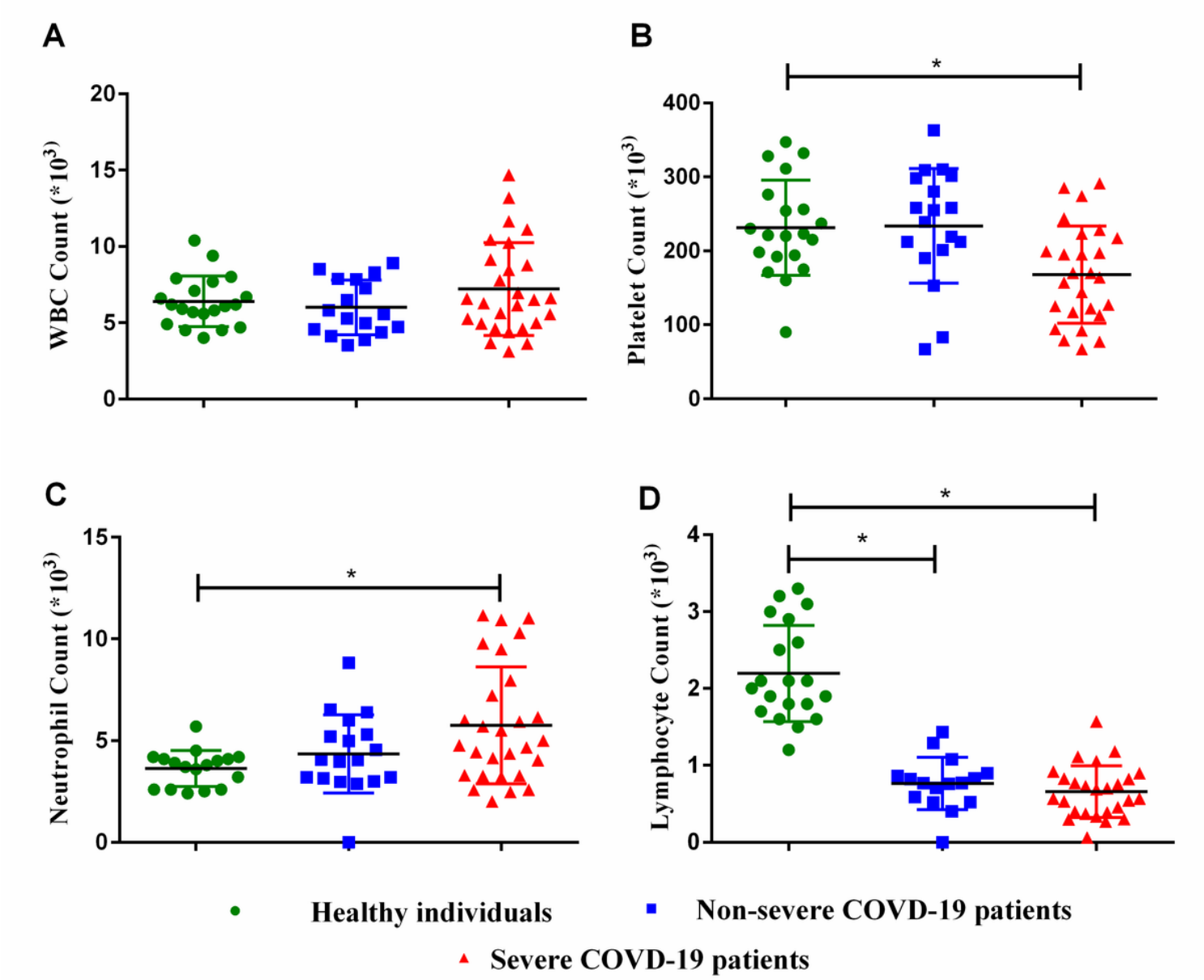

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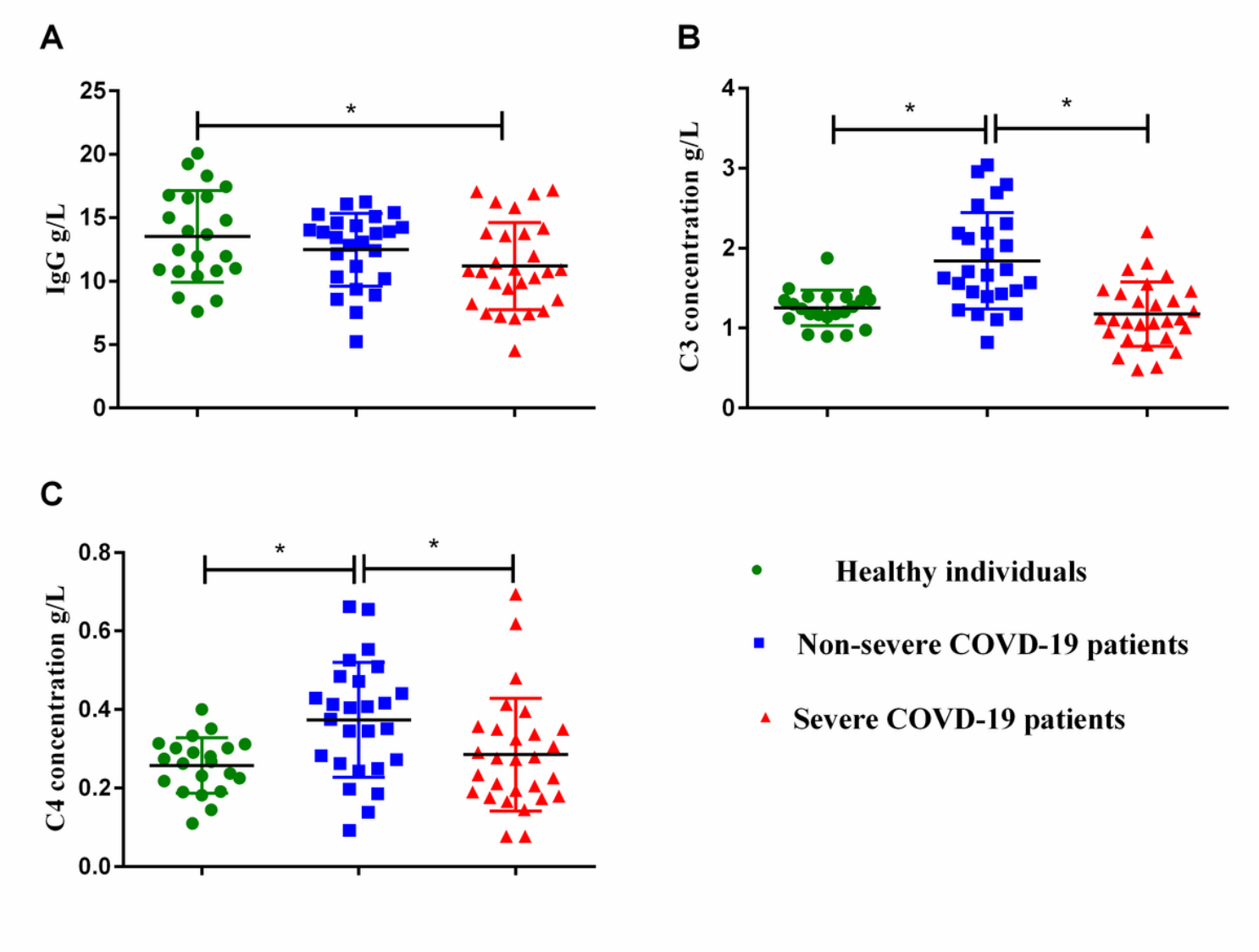
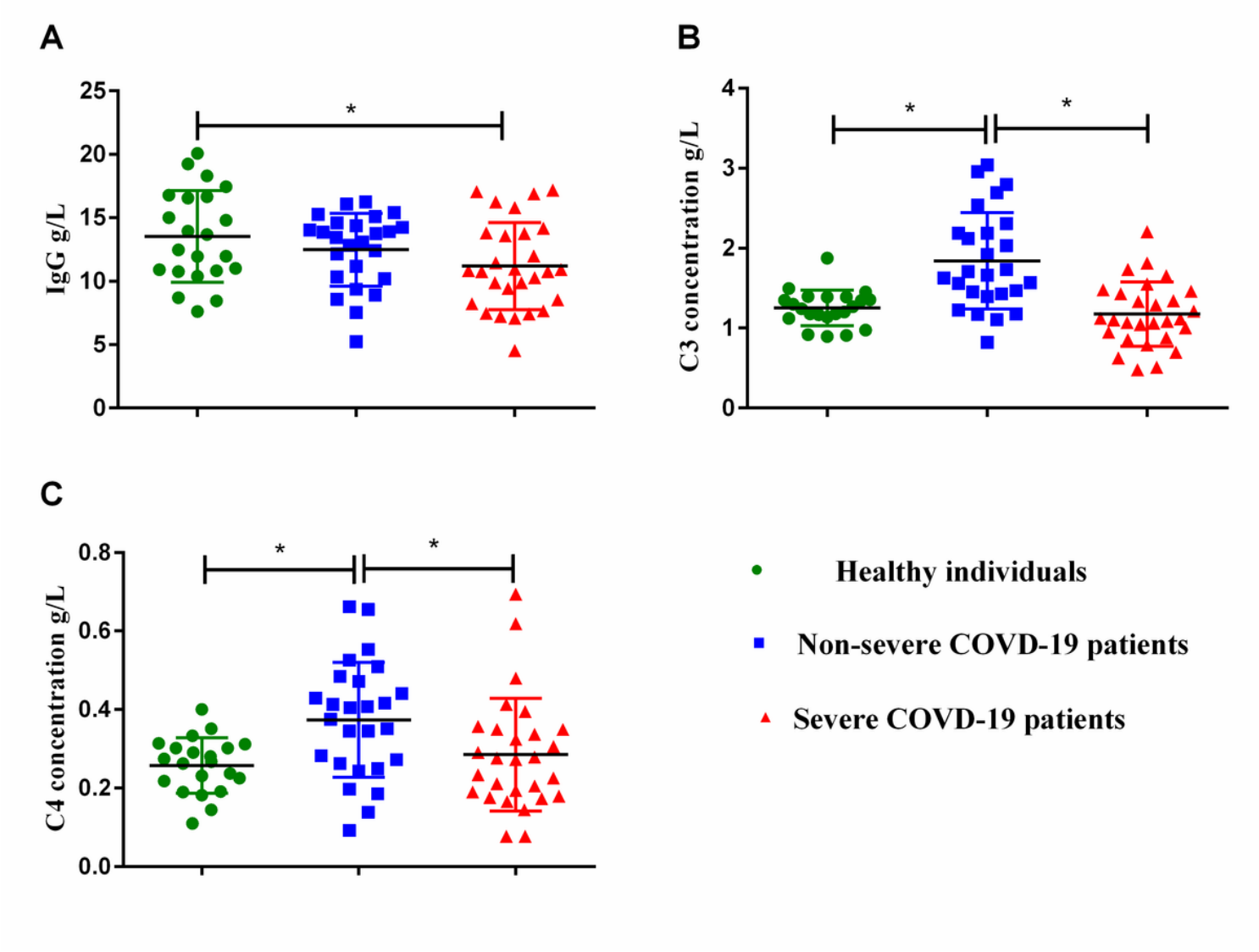


Figure 2

Nephelometry analysis results of C3, C4, and total IgG antibody in three investigated groups. (A) Total IgG, (B) C3 level, (C) C4 level. *P < 0.05.



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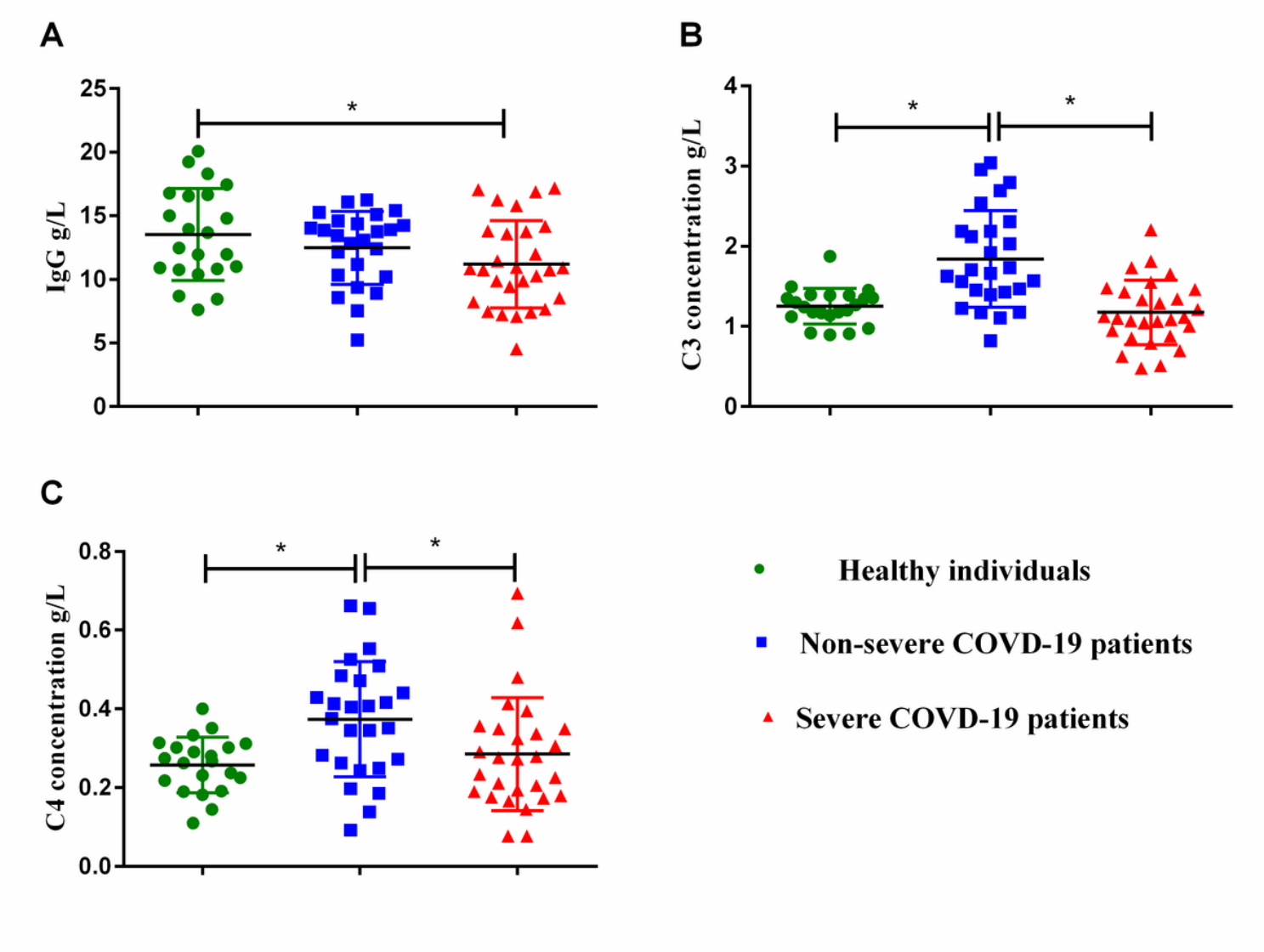

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