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Research article

Association between the expression of toll-like receptors, cytokines, and homeostatic chemokines in SARS-CoV-2 infection and COVID-19 severity

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ABSTRACT

The recent identification of the involvement of the immune system response in the severity and mortality of acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection highlights the importance of cytokines and chemokines as important factors in the clinical outcomes of COVID-19. However, the impact and roles of the BAFF/APRIL cytokine system, homeostatic chemokines (CXCL12, CXCL13, CCL19, and CCL21), as well as Toll-like receptor (TLR)-3/4 in COVID-19, have not been investigated. We sought to assess the expression levels and roles of TLR3/4, BAFF, APRIL, IFN-β, homeostatic chemokines (CXCL12, CXCL13, CCL19, and CCL21), SARS-CoV-2 IgG and IgM antibodies in patients with critical (ICU) and non-ICU (mild) COVID-19 and their association with mortality and disease severity. Significant high levels of TLR-4 mRNA, IFN-β, APRIL, CXCL13, and IgM and IgG antibodies were observed in ICU patients with severe COVID-19 compared to non-ICU COVID-19 patients and healthy controls. On the other hand, BAFF and CCL21 expression were significantly upregulated in non-ICU patients with COVID-19 compared with that in critical COVID-19 patients. The two groups did not differ in TLR-3, CXCL12, and CCL19 levels. Our findings show high expression levels of some inflammatory chemokines in ICU patients with COVID-19. These findings highlight the potential utility of chemokine antagonists as an immune-based treatment for the severe form of COVID-19. We also believe that selective targeting of TLR/spike protein interactions might lead to the development of a new COVID-19 therapy.

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1. Introduction

In December 2019, the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in China led to a pandemic. An elevated expression level of some inflammatory cytokines and chemokines in COVID-19 patients is related to ICU admission. Until now, only a few effective vaccines and repurposed treatments were available to mitigate the transmission of the virus [1]. Repurposing anti-inflammatory pharmaceuticals like corticosteroids [2], antiviral and antiparasitic treatments like ivermectin [3], and different immunotherapies like bamlanivimab [2] aimed against the spike protein of SARS-CoV-2 to limit virus-host interaction have been studied. The questionable efficiency of SARS-CoV-2 vaccines has evolved after several variants with gene mutations in the spike protein have emerged. Although mass production of vaccines is ongoing, some vaccines show low efficacy against some variants. Furthermore, the lack of vaccines worldwide has inspired scientists to further study the immune system behavior in response to the virus, along with the underlying mechanisms [4].

The innate part of the immune system plays a pivotal role of defense against microbial invasion. In viral infection, Toll-like receptors (TLRs), cytosolic RNA sensors, and secretory PRRs, are pattern recognition receptors (PRRs) thatplay an important role in recognizing viral proteins. As a result of viral recognition, immune components, including interferon (IFN)-β, stimulator of interferon genes (STING), and nuclear factor-kappa B (NF-κB), are activated and secreted through different pathways [5]. The type of innate immune response during any viral interactions plays a significant role in disease progression. Some innate immune mediators such as type I IFNs are produced early after the infection to limit and control viral replication early. The immediate response of the innate system not only shapes the response of the adaptive system but can also result in tissue damage if overactivated [6].

TLRs are a type of cellular proteins that play an essential function in the innate immune response. For example, TLR-1, 2, 4, 5, 6, and 10 are cell surface receptors, while other receptors such as TLR-3, 7, 8, and 9 are found within intracellular compartments. A variety of TLRs are expressed in diverse antigen-presenting cells (APCs) and are produced in response to different stimuli. Viral proteins may attach to TLR-2 or TLR-4, ssRNA attach to TLR-7 and TLR-8, dsRNA attach to TLR-3, and viral DNA attach to TLR-9 [5]. Bioinformatics analysis of three different ssRNA viruses revealed that the SARS-CoV-2 genome had the most abundant ssRNA fragments sensed by TLR-7 and TLR-8 [6]. The interaction of the viral spike (S) glycoprotein and APCs, where TLR-7 is expressed on the surface activates different proinflammatory cytokines e.g. interleukin (IL)-1, IL-6, tumor necrosis factor alpha (TNF-α), and type 1 IFN [6, 7]. One study found that TLR-3-coronavirus interaction can lead to the activation of inflammatory cytokines, including IL-6, TNF, and type 1 IFN [6]. Intriguingly, several recent studies have also investigated the involvement of cell surface TLRs, especially TLR4 in recognizing molecular patterns that are playing an essential role in the virus-induced inflammatory responses [8]. As a result, the immune system might react to this strong binding by excessive inflammation, especially in patients with severe symptoms [1, 8]. As a result, developing competitive TLR4 antagonists as immuno-pharmacological drugs would disrupt the TLR4-spike protein interaction, which could lead to an immunotherapeutic for COVID-19 [9,10].

Triggering chemokines at the site of infection plays a key role in the early activation and migration of immune cells, including B cells and T cells. B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) are cytokines that are members of the TNF superfamily and play an important role in producing antibodies, activating B cells, and cell differentiation [11, 12]. CXCL12 and CXCL13 are potent B cell chemoattractant chemokines, and CCL21 and CCL19 are T cell chemokines [13]. These chemokines are mainly activated in secondary lymphoid tissues. However, in non-lymphoid tissues, the lungs for example, expression of CXCL13, CCL19, and CCL21 can recruit lymphocytes to the lungs indicating local immune response to inflammation or infection [13]. Considerable evidence supports that other viruses such as RSV can trigger inflammation resulting in the inducible expression of chemokines at mucosal sites, including the lungs [14]. Similarly, after influenza infection, CXCL13, CCL19, and CCL21 have been found to be constitutively expressed in the lung, playing an essential role in local B cell and T cell protective immune responses to influenza virus [15], and possibly other respiratory viruses such as SARS-CoV-2. Therefore, increasing scientific data on early viral-host immune recognition will provide a scientific insight of the interaction between the host immune response and viral entry and infection. The impact and roles of the BAFF/APRIL cytokine system, homeostatic chemokines, as well as Toll-like receptor (TLR)-3/4 in COVID-19 have not been investigated. We aimed to assess the expression levels and roles of TLR-3/4, BAFF, APRIL, IFN-β, homeostatic chemokines (CXCL12, CXCL13, CCL19, and CCL21), SARS-CoV-2 IgG and IgM antibodies, as well as the viral load in critical (ICU) and non-ICU patients with COVID-19 and their associations with disease prognosis and in-hospital mortality. Our results highlight the potential utility of chemokine antagonists as an immune-therapy for the severe form of COVID-19. We also believe that selective targeting of TLR-4 could lead to therapeutic development for COVID-19.

2. Materials and methods

2.1. Study population and specimen collection and analysis

A total of 119 upper respiratory tract samples were included in this study, of which 100 samples were from patients with positive PCR for SARS-CoV-2 and 19 normal controls. the positive samples were collected from ICU-admitted patients (n = 50) and non-ICU patients (n = 50). Following a 1000 rpm centrifugation at 4 °C for 5–10 min, serum was used for inflammatory chemokines analysis and SARS-CoV-2 IgG and IgM antibodies using ELISA. All samples were obtained during the first week of hospital admission.

COVID-19 severity was defined according to the WHO Interim Clinical Guidance for "Clinical Management of COVID-19." The mild group included patients with any of the following sings: mild clinical symptoms or asymptomatic patient not showing viral pneumonia or hypoxia. The critical patients included patients with respiratory failure, patients on mechanical ventilation, shock, signs of organ dysfunction, and ICU.

The Institutional Review Board (IRB) of King Fahad Medical City reviewed the protocol and approved the study (IRB log number 20-526E). Due to the retrospective nature of the study and the use of only anonymized leftover samples without any personally identifiable data, signed informed consent was not required and was waived by the IRB. Demographic characteristics and patients' medical history were extracted from the electronic medical records. All of the basic characteristics of the enrolled patients are shown in Table 1.

2.2. RNA extraction

Under clean and sterile conditions, viral RNA was extracted from the nasopharyngeal specimens using an RNeasy Mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Briefly, 500 μl of lysis buffer and 300 μl of 70% sterile ethanol were mixed with the sample, followed by mixing, and then applied to an RNeasy Mini column. After cell lysis, the collection tubes were discarded, and 700 μl of wash buffer was added and centrifuged at 14000 rpm for 15 s. The membrane-bound RNA was then washed two times with 500 μl of wash buffer for 15 s and 2 min, respectively. Subsequently, the collection tubes were replaced, and the columns were centrifuged at 14000 rpm for 2 min. Finally, 50 μl of DNAse/RNAse-free water was added to each tube and incubated for 1 min at 23 °C. The Mini columns were centrifuged to collect the eluate total RNA in sterile 1.5 ml tubes. Total RNA concentration was measured using a NanoDrop Spectrophotometer (Biotech International).

2.3. Cellular RNA isolation, reverse transcription, and quantitative real-time PCR (RT-qPCR)

Total RNA was reverse-transcribed using reverse transcriptase and a cDNA synthesis kit (Qiagen, Germantown, MD, USA). Genomic DNA elimination and digestion were performed using RNase-Free DNase Set (Qiagen) based on the manufacturer's instructions. RT-qPCR was performed using predesigned TaqMan Gene Expression Assays (Applied Biosystems) for TLR-3 (Hs01551078 m1 TLR3), TLR-4 (Hs00152939 m1 TLR4), and MRPL32 (Hs00388301 m1 MRPL32) on a 7500 Fast Real-Time PCR System (Applied Biosystems). mRNA expression was normalized to MRPL32 as an endogenous control gene. 2-ΔΔCt method was used to estimate the relative changes in gene expression and presented as a fold-difference relative to the healthy control group.

2.4. Estimation of circulating cytokines, homeostatic chemokines and SARS-CoV-2 IgG and IgM levels

We determined the levels of cytokines and homeostatic chemokines in SARS-CoV-2-infected ICU-admitted patients (n = 50); SARS-CoV-2-infected, non-ICU-admitted patients (n = 50); and healthy individuals (n = 19). Human IFN-β, BAFF, APRIL, CCL21, CCL19, CXCL12, and CXCL13 were quantified using ELISA kits (DuoSet ELISA DY814-05, DY124-05, DY884B, DY366, DY361, DY350, and DY801, respectively; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The standard curve was utilized to calculate the concentrations (pg/ml) of cytokines and chemokines. SARS-CoV-2 IgG and IgM antibody concentrations (pg/ml) were quantified using ELISA kits (0601038 and 0601039; BGI Europe, Copenhagen, Denmark) by measuring the optical density at 450 nm. An automated ELISA washer and ELISA reader were used (Well Wash Versa Microplate Washer and Multiskan FC Microplate Photometer 5165010 & 51119000; Thermo Scientific, Vantaa, Finland).

2.5. Statistical analysis

Quantification of circulating cytokines, chemokines, and SARS-CoV-2 IgG and IgM levels was done via GraphPad 5.0 (GraphPad, San Diego, CA, USA). A one-way ANOVA followed by Tukey's multiple comparison test was used for data evaluation. 2-ΔΔCt method was used to estimate the relative changes in gene expression. Data are presented as mean ± standard deviation (SD). Statistically

Table 1
Patients demographic information and medical history.

Variables	All participants	Non-ICU	ICU	*P*-value
Demographics	*n* = 100	*n* = 50 (50%)	*n* = 50 (50%)	
Age (years)				
Median	56 ± 14	55 ± 13	57 ± 15	0.4779
Range	24–84	25–79	24–84	
Gender				
Male	56 (56%)	25 (50%)	31 (62%)	0.22
Female	44 (44%)	25 (50%)	19 (38%)	
Ethnicity				
Saudi	57 (57%)	28 (56%)	29 (58%)	0.83
Non-Saudi	43 (43%)	22 (44%)	21 (42%)	
Comorbidities				
Chronic lung diseases	13 (13%)	4 (8%)	9 (60%)	
Chronic Heart diseases	47 (47%)	24 (48%)	23 (46%)	
Metabolic diseases	38 (38%)	20 (40%)	18 (36%)	
Kidney diseases	8 (8%)	1 (2%)	7 (14%)	
Death rate	10 (10%)	-	10 (5%)	

significant values were set at $P < 0.05$.

3. Results

3.1. Demographics and characteristics of ICU and non-ICU patients

All patients with COVID-19 had a confirmed SARS-CoV-2 infection by real-time RT-PCR test results from upper respiratory tract samples. High viral loads were detected in all COVID-19 patients. The mean SARS-CoV-2 RNA Ct values and SDs were 25.4 ± 5.3 and 27 ± 4 for ICU (critical) and non-ICU (mild) patients with COVID-19, respectively; there were no statistically significant differences between the tested groups ($P = 0.092$). The basic characteristics of the enrolled patients are shown in Table 1.

3.2. Relative mRNA expression of TLR-4 differed in patients with critical (ICU) and non-critical COVID-19

The relative mRNA expression levels of TLRs (TLR-3 and TLR-4), normalized to MRPL32, were compared between the three groups: critical, mild, and healthy individuals. The expression levels of TLR-4 mRNA were highly expressed in the critical patients ($P < 0.0001$) than in the patients with non-critical COVID-19 (Table 2). No statistically significant values were observed in TLR-3 mRNA expressions among the two groups ($P = 0.5870$). TLR-3 and TLR-4 genes were considered to be differentially expressed if they had a fold-change >2.

3.3. ICU patients with COVID-19 exhibited higher levels of humoral immunity

We sought to see if stronger antibodies produced against SARS-CoV-2 are related to ICU admission in patients with COVID-19 disease. IgM antibody levels were higher in both ICU and non-ICU patients with COVID-19 disease (Figure 1A). and IgG (Figure 1B) antibody responses compared with those of healthy controls, as measured by an enzyme-linked immunosorbent assay (ELISA). However, ICU patients with COVID-19 showed a higher level of IgM and IgG antibodies than non-ICU patients with COVID-19.

3.4. Inflammatory chemokine expression levels were significantly increased in patients with COVID-19 requiring ICU admission

To examine the serum levels of several inflammatory chemokines in ICU and non-ICU patients with COVID-19, we measured the circulating levels of some chemokines using ELISA. The levels of IFN-β (Figure 2A), BAFF (Figure 2B), APRIL (Figure 2C), CCL21 (Figure 2D), CCl19, CXCL12, and CXCL13 (Figure 2E) all increased during SARS-CoV-2 infection compared to those in healthy controls. In addition, the levels of IFN-β, APRIL, CXCL13, and BAFF were significantly elevated in ICU COVID-19 patients than in non-ICU patients and healthy individuals. On the other hand, significant *p*-values in CCL21 levels were observed in the non-ICU and ICU patients with COVID-19. Our findings suggest that elevated levels of several inflammatory chemokines in COVID-19 patients are associated with ICU admission.

Chemotactic chemokine (CXCL12 and CCL19) levels did not differ between ICU and non-ICU patients with COVID-19.

CXCL12 is involved in immune cell activation, adhesion, and migration, as well as inflammation. We evaluated the systemic levels of CXCL12 and CCL19 in samples from COVID-19 patients. CXCL12 (Figure 3A) and CCL19 (Figure 3B) levels did not differ substantially between the two COVID-19 groups when compared to the healthy group; levels also did not differ significantly between the ICU and non-ICU COVID-19 patients.

3.5. APRIL and CXCL13 expression levels were not attributed to COVID-19 mortality

Although the expression levels of IFN-β, APRIL, and CXCL13 were significantly higher in ICU patients with COVID-19 than in non-ICU patients. We found no significant differences between survivors and non-survivors among ICU COVID-19 patients (Figure 4, A to C). Our finding indicates that APRIL and CXCL13 may not be considered as risk factors for mortality among ICU admitted COVID-19 patients.

4. Discussion

Measuring the levels of IgM and IgG is a crucial and sensitive test for the serological diagnosis of many viruses, such as SARS-CoV-1

Table 2
RT-qPCR analysis of TLR-3 and TLR-4 expression.

Gene	Fold-change (mean ± SD)		*P*-value
	Critical (ICU) patients with COVID-19	Patients with mild COVID-19	
TLR-3	29.01 ± 100.99	15.13 ± 51.49	0.5870
TLR-4	226.39 ± 126.16	88.78 ± 29.75	**<0.0001**

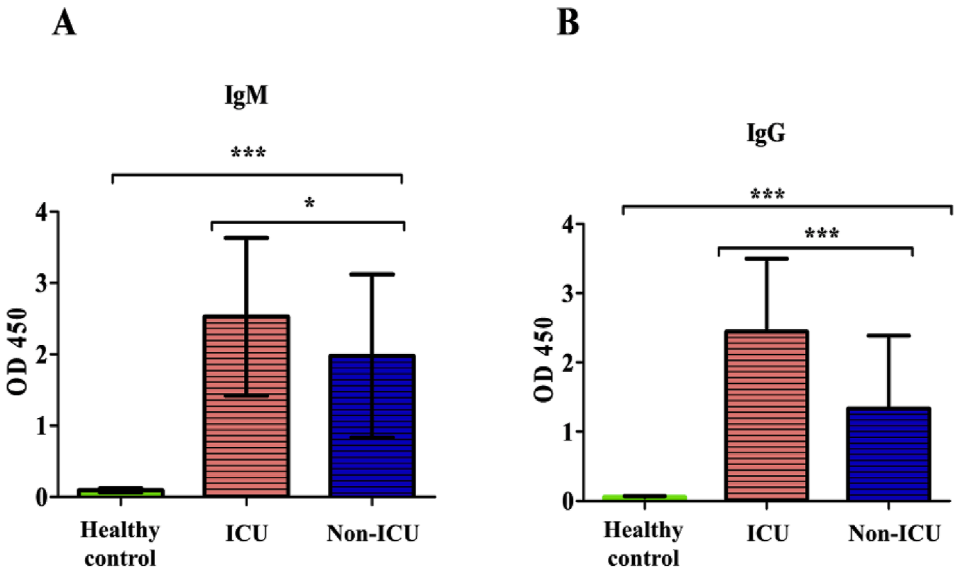

Figure 1. SARS-CoV-2 IgM and IgG antibodies were significantly higher in ICU COVID-19 **patients than in non-ICU** COVID-19 **patients and healthy individuals. (A)** SARS-Cov-2 IgM concentrations were considerably higher in ICU COVID-19 patients than in non-ICU patients and a healthy control group ($P^{***} = < 0.0001$). **(B)** The levels of viral IgG were significantly increased in the ICU COVID-19 patients compared with that in the non-ICU patients and healthy control group ($P^{***} = < 0.0001$). Values represent the means ± SD.

[16] and influenza [17] infections. However, in chronic hepatitis B, measuring IgA levels is a more sensitive test for diagnosis than IgM [18]. This diagnosis may provide more rapid treatment and protection against viral infections. In this study, we observed that the serum levels of SARS-CoV-2 IgM and IgG in patients with COVID-19 showed a statistical difference when compared to healthy controls. Detection of these specific antibodies against SARS-CoV-2 is considered a rapid test and a confirmative test for COVID-19 diagnosis and convalescence. IgM and IgG levels were higher in ICU patients than in non-ICU patients with COVID-19. In addition, ICU patients had higher antibody responses to IgG and IgM than non-ICU patients, suggesting that ICU patients were in the active phase of SARS-CoV-2 infection [19]. As reported in other studies, high levels of antibody responses in patients with COVID-19 were associated with COVID-19 severity [20, 21, 22]. In addition, a high level of B cell activation and proliferation in patients with severe COVID-19 is linked with poor clinical outcomes [23].

IgM is usually detected early on the fourth day after SARS-CoV-2 infection and starts to increase four days after the onset of symptoms, providing a rapid response [24]. In contrast, IgG is detectable after seven days and increases later (after ~14 days), providing a long-term humoral response. IgG levels are typically higher in severe rather than in mild cases, as well as in critical COVID-19 cases [25]. In other studies on patients with COVID-19, [24, 26]. IgM was detectable 4–6 days after the onset of symptoms, and its level was maintained for up to 3 weeks, before declining. Detectable levels of IgG and IgM antibodies among COVID-19 patients were attributed to a recent SARS-CoV-2 infection and a class switch to high-affinity IgG antibodies following viral infection and during the recovery phase. In the current study, levels of SARS-CoV-2-specific IgM were lower in the non-ICU group than those in the ICU group, suggesting a correlation of lower IgM with low disease severity, and indicating that these patients may gradually recover. Several published reports on corona viruses have demonstrated the involvement of antibody-dependent COVID-19 disease enhancement (ADE) in the pathogenesis of SARS-CoV-2 infection and the complexity of the role of antibodies in COVID-19 severity [27, 28, 29]. Our findings indicate that ADE may occur in ICU patients with COVID-19. Overall, the levels of IgG and IgM are important as they are indicators for disease severity and may potentially be used for disease prognosis.

Currently available data show that TLR-2 and TLR-4 are associated with hyperinflammation and severe COVID-19 [30]. TLR-4 suppresses the production of type 1 IFN and activates neutrophil extracellular traps (NETs). NET formation has been linked to sustained inflammation and the severity of COVID-19, suggesting the involvement of TLR-4 in the pathogenesis of COVID-19. In this study, the expression levels of TLR-4 mRNA were significantly elevated in ICU COVID-19 patients than in non-ICU COVID-19 patients [7]. However, the expression of TLR-3 mRNA did not show any significant difference between the two groups of patients. We also believe that selective targeting of TLR-4 could lead to the development of a new treatment for COVID-19. The innate immune system responds to viral infections immediately by producing INF mediator [7, 29]. Compared to the non-ICU patients with COVID-19 and healthy controls, the concentration of IFN-β in ICU COVID-19 patients was considerably elevated in our study. Additionally, in agreement with another study, an elevated level of IFNs was observed in hospitalized patients with COVID-19 [31]. The expression of type I IFN rapidly declined after the first three days of hospitalization. In our study, there was no statistical difference in IFN-β levels between survivors and non-survivors among ICU COVID-19 patients. A previous study contradicts our finding; they showed that survivors had a higher level of IFN-β than non-survivors [32]. Another study demonstrated that early treatment with IFN-α [33] and IFN-β [34] reduced hospital mortality; however, late therapy resulted in an increased mortality rate and delayed recovery. Thus, the timing of IFN production is important for patients with COVID-19 [33,34]. Taken together, the impaired, reduced, or delayed

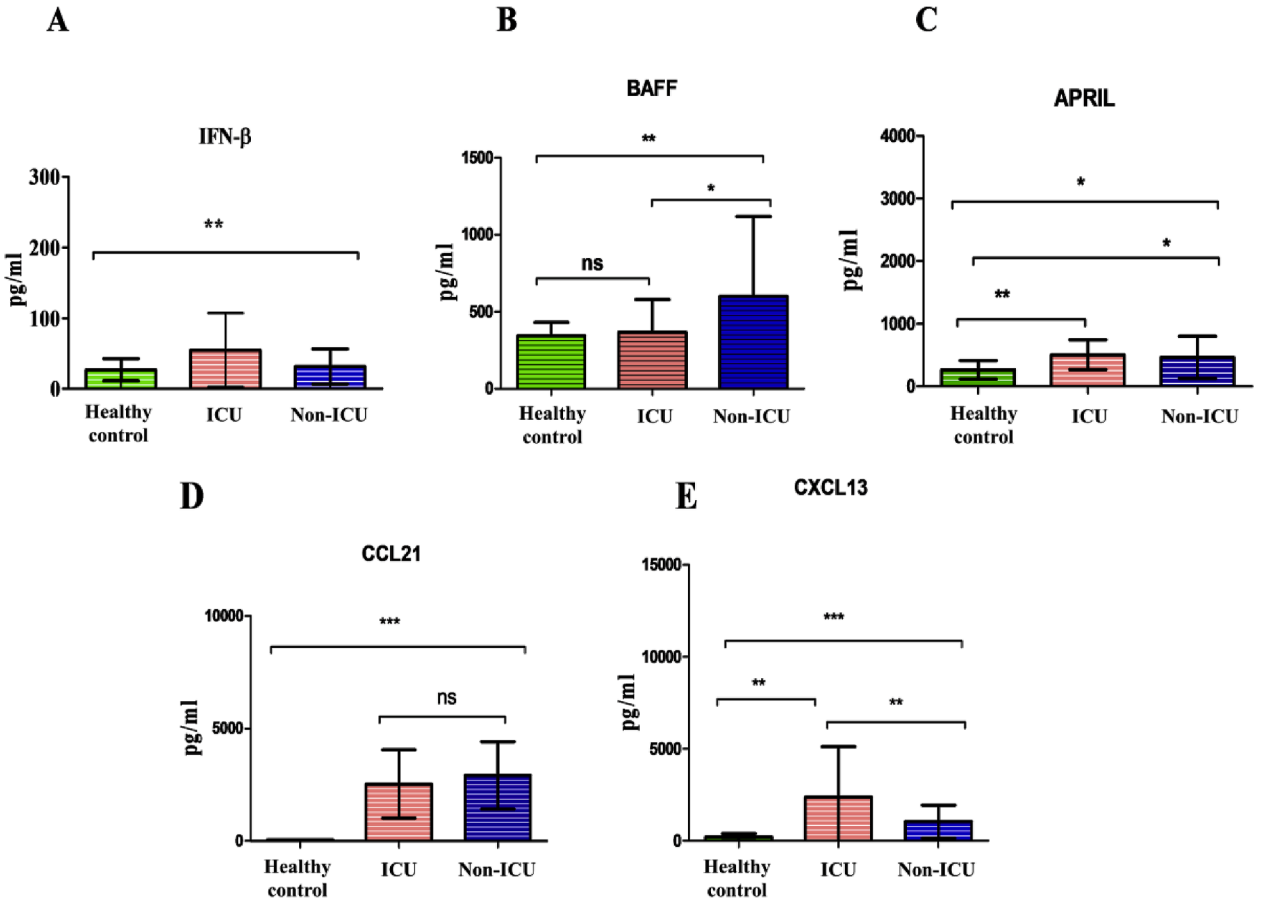

Figure 2. Blood IFN-β, BAFF, APRIL, CCL21, and CXCL13 levels in ICU and non-ICU patients with COVID-19 and healthy controls. **(A)** The concentration of IFN-β was significantly increased in ICU COVID-19 patients in comparison to that in the non-ICU COVID-19 patients and healthy control group (P **$= < 0.001$). **(B)** BAFF was significantly higher in the non-ICU COVID-19 patients compared with that in the ICU COVID-19 and healthy control group (P **$= < 0.001$). **(C)** The levels of APRIL were significantly elevated in the ICU COVID-19 patients compared with that in the non-ICU COVID-19 patients and healthy control group (P*$= < 0.01$). **(D)** The levels of CCL21 were elevated in the non-ICU COVID-19 patients compared with that in the ICU COVID-19 patients and healthy control group (P ***$= < 0.0001$). **(E)** CXCL13 was elevated in the ICU COVID-19 patients compared with that in the non-ICU COVID-19 patients and healthy control group (P***$= < 0.0001$). Values represent the means ± SD.

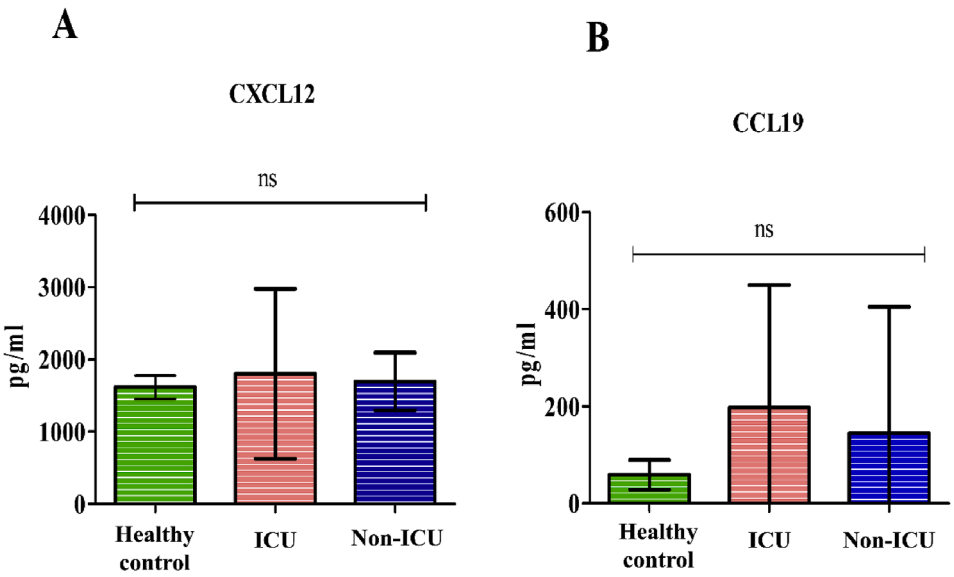

Figure 3. Concentrations of CXCL12 and CCL19 in ICU and non-ICU patients with COVID-19 and healthy controls. (A) CXCL12 and (**B**) CCL19 levels in levels in ICU and non-ICU patients with COVID-19 and healthy controls. There was no statistical significance (ns) in the serum concentrations of CXCL12 in ICU and non-ICU COVID-19 patients compared with that in the healthy control group. Values represent the means ± SD.

expression of IFNs might be attributed to a long viral incubation time, viral persistence at the site of infection, and no viral resistance; this can lead to immunopathology and a poor outcome in SARS-CoV-2 infection.

Following viral infection, BAFF and APRIL cytokines are expressed to play an important role in B cells response and antibody production [35]. Furthermore, increased levels of BAFF, but not APRIL, have been detected in the bronchoalveolar lavage (BAL) fluid of newborns with upper respiratory tract infections including RSV virus [36]. Furthermore, it has been shown that levels of BAFF protein, but not APRIL, were highly expressed post-RSV infection in mice [14]. A similar increase was shown in cases of H1N1influenza, bocavirus, rhinovirus, metapneumovirus, and mycoplasma pneumonia infections (as reviewed in [35]). These findings contradict those of Reed et al. [37], which emphasized the important role of APRIL in RSV infections. In support of our findings, few studies have investigated the role of BAFF and APRIL in COVID-19 patients. It was demonstrated that expression levels of BAFF in the plasma were higher in COVID-19 patients with positive association with B cell counts. APRIL levels, on the other hand, were elevated in recovered COVID-19 patients, while the expression pattern of both cytokines was linked with the expression of IFN type I [38]. A recent study has found that BAFF levels, but not APRIL were elevated significantly in a patient with severe COVID-19 than in the mild group, indicating a strong B cell response [39, 40]. However, in our study, we found that BAFF levels were higher in non-ICU than in ICU COVID-19 patients and the sera levels of APRIL were higher in ICU patients with COVID-19, hypothesizing that the serum level may be positively associated with the viral load of SARS-CoV-2. Even though patients with COVID-19 had higher BAFF plasma levels, that are known to activate B cells response, antibody titers in infected patients were decreased over time [40]. The role of the BAFF and APRIL cytokines following SARS-CoV-2 infection is still not fully investigated. Therefore, further studies are required to explain how BAFF/APRIL can induce B cell response and antibody production in the lungs of COVID-19 patients after SARS-CoV-2 infection.

The adaptive immune responses and homing of B and T cell responses during SARS-CoV-2 infection is better understood measuring the levels of homeostatic chemokines, including CXCL12, CXCL13, CCL19, and CCL21 in the sera of COVID-19 patients admitted and non-admitted to ICU. Migration of immune cells to the site of infection or inflammation is often mediated by chemokines and their cogent receptors [41]. CXCL12 and CXCL13 are potent B cell chemokines that can facilitate trafficking of B cells after interaction with their receptors, CXCR4 and CXCR5, respectively. CCL19 and CCL21 can lead to T cell migration after binding with their receptor CCR7 [41].

Hua et al. [42] reported that CXCL12 levels increased after SARS-CoV-2 infection relative to healthy individuals, but there were no significant differences among mild, severe, and fatal COVID-19 cases, and the levels of CXCL12 remained fixed over the different time points tested. In line with this, we found that the CXCL12 serum levels were elevated, but not significantly different between ICU and non-ICU patients with COVID-19. Taken together, CXCL12 in the sera of patients with COVID-19 should not be used as a biomarker to assess COVID-19 severity.

CXCL13 levels in the serum have been reported to increase significantly in severe COVID-19 patients higher than patients with mild or moderate disease and healthy asymptomatic groups [43]. Furthermore, CXCL13 levels were associated with elevated germinal center (GC) B cell responses and virus-specific IgG levels. A recent study reported that CXCL13 expression levels were increased in COVID-19 patients who did not survive relative to the decrease observed post-SARS-CoV-2 infection in patients who did survive; CXCL13 expression was associated with elevated specific antibodies to RBD and S1 antigens after SARS-CoV-2 infection [44]. Another study found that CXCL13 was the most relevant indicator for requiring ICU admission and predictor of death during a follow-up of

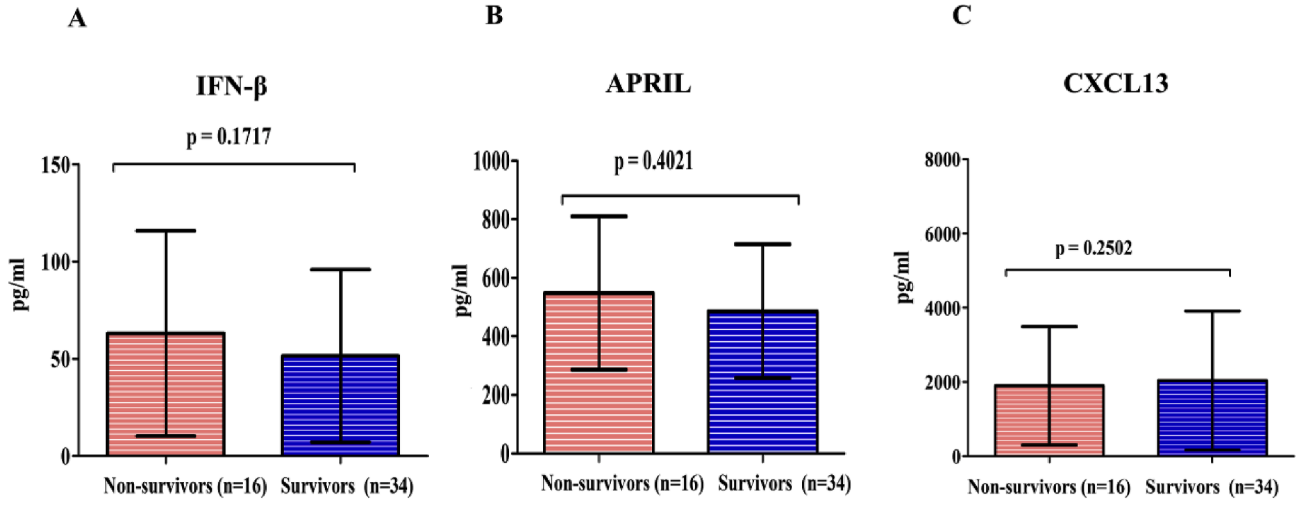

Figure 4. Serum levels of IFN-β, APRIL, and CXCL13 in survivors and non-survivors among ICU patients with COVID-19. (A) The concentration of IFN-β was not changed in the non-survival group in comparison to that in the survival group (P = 0.1717). **(B)** There was no statistical significance in the serum concentrations of APRIL between non-survival and survival group (P = 0.4021). **(C)** The levels of factor P were not significantly changed in the non-survival group in comparison to that in the survival group (P = 0.2502). Values represent the means ± SD.

COVID-19 patients [45]. Similarly, we found that CXCL13 serum levels was significantly increased in ICU patients in comparison to those in non-ICU patients with COVID-19 and healthy controls, suggesting the diagnostic utility of serum CXCL13 measurements as a predictive biomarker for disease severity.

CCL19 plasma levels were elevated significantly in COVID-19 patients with mild, moderate, and critical disease when compared to healthy individuals in a previous study [46]. Furthermore, CCL19 levels were elevated significantly in the mouse lung post-SARS-CoV-2 infection compared to those in controls, causing severe lung inflammation as well as impaired lung function [47]. CCL19 expression levels were increased in ICU COVID-19 patients and the levels of CCL19 were associated with a high mortality rate [48]. However, in this study, no significant differences in CCL19 levels between ICU and non-ICU patients were observed in comparison to the healthy group. This suggests that the expression of CCL19 in the sera of COVID-19 patients should not be used to predict COVID-19 severity.

In previous reports, CCL21 serum levels were increased significantly after 12 days of SARS-CoV-2 infection in deceased COVID-19 patients [49]. in this study, CCL21 expression levels were highly increased in the ICU and non-ICU patients compared to those in the healthy control group, and there were no significant differences between ICU and non-ICU patients, suggesting that measuring CCL21 levels may not be utilized as an early predictive biomarker for COVID-19 severity.

Some limitations were encountered during the course of this study. Measurements of cytokine and chemokine expression in the blood of COVID-19 patients may not reflect the host lung response against SARS-CoV-2. Additionally, serum specimens of COVID-19 patients were collected at one-time point; thus, it is difficult to know and predict the kinetics of the cytokines/chemokines post-SARS-CoV-2 infection. Indeed, collecting samples at different time points may contribute to defining the peak kinetics for the tested chemokines and cytokines. Furthermore, some patients in the critical group had chronic diseases prior to ICU admission. Further studies should determine the expression levels of cytokines and chemokines in the lower respiratory tract to investigate the host immune response to SARS-CoV-2 infection.

In conclusion, we found an elevated expression in the serum levels of IgM, IgG, IFN-β, APRIL, and CXCL13 in ICU patients compared to those in non-ICU patients. In contrast, levels of BAFF and CCL21 were significantly increased in non-ICU patients compared with ICU patients and healthy controls. No significant correlation was observed between increased expression of IFN-β, APRIL, and CXCL13 and ICU COVID-19 hospital deaths. Increased levels of TLR-4, IFN-β, APRIL, and CXCL13 could be used as predictive biomarkers to assess COVID-19 severity and, consequently, the targeting of these molecules may provide new therapeutic treatment for COVID-19 patients.

Declarations

Author contribution statement

Wael Alturaiki and Bandar Alosaimi: Conceived and designed the experiments.

Maaweya E. Awadalla, Haitham Alkadi, Faris Q. Alenzi, and Ayman Mubarak: Performed the experiments; Wrote the paper.

Saad Alamri, Wael Alturaiki, Abdulkarim Alfaez, Mona Awad Alanazi, Brian F. Flanagan, and Haitham Alkadi: Analyzed and interpreted the data; Wrote the paper.

Wael Alturaiki, Bandar Alosaimi, Mona Awad Alanazi and Ayman Mubarak: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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