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# Diminution of STING Expression in the Blood of Patients with Severe or Critical COVID-19 Pneumonia

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**Keywords**
COVID-19 · SARS-CoV-2 · STING · IRF3 · Cytokine storm

**Abstract**

***Introduction:*** Cytokine storm and critical COVID-19 pneumonia are caused in at least 10% of patients by inborn errors of or auto-Abs to type I IFNs. The pathogenesis of life-threatening COVID-19 pneumonia in other patients remains unknown. ***Methods:*** This study was conducted at Masih Daneshvari Hospital, Tehran, Iran. In the period of study, 75 confirmed cases of COVID-19 with presentations ranging from mild upper respiratory tract infection to lower respiratory tract infection, including moderate, severe, and critical disease, were recruited. Expression of STING mRNA was measured in peripheral blood mononuclear cells (PBMCs) and compared between patients with different severity and outcome. ***Results:*** There was a significant negative correlation between age and STING expression level ($p$ value = 0.010). Patients with "severe to critical" illness had a 20-fold lower STING expression level compared to the "mild to moderate" group ($p$ value = 0.001). Also, the results showed lower ex-

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pressions of STING in the patients admitted to the ICU (*p* value = 0.015). Patients who finally died had lower expression of STING at the time of sampling (*p* value = 0.041). ***Conclusion:*** STING mRNA expression in PBMCs was significantly lower in older COVID-19 cases, the patients with more severe illness, who needed intensive care, and who eventually died.



## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a single-stranded RNA virus belonging to the coronavirus family, was first isolated from the bronchoalveolar fluid of a patient with acute respiratory distress syndrome (ARDS) in Wuhan, China [1]. This virus causes an acute viral illness that was named coronavirus disease 2019 (COVID-19) [2]. By June 17, 2022, SARS-CoV-2 has infected more than 535 million people and caused at least 6.3 million deaths worldwide [3]. Although SARS-CoV-2 infection is often silent and COVID-19 clinical manifestations are often mild due to an infection restricted to the upper respiratory tract, the disease can progress into pneumonia, which can lead to hospitalization of 10–20% of patients [4]. In about 2% of patients, respiratory failure does occur and death is observed globally for 1% of patients (infection fatality ratio). Moderate pneumonia is not hypoxemic and can be treated ambulatorily. Severe pneumonia requires oxygen, and critical pneumonia requires high-flow oxygen or intubation and ventilation. Involvement of other organs such as myocarditis, acute renal injury, and septic shock is not uncommon [5].

The pathophysiology of COVID-19 has not yet been accurately identified. Yet, there is one major epidemiological risk factor: age. The risk of critical pneumonia or death doubles every 5 years from childhood onward [6, 7]. There is a 100-fold difference in risk between the ages of 20 and 60 years. The other epidemiological risk factors are much more modest, with ORs typically <1.5 and always <2 [6]. The risk of life-threatening disease is about 1.5 times greater in men than in women [6–8]. The SARS-CoV-2 infection triggers innate and adaptive immune responses [9]. Existing data from severe forms of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and SARS-CoV, as well as recent accumulating evidence concerning SARS-CoV-2, suggest that an inappropriate response of host immunity to the virus contributes to disease [10–12].

Recently, inborn errors of and auto-Abs to type I IFNs were found to underlie at least 10% of critical cases [13, 14]. These patients are clinically and immunologically undistinguishable from the others [15]. This led to the proposal of a two-step mechanism of disease, with insufficient type I IFN during the first week of the disease being responsible for leukocyte-driven inflammation in the lungs and elsewhere during the second and third weeks of infection [6]. Indeed, a unique pattern of immune dysregulation in these patients is cytokine production and hyperinflammation [9]. This uncontrolled response leads to an overreaction of the immune system and a cytokine storm [10]. The cytokine storm is divided into two stages: the first stage is an immune deficiency state in which the body does not react timely and sufficiently against the pathogen. Some studies suggest that the first stage is the result of impaired IFN responses characterized by a low level of IFN activity and downregulation of IFN-stimulated genes (ISGs). The subsequent secondary stage, which results in clinical symptoms of the cytokine storm, is an overactive immune response compensating for the virus clearance failure. The characteristic of this stage is excessive secretion of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-12, and tumor necrosis factor-α (TNF-α) and other chemokines such as CXCL10 and CCL2 [16–19].

Microbial sensors are an important part of the innate immune system, contributing to the sensing of nucleic acids [20]. These sensors are divided into two major groups: endolysosomal nucleic acid sensors and cytosolic nucleic acid sensors. Endolysosomal sensors are produced in plasmacytoid dendritic cells (pDCs), conventional dendritic cells (cDCs), monocyte/macrophages, and B cells. These sensor include TLR3, TLR7, and TLR8 for RNA and TLR9 for DNA. Cytosolic sensors are grouped as RNA sensors and DNA sensors. There are 3 cytosolic RNA-specific sensors: RIG-1, MDA5, and LGP2. Cytosolic DNA sensors include DAI, RNA polymerase III, IFI16, and four other sensors [20, 21]. Inborn errors of TLR3-dependent production of type I IFN can underlie the critical COVID-19 [14]. TLR3 is an endosomal sensor of dsRNA that regulates the tonic, baseline levels of type I IFNs [22]. In vitro, RIG-I and MDA5 have also been involved in the recognition of dsRNA from SARS-CoV-2 [23]. No inborn errors of this pathway have yet been reported in patients with COVID-19. STING (stimulator of interferon genes) is an endoplasmic reticulum localized protein involved in initiating the IFN-I expression cascade in response to mislocalized nucleic acids in the cytosol [24, 25]. STING is a required factor for RIG-1, RNA polymerase III, and IFI16-dependent type 1 INF production signaling path-

ways [21]. Viral nucleic acid and other cytosolic DNAs such as cancerous cells or mitochondrial DNA (mtDNA) can trigger a cyclic GMP-AMP synthetase (cGAS) dependent pathway which results in the production of cyclic GMP-AMP (cGAMP) as the second messenger. cGAMP then binds to STING on the endoplasmic reticulum and translocates it to the Golgi apparatus, where it activates TANK-binding kinase-1 (TBK1) by autophosphorylation. This cascade goes on and activated TBK1 phosphorylates interferon regulatory factor 3 (IRF3) and nuclear factor κB (NF-κB), which then translocate to the nucleus to induce transcription of inflammatory genes such as IFNs [24–31]. IFN-I has potent antiviral activity, but delayed IFN-I production leads to higher expression of proinflammatory cytokines and chemokines (see above) [32]. As a first approach to the testing that inborn errors of STING can underlie critical COVID-19, we tested the hypothesis that STING expression in peripheral blood mononuclear cells (PBMCs) may correlate with COVID-19 severity [33, 34]. We analyzed the expression of STING in 75 COVID-19 patients along with their clinical characteristics.

## Materials and Methods

*Patients*

This study was conducted at Masih Daneshvari Hospital, Tehran, Iran. In the period of study, 75 confirmed cases of COVID-19 with presentations ranging from asymptomatic to upper respiratory tract infection and lower respiratory tract infection were recruited. Patients with a history of autoimmune disease or immunocompromised state were excluded. Diagnosis of COVID-19 was confirmed by the real-time reverse transcription-polymerase chain reaction (RT-PCR) for SARS-CoV-2 on nasopharyngeal samples, as previously described [35].

For the purpose of this study, we categorized the patients by disease severity into four groups as mild, moderate, severe, and critical. The lowest amount of oxygen saturation in the course of the disease was used to determine the severity of the disease based on the US National Institutes of Health (NIH) guideline for COVID-19. Cases with normal chest imaging were classified as mild. Patients with abnormal chest imaging and $O_2$ saturation ($O_2$ sat) equal to or higher than 94% were categorized as moderate and the cases with $O_2$ sat less than 94% were classified as severe disease. The patients who needed noninvasive or mechanical ventilation; or critical care were categorized as critical group [36]. Also, at rest, $O_2$ saturation in the room air at the time of sampling was recorded.

The study was approved by the Research Medical Ethics Committee of Shahid Beheshti University of Medical Sciences (Approval number: IR.SBMU.NRITLD.REC.1399.061). The study was conducted in accordance with the Declaration of Helsinki and institutional ethics guidelines. Written informed consent was obtained from all the patients for the use of clinical data and blood samples.

One fresh blood sample was obtained from any patient and PBMCs were separated up to 6 h after sampling. On the same day, another blood sample was obtained to evaluate C-reactive protein (CRP) and complete blood count (CBC) parameters such as white blood cell (WBC), polymorphonuclear (PMN) cell, lymphocyte, and monocyte count.

*Extraction of Total RNA*

Total RNA extraction was carried out using RiboEX solution (GeneAll Biotechnology Co., Ltd., Songpa-gu, South Korea) according to the manufacturer's instructions and followed by the treatment with DNase I. The quantity of extracted RNAs was evaluated with Nanodrop (Thermo Scientific™ NanoDrop 2000) spectrophotometer analyses. The absorption ratio in 260/230 nm and 260/280 nm was assessed and the ratio between 1.8–2.2 and 1.7–1.9 was considered as proper values. Then nucleic acids were separated using agarose gel 1% electrophoresis.

*Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)*

Complementary DNA (cDNA) was designed and synthesized using BioFact™ RT-Kit (BioFACT, Daejeon, Korea) as directed by the manufacturer's suggested protocol in a 10 μL reaction mixture including 1 μL oligo dT primer, 1 μL random hexamer primer, 1,000 ng/μL RNA, and 10 μL master mix, this mixture was then incubated at 65°C for 5 min and incubate mixture 1 min on ice after adding the master mixture incubate 50°C for 60 min and later 70°C for 10 min.

*Quantitative Real-Time PCR*

In this investigation the STING expression was evaluated in COVID-19, To evaluate the expression levels the synthetized cDNA was subjected to quantitative real-time PCR using LightCycler 480, TB Green™Premix Ex Taq™ II (TliRNaseH Plus) (RR820R, TAKARA BIO, Japan) as directed by the manufacturer's instructions. The master mix including water, primers, and SYBR Green was prepared for all the reactions and an additional sample as the negative control (NTC). The qPCR reaction mixture was preheated to 95°C for 10 s, followed by 40 cycles consisting of 95°C for 15 s, 60°C for 30 s, and 72°C for 20 s. Specific primers to detect the expression level of STING include sense primer 5′-TGTCATCTGCAGGTTCCTGGT-3′ and antisense primer 5′-GCCATGTCACAATACAGTCAAGC-3′. β-Actin was used as an internal control. The relative expression levels of STING were normalized by β-actin.

*Statistical Analysis*

For data analysis, we used SPSS (version 26.0; IBM, Armonk, NY, USA) and GraphPad Prism (version 8.4.3; GraphPad, La Jolla, CA, USA). We compared STING expression levels between different groups with the Kruskal-Wallis test. To compare STING expression between two groups, the Mann-Whitney U test was used. We used Students' *t* test to compare peripheral blood parameters between mild to moderate and severe to critical groups. We analyzed the relation between patients' age, weight, body mass index (BMI), and peripheral blood parameters with STING expression level by Spearman's correlation. *p* value <0.05 considered significant.

**Table 1.** Demographic and characteristics of 75 COVID-19 cases

| | Patients, *n* (%) | | | | |
|---|---|---|---|---|---|
| | total (*n* = 75) | mild[1] (*n* = 5) | moderate (*n* = 13) | severe (*n* = 18) | critical (*n* = 39) |
| Age | | | | | |
| ≤60 years | 56 (74.7) | 5 (100) | 12 (92.3) | 15 (83.3) | 24 (61.5) |
| >60 years | 19 (25.3) | 0 (0.0) | 1 (7.7) | 3 (16.7) | 15 (38.5) |
| Gender | | | | | |
| Male | 45 (60) | 5 (100) | 7 (53.8) | 8 (44.4) | 25 (64.1) |
| Female | 30 (40) | 0 (0.0) | 6 (46.2) | 10 (55.6) | 14 (35.9) |
| Underlying disease | | | | | |
| Hypertension | 12 (16.9) | 0 (0.0) | 1 (7.7) | 0 (0.0) | 11 (29.7) |
| Diabetes mellitus | 10 (14.1) | 0 (0.0) | 1 (7.7) | 4 (23.5) | 5 (13.5) |
| Chronic heart diseases | 3 (4.2) | 1 (25.0) | 0 (0.0) | 0 (0.0) | 2 (5.4) |
| Chronic lung diseases | 4 (5.6) | 0 (0.0) | 1 (7.7) | 3 (17.6) | 0 (0.0) |
| ICU admission | 39 (52.0) | 0 (0.0) | 0 (0.0) | 1 (5.6) | 38 (97.4) |
| $O_2$ saturation[2] ≤90% | 37 (49.3) | 0 (0.0) | 0 (0.0) | 7 (38.9) | 30 (76.9) |
| IVIG treatment | 20 (26.7) | 0 (0.0) | 0 (0.0) | 1 (5.6) | 19 (48.7) |
| Final outcome | | | | | |
| Recovered | 59 (78.7) | 5 (100) | 13 (100) | 17 (94.4) | 24 (61.5) |
| Died | 16 (21.3) | 0 (0.0) | 0 (0.0) | 1 (5.6) | 15 (38.5) |

ICU, intensive care unit; IVIG, intravenous immunoglobulin. [1] Based on the lowest amount of oxygen saturation in the course of the disease and NIH guideline (see the text). [2] At the time of sampling.

**Table 2.** Laboratory data of 75 COVID-19 cases

| Laboratory data | Total (*n* = 75) | Mild to moderate[1] (*n* = 18) | Severe to critical (*n* = 57) | *p* value |
|---|---|---|---|---|
| CBC | | | | |
| WBC, mean±SD, cells/mm$^3$ | 10,557.0±7,119 | 6,783.3±2,329 | 11,770.0±7,629 | 0.000 |
| PMN %, mean±SD | 69.1±17 | 58.7±14 | 72.4±17 | 0.003 |
| PMN count, mean±SD, cells/mm$^3$ | 7,958±6,686 | 4,302.8±3,165 | 9,132.5±7,103 | 0.000 |
| Lymphocyte %, mean±SD | 21.8±15 | 30.9±12 | 18.8±15 | 0.003 |
| Lymphocyte count, mean±SD, cells/mm$^3$ | 1,752±1,775 | 1,824.3±608 | 1,728.9±2,016 | 0.844 |
| Monocyte %, mean±SD | 8.3±10 | 8.9±3 | 8.1±12 | 0.773 |
| Monocyte count, mean±SD, cells/mm$^3$ | 682±654 | 550.4±272 | 724.7±734 | 0.329 |
| CRP, mean±SD, mg/L | 28.9±23 | 23.3±28 | 31.2±21 | 0.217 |

WBC, white blood cell; PMN, polymorphonuclear leukocyte; CRP, C-reactive protein. [1] Based on the lowest amount of oxygen saturation in the course of the disease and NIH guideline (see the text).

## Results

### *Patients' Characteristics*

A total of 75 patients were included in the study, 45 (60%) males and 30 (40%) females. The mean age of patients was 48.3 years (median 49.5, range 14–86 years). Twenty-nine (40.8%) individuals had minimally one underlying disease: ten (14.1%) diabetes mellitus, 3 (4.2%) chronic heart diseases, 12 (16.9%) hypertension, 4 (5.6%) chronic pulmonary disease, and 15 (21.4%) patients were obese (BMI ≥ 30). Concerning disease severity, the cases were divided into four groups: mild (*n* = 5, 6.7%), moderate (*n* = 13, 17.3%), severe (*n* = 18, 24.0%), and critical (*n* = 39, 52.0%). Among the 60 years older patients, 15 (78.9%) cases had a critical illness. Thirty-seven (49.3%) individuals had $O_2$ saturation of lower than or equal to 90% at the time of sampling. Thirty-nine (52.0%) individuals were admitted to the ICU in the course of the disease. Twenty

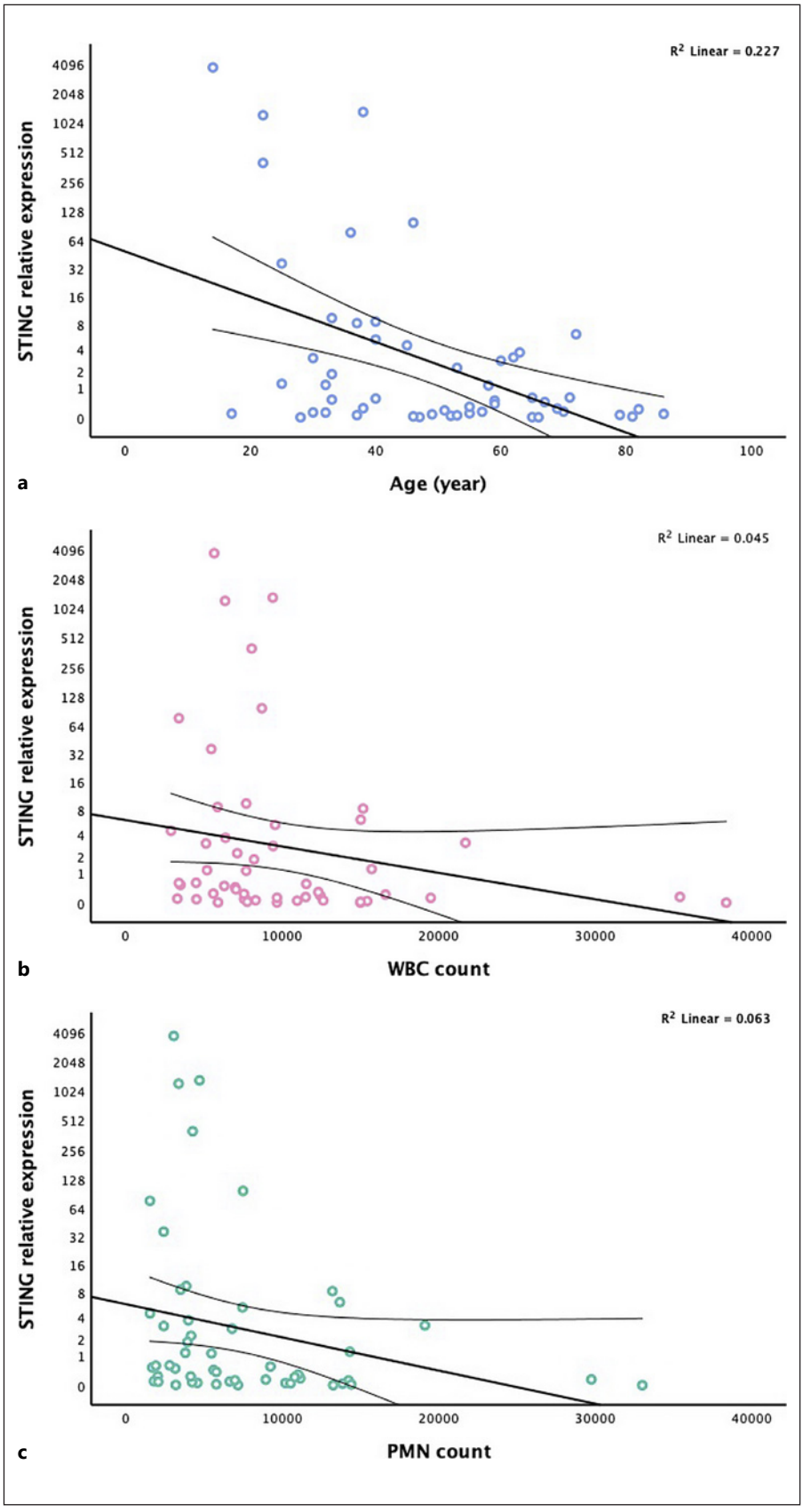


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**Fig. 1. a** Correlation of STING expression and Age was evaluated by Spearman's correlation. **b** Correlation of STING expression and WBC count was tested by Spearman's correlation. **c** Correlation of STING expression and PMN count was tested by Spearman's correlation. WBC, White blood cell; PMN, Polymorphonuclear leukocyte.

(26.7%) of patients received intravenous immunoglobulin (IVIG) before sampling. Demographics and characteristics of patients were summarized in Table 1.

*Laboratory Parameters*

Regarding the CBC parameters, there was a significant reduction in the percentage of lymphocyte in severe to critical group compared to mild to moderate group. The percentage of PMN, as well as WBC and PMN count, significantly increased in severe to critical cases. Also, in severe to critical cases, the monocyte/lymphocyte ratio was notably higher ($p$ value = 0.014). Laboratory data of patients are summarized in Table 2.

*Peripheral Blood Cells Changes following IVIG Therapy*

Patients who received IVIG had a notably higher WBC count in contrast with others ($p$ value = 0.021). Also, there was a significant increase in PMN count in patients who received IVIG ($p$ value = 008). There was no significant difference in monocyte count and lymphocyte count in patients who received IVIG compared to patients who did not ($p$ value = 0.503 and 0.281, respectively).

*STING Expression and Patients' Characteristics*

There was a significant negative correlation between age and STING expression level (Spearman' $\rho = -0.355$; $p$ value = 0.010) (shown in Fig. 1a). There was no significant correlation between STING expression level and patients' weight and BMI.

*STING Expression and Peripheral Blood Cells*

We evaluated the STING expression correlation to CBC parameters. STING relative expression was negatively correlated to WBC count (Pearson $r = -0.359$; $p$ value = 0.009). Also, there was a significant negative correlation between STING relative expression and PMN count (Pearson $r = -0.389$; $p$ value = 0.004). There was no significant correlation between STING expression and lymphocyte and monocyte counts (shown in Fig. 1b, c).

*STING Expression in Different Disease Severity and Outcome*

The more severe the disease, the lower expression of the STING was observed. Regarding NIH classification, there was no significant difference in STING expression in four groups of disease severity ($p$ value = 0.109) (shown in Fig. 2a). For better analysis, we combined the patients with mild and moderate diseases in one group and the patients with severe and critical diseases in another group. Comparing STING expression levels in "mild to moderate" and "severe to critical" groups revealed a 20-fold reduction in expression levels in "severe to critical" patients ($p$ value = 0.001) (shown in Fig. 2b). Also, we examined the expression of STING in patients with $O_2$ saturation lower than or equal to 90% at the time of sampling compared to patients with $O_2$ saturation 90% or higher. The results showed lower expressions of STING in patients with $O_2$ saturation lower than 90%. The geometric mean declined almost 5 times in the $O_2 \leq 90$ group compared to the $O_2 > 90$ group ($p$ value = 0.082) (shown in Fig. 2c). Concerning ICU admission, the STING expression was significantly lower in patients admitted to the ICU. There was an almost 9-fold decline in the ICU group compared to the non-ICU group ($p$ value = 0.015) (shown in Fig. 2d). Patients who finally died had lower expression of STING at the time of sampling. The reduction in STING expression was almost 1/11 ($p$ value = 0.041) (shown in Fig. 2e). The geometric mean fell by almost 6 times in the patients with a history of IVIG therapy compared to the others. Although there was not any statistically significant difference in the level of STING expression concerning prior IVIG therapy (shown in Fig. 2f).

## Discussion

Our knowledge about the pathophysiology of COVID-19 is incomplete but growing. Recent findings hypothesize that virus-induced immune system dysregulation can lead to macrophage activation syndrome and cytokine storm [18]. The cGAS-STING pathway is a DNA sensor and has an important role against DNA virus infections such as herpesviruses, adenoviruses, cytomegalovirus, and papillomavirus [37, 38]. It seems that the cGAS-STING pathway can be triggered by host damage-associated molecular patterns (DAMPs) such as mtDNA released as by-products of SARS-COV-2 reproduction (similar to Dengue virus as another RNA virus) and tissue injury [38–40]. STING also participates in defending against RNA virus infections through a non-conventional pathway executed by retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) [41]. Furthermore, retroviruses such as the human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), and murine leukemia virus also activate the cGAS-STING pathway due to the reverse transcriptase enzyme activity which results in the proviral DNA formation. Finally, STING can be activated by viral membrane fusion, independent of its DNA sensing capability [38, 42, 43].

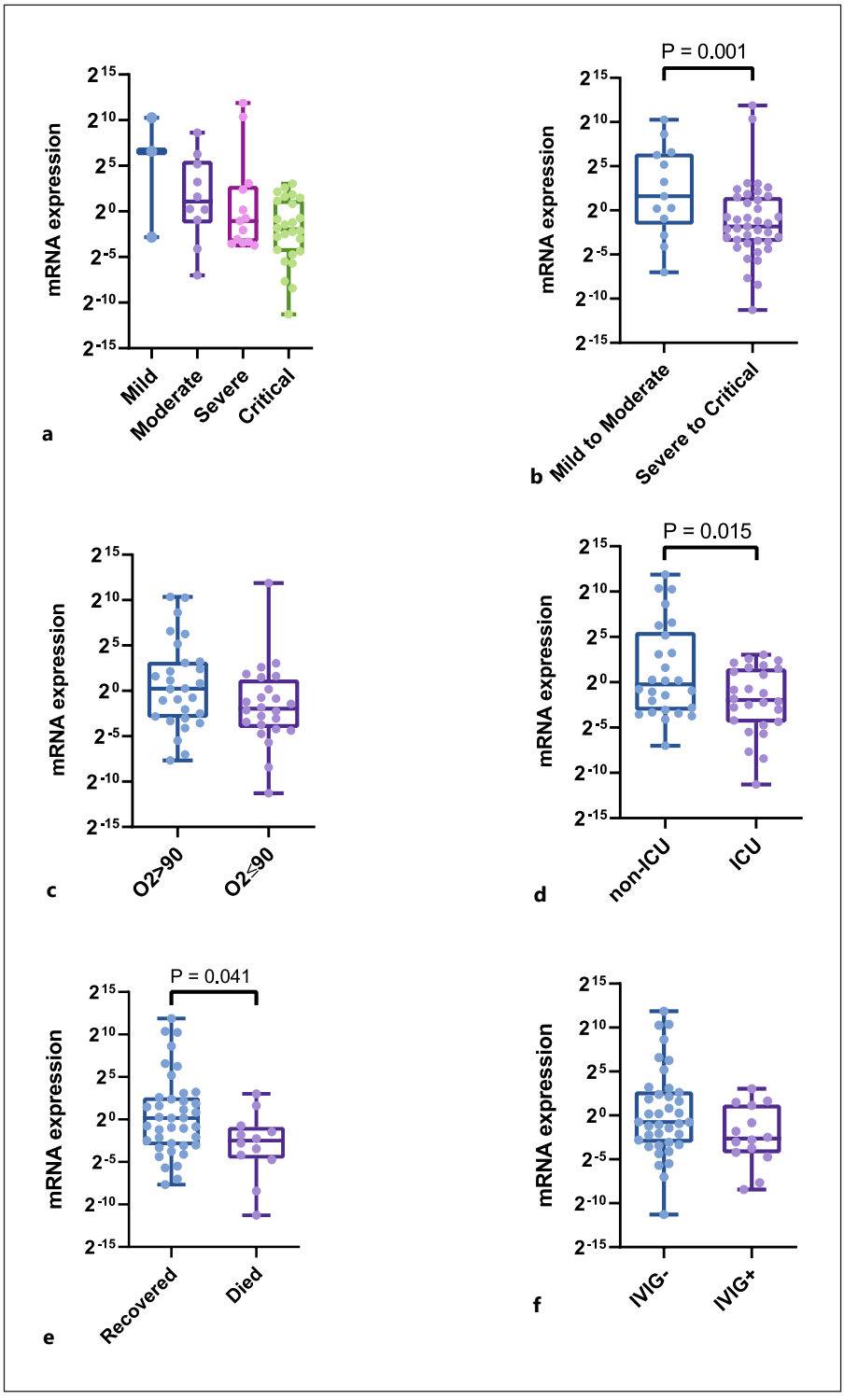


**Fig. 2.** **a** Comparison of STING expression levels among mild, moderate, severe, and critical COVID-19 patients according to NIH classification was analyzed with a Kruskal-Wallis test. Mann-Whitney U test was used for. **b** Comparison of STING expression levels among "mild to moderate" and "severe to critical" COVID-19 patients according to NIH classification. **c** Comparison of STING expression levels between patients with $O_2$ Saturation equal or lower than 90% and higher than 90% at the time of sampling. **d** Comparison of STING expression levels between patients who were admitted to ICU and patients who did not. **e** Comparison of STING expression levels between patients recovered and patients who died and, **f**: Comparison of STING expression levels between patients who received IVIG and patients who did not.

Berthelot et al. [33, 34, 44] hypothesized that delayed hyperactivation of STING contributes to cytokine storm and severe COVID-19. They used several shreds of evidence to support their idea. The high metabolic demand of flight in bats (with a high capacity to coexist with coronaviruses) causes much DNA damage and the release of self-DNA into the cytoplasm. Thus, to prevent overactivation of STING and excessive inflammatory response,

STING activation is decreased [45]. Also, they showed a STING-associated mutation that results in hyperactivation of type 1 IFN and induces SAVI disease (STING-associated vasculopathy with onset in infancy). SAVI presents with pulmonary inflammation, vasculitis, and endothelial-cell dysfunction in affected children, mimicking many signs and symptoms of COVID-19 [46]. Furthermore, STING is mostly expressed in humans in three subsets of cells: pulmonary alveolar epithelial cells, endothelial cells, and spleen cells. Interestingly, they are also the most important cells in COVID-19 pathogenesis. Also, they assumed STING overactivation and polymorphisms are associated with aging and metabolic disorders such as obesity and cardiovascular diseases and it can explain the impact of comorbidities on the development of severe COVID-19 [47–49].

To test the role of STING in COVID-19, we performed this study. As we reported above, such as Berthelot et al.'s statements [34], there is a correlation between STING expression and disease severity in COVID-19 patients. In severe to critical patients, an expectant decrease in STING expression regardless of worsening of the patient's status was observed. This sudden alteration may be because of various reasons we discuss below.

Some studies explained various negative feedback mechanisms of STING, which are suitable explanations to justify our results. Mudla et al. [50] demonstrated that IFN-I action is controlled by a regulatory network composed of a fast-acting positive feedback loop and a delayed negative feedback loop. Also, Maekawa et al. [51] in an animal model of Medaka fish, showed that long-term and excessive IFN stimulation leads to reduced sensitivity to the IFN signal through a negative feedback loop. Some studies reported that tripartite motif protein (TRIM) 29 and 30α, ubiquitin E3 ligase, acts as a negative regulator of innate immune response in the STING-TBK1-IRF3 signaling pathway [52, 53]. Based on some studies, STING-mediated negative feedback of interferon-inducible protein 16 (IFI16) restricts IFN-I overproduction during antiviral immunity to prevent autoimmune diseases [20, 49, 54]. Interferon-inducible human oligoadenylate synthetase-like (OASL), another STING pathway regulator, directly binds to cGAS independent of dsDNA, resulting in a non-competitive inhibition of the second messenger cGAMP production [55]. Also, the STING pathway consequently increases the expression of both RIG-I and IL-6. Wu et al. [56] showed that STING degradation, which is mediated by RIG-I and IL-6, can be considered as another negative feedback mechanism. These findings increase the possibility of the existence of a negative feedback loop in the STING pathway. In the early phase of COVID-19, STING downstream activation results in cytokine release following IFN secretion to confront virus replication. At a point, a negative feedback loop starts to decrease the STING pathway to inhibit autoimmunity and cytokine storm. On the other hand, auto-Ab to INF or inborn errors might underline low STING expressions [57, 58].

Other mechanisms of cytokine storm without the interference of the STING pathway are notable subjects we should consider. After virus recognition, downstream transduction pathways, which are crucial for a proper antiviral response, such as IRF3 (IFN regulatory factor-3), nuclear factor κB (NF-κB), Janus kinase/signal transducer, and activator of transcription (JAK/STAT) signaling pathways, are activated [59–62]. IRF3-NF-κB production was mentioned as a part of the STING pathway. JAK/STAT is activated by the cytokine IL-6, which has been reported to be dramatically increased in COVID-19 patients [63–67]. In turn, the activation of the JAK/STAT pathway has been reported to stimulate the production of IL-6, thus establishing a positive inflammatory feedback loop [68]. As mentioned, the STING cascade is not the only pathway in the inflammatory response in COVID-19 patients. Thus, it may be a reason for less STING expression even though cytokine storm results in severe symptoms in COVID-19 patients. On the other hand, STING not only is involved in INF secretion but also is concerned with the antiviral response by induction of autophagy. The STING-induced autophagy results in the clearance of both cytosolic DNA and DNA of viruses [38, 69]. Thus, in COVID-19 patients, STING expression may increase to induce autophagy and other unknown STING functions except for IFN production [42].

SARS-COV-2 can evade the STING pathways, antagonize the IFN pathway, and thus, escape recognition [70–72]. It is suggested that SARS-CoV-2 can suppress the IFN-I response using similar mechanisms as SARS-CoV. Many viral proteins encoded by the virus, namely Nsp1, Nsp3d/Papain-like protease (PLpro), Nsp7, Nsp15/EndoU, Nsp16, ORF3, ORF6, ORF8, ORF9b, M, and N, can modulate innate antiviral response [73, 74]. Our study showed that patients with severe symptoms have less STING expression level compared to patients with mild disease. It can be proposed that in mild to moderate cases, STING activation results in virus elimination and prevents more cellular damage and severe symptoms. In favor of this theory, a recent study performed by Humphries et al. [75] shows that intranasal administration of a STING agonist before or even after virus infection can protect

mice from severe respiratory disease. But in patients with severe symptoms, as the virus impedes the STING pathway, it extensively replicates and harms cells.

It should be considered that some medicines such as Aspirin, Vitamin D, corticosteroids, and IVIGs can reduce the STING expression [76–78]. As the result shows, IVIG does not contribute to monocyte and lymphocyte count changes hence it may not have any significant influence on STING expression level.

Based on our results, the STING expression level is lower in elderly patients. So, the lower STING expression in the severe to the critical group may be partly influenced by their older age. Consistently, some studies have shown that the IFN production in DCs of lungs and blood is impaired in the elderly population in contrast to high basal levels of proinflammatory cytokines and chemokines in the blood [79].

Also, some individuals may have low STING expression levels in PBMC in physiological situations and consequently pathology situations. Severe symptoms in these patients may be due to this low STING expression and subsequent extensive virus replication. More viral load results in more cellular damage until other IFN secretion mechanism gets activated. STING expression level changes may not be exactly connected to the infection phase and may mostly be due to genetic variation between patients which results in different STING expression levels and different prognosis [14, 80].

To our knowledge, this study is the first original work concerning the link between STING and the severity of COVID-19 disease. Our study had some limitations. We did not know about the patients' basal STING levels in the normal situation. Also, we did not measure STING expression level at separated phases of the disease for each patient (during infection progress, recovery, and after recovery). A better alternative would be measuring STING expression in lung tissue. This hypothesis can be proved by performing an autopsy on patients with COVID-19 related death.

## Statement of Ethics

The study was conducted in accordance with the Declaration of Helsinki and institutional ethics guidelines. The study protocol was reviewed and approved by the Research Medical Ethics Committee of Shahid Beheshti University of Medical Sciences, approval number: IR.SBMU.NRITLD.REC.1399.061. Written informed consent was obtained from all the patients for the use of clinical data and blood samples.

## Conflict of Interest Statement

The authors have no conflicts of interests to declare.

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## Author Contributions

Mitra Rezaei and Majid Marjani designed the research study. Mitra Rezaei, Hadiseh Mohammadpour, Farinaz Nasr Azadani, Niloufar Bineshfar, Alireza Mirahmadi, and Zahra Amanzade performed the research. Alireza Mirahmadi and Niloufar Bineshfar wrote the manuscript draft. Seyed Ali Ziai and Niloufar Bineshfar analyzed the data. Mihan Pourabdollah Toutkaboni, Payam Tabarsi, Jean Laurent Casanova, and Davood Mansouri contributed essential reagents or tools. Majid Marjani, Jean Laurent Casanova, and Davood Mansouri revised the paper.

## Data Availability Statement

The data in this study was obtained from Shahid Beheshti University of Medical Sciences where restrictions apply. Such dataset may be requested from the corresponding author, Majid Marjani.

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