Low systemic IFN response and high viral load are associated with COVID-19 disease severity in unvaccinated patients in Kenya, 2022-2023

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Abstract

Cellular and humoral responses, as well as virus replication kinetics, may affect the severity of COVID-19. This study examined systemic and mucosal immune responses as well as viral load in unvaccinated SARS-CoV-2 patients. Forty-eight COVID-19-positive, grouped into asymptomatic, moderate and severe disease, and 48 COVID-19-negative individuals at Moi Teaching and Referral Hospital in Kenya were included. Severe patients showed higher viral loads and systemic anti-spike IgG compared to moderate and asymptomatic individuals. Asymptomatic individuals had higher mucosal anti-spike IgG and receptor binding domain (RBD) levels compared to severe patients. Systemic IFN-α mRNA transcript was expressed at higher levels in asymptomatic individuals compared to patients with severe COVID-19 and healthy individuals. Severe patients had significantly lower expression of IFN-γ mRNA transcript levels in both blood and mucosa, as well as significantly lower systemic IFI-16 mRNA transcript levels. These results suggest that mucosal anti-spike and RBD IgG may offer protection, while systemic antibodies indicate disease progression. Suppressed interferon responses, both mucosal and systemic, were linked to severe disease. To conclude, viral load, IFN, anti-viral, and systemic IgGs could help predict COVID-19 outcomes and aid in developing personalized treatment strategies.

Keywords: COVID-19 severity$_1$, IgG$_2$, viral load$_3$, inteferon$_4$, mucosal$_5$, systemic$_6$

INTRODUCTION

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is often of moderate severity, although severe and life-threatening symptoms are not uncommon [1]. While much has been learned with regards to severe COVID-19 [2], [3], there is still a need for a better understanding of the risk factors associated with severe disease. In particular, there is a lack of knowledge regarding the viral kinetics and immune response to infection of the SARS-CoV-2 Omicron variant in unvaccinated individuals. While SARS-CoV-2 vaccines strongly protect against severe COVID-19, for all existing viral variants, including after Omicron [4]Large populations worldwide are not yet vaccinated, highlighting the importance of understanding risk factors for Omicron-mediated severe COVID-19 in unvaccinated populations.

The risk of developing severe COVID-19 is associated with several host factors, including age, sex, and underlying comorbidities such as diabetes, hypertension and pulmonary, kidney, liver and heart diseases [5]Severe COVID-19 has furthermore been associated with viral load and antiviral immune responses, in particular, antibody and type I and II interferon responses [6]. Patients developing a more severe illness often present with higher nasopharyngeal SARS-CoV-2 RNA levels than patients developing milder disease [7], [8], [9]. To effectively protect the host against viral infections, type I interferons (type I IFN; mainly IFN-α and IFN-β) and type II IFN-II (IFN-γ) are essential [10]. Type I IFNs, produced in response to viral infection, activate hundreds of genes known as interferon-stimulated genes (ISGs) [11], [12]with a variety of antiviral functions. Importantly, type I IFNs seem to play a major role in protection against severe COVID-19 [13] indicating that it is also important against the Omicron variants. IFN-γ and other cytokines are secreted by activated T cells and NK cells [14], which contributes to the activation of antiviral immune responses, thereby potentially also contributing to protection against severe disease. Immune features linked to abortive/transient compared to sustained infection have been identified, including an early mucosal IFN response in mild infections [15].
As of 2024, Kenya had 344,130 confirmed COVID-19 cases, with 5,689 deaths [16]. Here, to better understand risk factors for severe Omicron-caused COVID-19, we investigated viral load, antibody levels, and innate immune responses in 48 SARS-CoV-2-infected unvaccinated individuals, diagnosed at the Moi Teaching and Referral Hospital (MTRH) in Eldoret, Kenya, from May 2022 to February 2023. Individuals were grouped by severity: asymptomatic, moderate and severe disease. Samples were analyzed for SARS-CoV-2 RNA levels, antibody responses and IFN-α, IFN-β, IFN-γ, and IFI-16 gene expression levels.

METHODS

Ethical approval

This study was approved by the Institutional Research and Ethics Committee of the Moi University, Faculty of Health Sciences (FAN 0003660, Eldoret, Kenya). All COVID-19 patients, asymptomatically infected and healthy controls included in the study, provided written informed consent for participation.

Study subjects and sampling

In this cross-sectional hospital-based study, 48 COVID-19-diagnosed individuals at the Moi Teaching and Referral Hospital (MTRH) in Eldoret, Kenya, were included. Patients from COVID-19 isolation facilities and normal wards and travelers who tested positive for SARS-CoV-2 at the MTRH testing center were asked to participate. Healthy individuals (n = 48) with no ongoing COVID-19 were included as controls.

The participants were scored into three categories stratified according to disease severity [37]; asymptomatic disease (needing no medical attention; n=16); moderate disease (requiring hospitalization but not intensive care; n=16), and severe disease (requiring intensive care; n=16). Participants were included from May 2022 to February 2023. Naso-oropharyngeal (NP/OP) and peripheral blood samples were collected.

The study employed a purposive sampling technique. The hospital recruited symptomatic patients prior to their admittance to the wards and the COVID-19 Isolation Center. The MTRH COVID-19 testing facility recruited asymptomatic individuals from travelers who tested positive for SARS-CoV-2. Once the initial participant satisfied the inclusion requirements, every third COVID-19-positive patient and every fifth COVID-19-negative traveler were added to the study until a total of 48 patients were enrolled.

Inclusion-exclusion criteria

The following three (3) requirements were met by COVID-19 cases: a positive RT-qPCR SARS-CoV-2 test, clinical symptoms confirmed by a doctor, and the capability to participate as assessed by the ability to agree. Similar to the positive subjects, the COVID-19 negative group had a history of underlying illnesses and tested negative for RT-qPCR SARS-CoV-2.

COVID-19-vaccinated persons, pregnant women, and those with certain chronic diseases like HIV and TB were not included. Chronic noncommunicable diseases were recorded, including diabetes, hypertension, asthma, and disorders of the heart, kidneys, and liver.

Viral load quantification

NP/OP samples (n=48) were obtained at study enrollment and stored at -80^0C until analysis. RNA extraction was carried out using the QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany). The High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific®, Uppsala,

Sweden) was then used for cDNA synthesis. qPCR for semi-quantification of SARS-CoV-2 RNA levels, using a standard curve of serially diluted plasmid, was performed as earlier described [8] using a CFX96 Real-Time PCR (Bio-Rad, Solna, Sweden).

Quantification of type I IFN, type II IFN and IFI-16 mRNA levels

RNA was extracted from NP/OP and blood using an ISOLATE II RNA Mini kit (Meridian Bioscience, Cincinnati, USA). The concentration of RNA was measured using a DS-11 FX/FX+ integrated UV-vis spectrophotometer (DeNovix, Wilmington, USA). Subsequently, the iScript cDNA Synthesis kit (Bio-Rad) was utilized to synthesize cDNA from 1 mg of RNA. cDNA and primers specific for IFN-α, IFN-β, IFN-γ, and IFI-16, and reference genes actin and GADPH were then added to iQ SYBR Green Supermix (Bio-Rad) and the qPCR reaction was run on a CFX96 Real-Time PCR system (Bio-Rad). mRNA expression values in each severity group were normalized against mRNA expression in the control group.

Quantification of SARS-CoV-2 spike, receptor binding domain and nucleocapsid-specific IgGs

NP/OP and blood samples were 10-fold diluted and then analyzed for anti-spike, anti-receptor-binding domain (RBD), and anti-nucleocapsid protein IgG using the V-PLEX SARS-CoV-2 Panel 2 kit (Meso Scale Diagnostics, Maryland, USA), according to the manufacturer´s instructions.

Statistical analysis

Descriptive statistics with frequency analysis are presented for categorical variables. Means with standard deviation (SD) are presented for normally distributed continuous variables, and medians with interquartile range (IQR) are presented for non-normal continuous variables. Chi-square, unpaired t-test, Mann-Whitney U-test, one-way analysis of variance (ANOVA) or Kruskal-Wallis tests were utilized as appropriate to compare between groups. Correlation coefficients between continuous variables were calculated using the Spearman test. Statistical analysis was performed using GraphPad Prism version 9.0 (GraphPad Software, La Jolla, CA, USA). A p-value <0.05 was considered statistically significant.

RESULTS

Description of the cohorts

The COVID-19 groups were well-matched regarding age and biological sex. The body temperature did not differ between the groups, but the level of oxygen saturation was significantly lower in the severe COVID-19 cases compared to the other groups (**Supplementary Table 1**). Patients with severe COVID-19 were more likely to have comorbid conditions compared to moderately symptomatic patients and asymptomatic individuals including diabetes (50%, 37.5% and 18.75%), liver diseases (50%, 37.5% and 50%), pulmonary disease (25%, 25% and 12.5%), chronic kidney disease (31.25%, 12.5% and 6.25%), heart disease (25%, 18.75% and 18.75%), asthma (43.75%, 25%, 6.25%), and heart disease (25%, 18.75%, 18.75%).

Patients with severe COVID-19 had high systemic anti-SARS-CoV-2 IgG titers early after symptom onset

Blood and NP/OP samples were collected during the acute phase of infection, with days post-symptom onset recorded for study subjects. We analyzed blood, i.e., systemic IgG responses. In general, the highest levels of systemic anti-spike IgG, anti-RBD-IgG, and anti-N-IgG were observed in patients with severe COVID-19 **(Figure 1, Supplementary Table 2).** Anti-spike levels were significantly higher in patients with severe COVID-19 compared to asymptomatic infected participants (**Figure 1a**) and anti-RBD IgG levels were significantly higher in patients with severe COVID-19 compared to moderate COVID-19 (**Figure 1b**). Anti-nucleocapsid (N) IgG levels were significantly higher in patients with severe COVID-19 compared to both moderate and asymptomatic groups (**Figure 1c**).

While systemic IgG titers in SARS-CoV-2 positive individuals were generally higher later on at days 7-9 post-symptom onset, there were no statistically significant differences comparing titers between early and late post-symptom onset in patients with moderate or severe COVID-19 **(Figure 2, Supplementary Table 3)**.

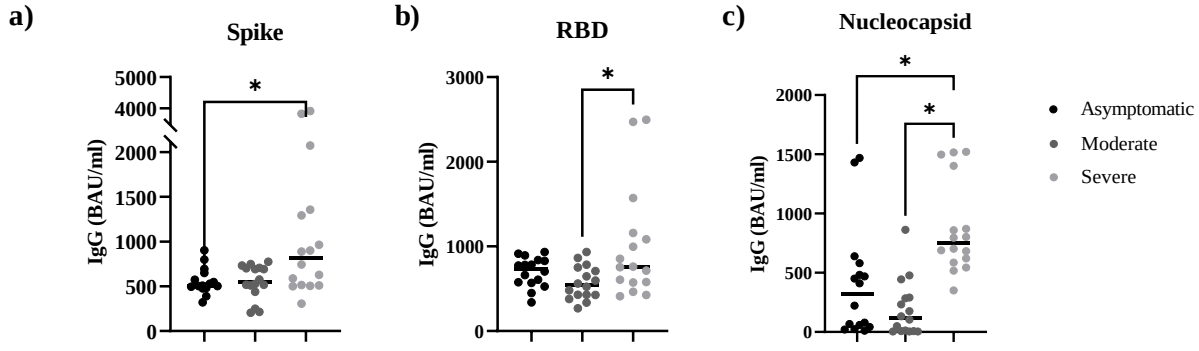

Figure 1. Anti-SARS-CoV-2 IgG levels in the blood of SARS-CoV-2-infected individuals.
IgG levels of Spike (a), RBD (b), and Nucleocapsid (c) were measured in blood from individuals with asymptomatic, moderate, and severe COVID-19. The horizontal line represents the median.

Statistical differences among the three groups were calculated using Kruskal-Wallis with Dunn's multiple comparisons between groups. * = $p < 0.05$.

COVID-19 patients had low airway mucosal anti-spike and anti-RBD IgG responses

A large majority of the participants were negative for SARS-COV-2 spike and RBD IgG in the mucosal area (**Figure 3a and b, Supplementary Table 4**). Three (19%) of the asymptomatically infected participants were positive for mucosal (NP/OP samples), anti-spike IgG and anti-RBD IgG **(Figure 2a and b)**. A subset of patients with severe COVID-19, but none with moderate COVID-19, were positive for mucosal anti-N IgG (**Figure 3c, Supplementary Table 4**). In the asymptomatic infected individuals, a majority were positive for anti-N IgG (**Figure 3c, Supplementary Table 4**). There were insufficient positive mucosal samples to allow for a meaningful comparison of titers stratified over time after symptom onset.

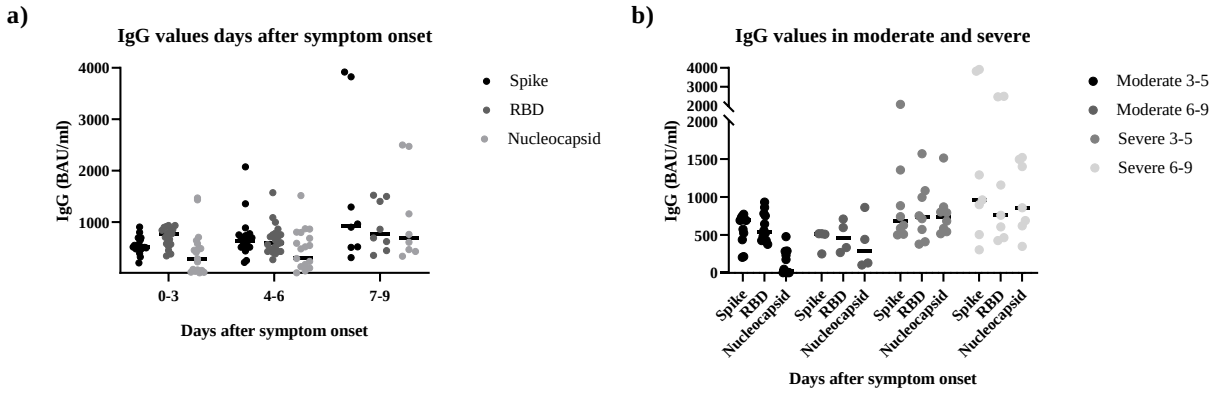

Figure 2: Anti-SARS-CoV-2 IgG levels in blood stratified by days post-symptom onset for patients with moderate and severe disease. IgG levels of Spike, RBD, and Nucleocapsid were measured in blood **(a and b)** from individuals with moderate and severe COVID-19 and the data were divided based on days after symptom onset (a) or severity and days after symptom onset (b). The horizontal line represents the median. Statistical differences among the three groups were calculated using Kruskal-Wallis with Dunn's multiple comparisons between groups. * = $p < 0.05$.

Asymptomatically infected individuals had strong mucosal and systemic interferon responses

NP/OP (mucosal) and blood (systemic) IFN-α, IFN-β, IFN-γ, and IFI-16 mRNA levels were quantified. Systemic IFN-α, IFN-β, and IFI-16, but not IFN-γ, responses were significantly higher in asymptomatic infected individuals compared to in patients with severe COVID-19 (**Figure 4a-d**). We observed significantly stronger mucosal antiviral responses in asymptomatically infected individuals compared to patients with severe COVID-19 (**Figure 4e-h**). Except for mucosal IFI-16 mRNA levels, no statistically significant difference was observed between patients with severe and

moderate symptoms (**Figure 4h**). There was a moderate positive association observed for IFN-α, IFN-β and IFI-16 mRNA levels in blood and NP/OP samples, with IFN-γ gene expression exhibiting a strong positive association (r=0.8, p<0.05) (**Supplementary Figure 1**).

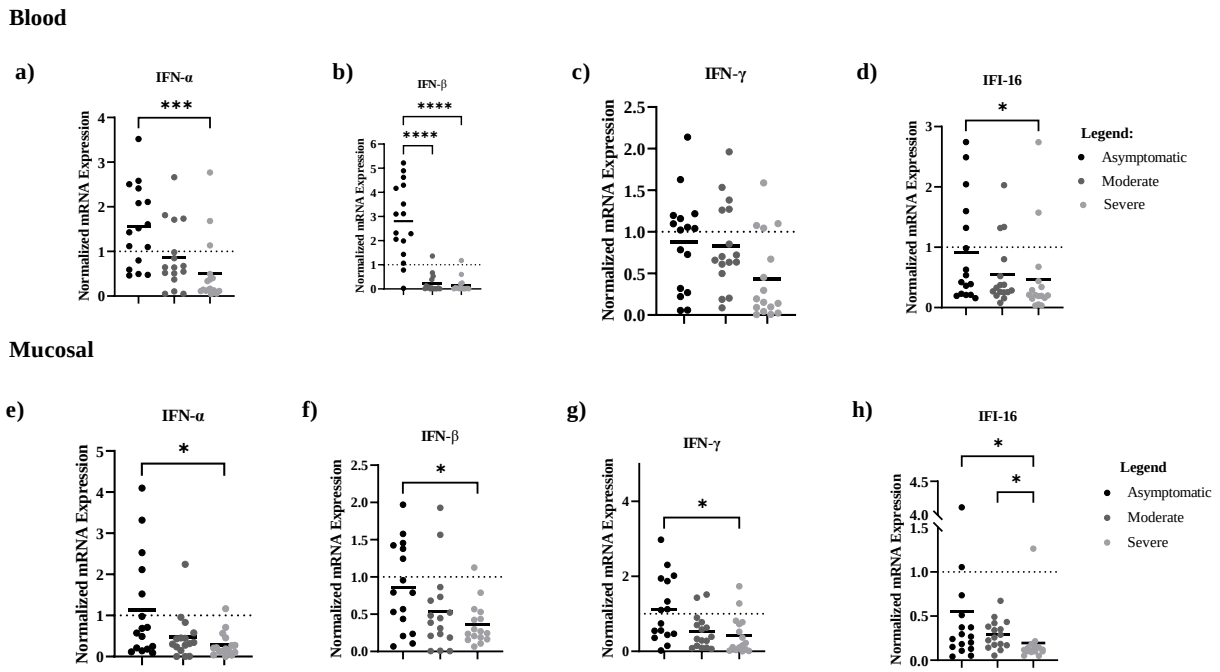

Figure 4: Interferon and interferon-stimulating gene responses to SARS-CoV-2-infected individuals.

mRNA transcript levels for blood IFN-α **(a)**, IFN-β **(b)**, IFN-γ **(c)**, and IFI-16 **(d)** and mucosal NP/OP samples IFN-α **(e)**, IFN-β **(f)**, IFN-γ **(g)**, and IFI-16 **(h)** from individuals with asymptomatic, moderate, and severe COVID-19. The horizontal line represents the median. Statistical significance among the three groups was calculated using Kruskal-Wallis with Dunn's multiple comparisons between groups. * = $p < 0.05$.

Higher SARS-CoV-2 viral load in patients with severe compared to moderate and asymptomatic COVID-19

In general, a higher SARS-CoV-2 viral load was observed in severe patients compared to moderate and asymptomatic infected individuals **(Figure 5a-c)**. Intergroup analysis showed statistically significant differences between the asymptomatic and severe patients (p=0.018) **(Figure 5a)**. The viral load in SARS-CoV-2-infected individuals with moderate or severe COVID-19 was further significantly higher at days 7-9 compared to 0-3 after symptom onset (p=0.01) **(Figure 5b)**.

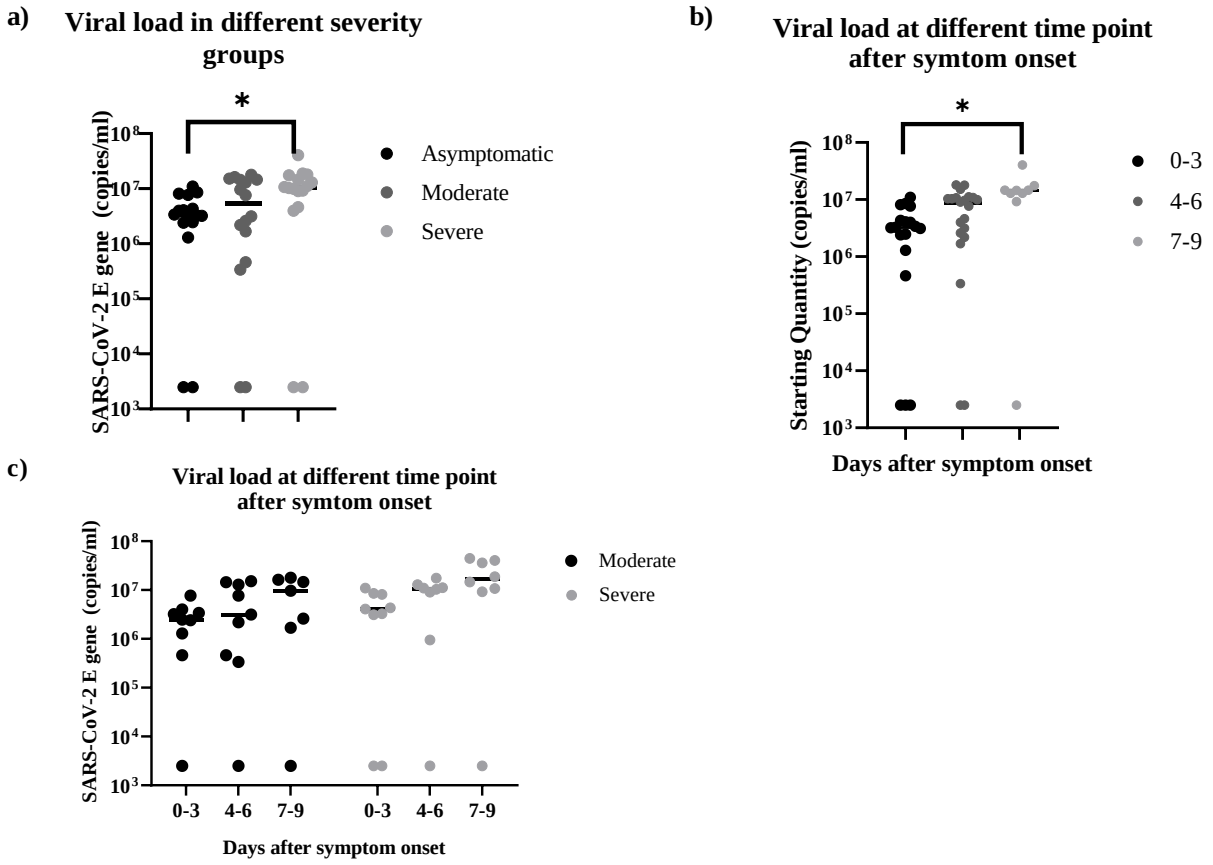

Figure 5: SARS-CoV-2 viral load in different COVID-19 severity groups and days after symptom onset.

SARS-CoV-2 viral load was measured by qPCR in NP/OP samples from individuals with asymptomatic, moderate, and severe COVID-19. Data was divided based on severity **(a)**, on days after symptom onset for patients with moderate and severe disease **(b)**, and days after symptom onset stratified within moderate and severe disease groups **(c)**. The horizontal line represents the median. Statistical differences among the three groups were calculated using Kruskal-Wallis with Dunn's multiple comparisons between groups. * = $p < 0.05$.

DISCUSSION

While much has been learned over the last five years regarding SARS-CoV-2 and COVID-19, less is known regarding immune responses and correlates to disease severity in unvaccinated individuals infected with Omicron.

The cohort in this study exhibited a range of different comorbidities and there was an association between underlying comorbidities and risk of severe COVID-19. Compared to moderately symptomatic and asymptomatic individuals, patients with severe COVID-19 had higher rates of chronic diseases, including diabetes, liver disease, lung illness, chronic kidney disease (CKD), heart disease, and asthma. For example, 50% (n=8) of severe cases had diabetes, compared to moderate (37.5%, n=6) and asymptomatic individuals (18.75%, n=3) Similarly, 31.25% (n=5) of severe patients had CKD, a higher rate compared to in the moderate (12.5%, n=2) and asymptomatic (6.25%, n=2) groups.

These results are in line with earlier studies where patients with concomitant complications, specifically diabetes, cardiovascular diseases, and chronic respiratory illnesses, were more likely to acquire severe forms of COVID-19 [17]. The mechanisms underlying these associations are multifactorial. Immune dysregulation, weakened inflammatory responses, and elevated ACE2 receptor expression are common outcomes of chronic illnesses, and these factors may enhance SARS-CoV-2 infection [18], [19]. Furthermore, comorbidities might worsen the cytokine storm caused by severe COVID-19, resulting in multi-organ dysfunction [20].

Higher systemic RBD-specific IgG antibodies were observed in comparison to nucleocapsid and spike in all SARS-CoV-2 patients. Patients with severe COVID-19 had significantly higher systemic spike, nucleocapsid and RBD-specific IgG levels as compared to moderate and asymptomatic groups. In contrast, mucosal IgG titres were higher in asymptomatic individuals and patients with moderate severity as compared to severe patients.

The highest systemic IgG levels were observed during days 7-9 from symptom onset, in contrast to during days 0-3 for the mucosal response (NP/OP), in all SARS-CoV-2-infected individuals. Strong nasal antibody response (anti-RBD IgG) has been linked to the remission of systemic symptoms (e.g., fatigue, fever, headache, disorientation, joint or muscle pain, enlarged lymph nodes) [21]

In a previous study, the percentage of patients with positive virus-specific IgG reached 100% about 17–19 days following the beginning of symptoms [22]. Conversely, individuals with mild SARS-CoV-2 infection showed transient, delayed, or absent Spike protein-specific blood IgG production, which was followed by a late or negative S protein-specific serum IgG response [23]. Systemic titers of S protein-specific IgG are reflected in mucosal S protein–specific IgG titers [24], [25]. A possible explanation for a high humoral immune response in immunological pathology has been suggested: it may enhance antigen uptake and stimulate pro-inflammatory monocytes in the lungs,

according to preclinical SARS-CoV infection models and correlative evidence from the outbreak [26], [27], [28].

This study further investigated how various IFN-associated gene transcript levels may be utilized to understand the immune response and pathophysiology of COVID-19. Severe patients had the lower systemic and mucosal IFN-α, IFN-β, IFN-γ and IFI-16 transcript levels compared to moderate and asymptomatic patients. Systemic mRNA levels of IFN-α, IFN-γ and IFI-16 in SARS-CoV-2 patients correlated positively with mucosal mRNA gene expression levels and viral load. In contrast, asymptomatic individuals showed a negative correlation between mucosal and systemic IFN-α and IFN-β mRNA with viral load.

Consistent with these results, a previous study showed reduced IFN expression and pro-inflammatory response in the peripheral blood of critically ill COVID-19 patients [29]. Another study showed that while high levels of chemokines were present for the recruitment of immune cells, the host response to SARS-CoV-2 was unable to activate a robust IFN-I and -III response [30]. Similarly, [31] found that there was no discernible variation in the levels of interferon (IFN) between the upper respiratory tracts of COVID-19 patients and healthy persons. However, elevated levels of inflammatory cytokines, specifically IFN-I and IFN-III, were seen in the broncho-alveolar lavage fluid of these individuals. The findings suggest that SARS-CoV-2 suppresses the synthesis of interferon in the upper respiratory tract, hence reducing the immune response and enhancing the survival of the virus. However, an overactive immune response and the overexpression of damaging interferons are triggered by the time the virus reaches the lower respiratory tract [32].

This study compared the viral load of COVID-19 patients of various severities and asymptomatic individuals, where severe SARS-CoV-2 patients had the highest viral load. It is also worth noting that SARS-CoV-2 viral load in all individuals was highest on days 7-9 post-symptom onset. A previous study found that the viral load in the nasopharyngeal specimens of severe cases was approximately 60 times higher than that of mild cases and that there was a sustained positive correlation over the initial 12 days of infection, suggesting that higher viral loads might be associated with severe clinical outcomes. While the NP/OP virus load was comparable between groups, they also discovered elevated blood levels of SARS-COV-2 [33].

Another study of patients who were hospitalized at a hospital in Zhejiang province in China found that patients with severe illness had a higher viral load in their respiratory samples but not in their stool or serum samples after approximately 22 days [34]. Most previous studies thus[35]have found a positive correlation between COVID-19 severity and higher viral load for pre-omicron SARS-CoV-2 variants. The results presented here are consistent with this and emphasize the importance of quantifying viral load to identify individuals more likely to develop severe diseases. A limitation of this study is the lack of sequencing data to validate the assumed SARS-CoV-2 Omicron variants in the samples collected. However, during the time of sample collection, the vast majority of SARS-CoV-2 in the region were of the Omicron variant [36]. Another limitation is that there was no data on putative previous SARS-CoV-2 infections in the enrolled cohort, which could affect the

interpretation of the results.

To conclude, we report that mucosal anti-spike and RBD IgG titers appear protective against COVID-19, while systemic anti-spike and nucleocapsid IgG titers were linked to disease severity. Both mucosal and systemic interferon responses were suppressed in patients with severe disease who also exhibited higher SARS-CoV-2 virus load and higher systemic antibody levels. Taken together, these findings suggest that the outcome of SARS-CoV-2 Omicron infection of unvaccinated individuals is linked to the capacity to rapidly induce a strong mucosal and systemic innate antiviral response, likely limiting viral replication and spread, and reducing the pathogenic effects of the Omicron variant. These results could further help in predicting COVID-19 outcomes and aid in developing personalized treatment strategies.

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Author Contributions
RMA: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Software; Visualization; Writing-original draft; Writing-review & editing.
KP: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing-review & editing; Supervision
IN: Conceptualization; Data curation; Formal analysis; Investigation; Project administration; Writing-review & editing; Supervision
SKM: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Writing-review & editing
JK: Conceptualization; Investigation; Methodology; Project administration; Writing-review & editing
JN: Conceptualization; Resources; Data curation; Formal analysis; Investigation; Methodology; Supervision; Project administration; Writing-review & editing
ML: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Conflict of Interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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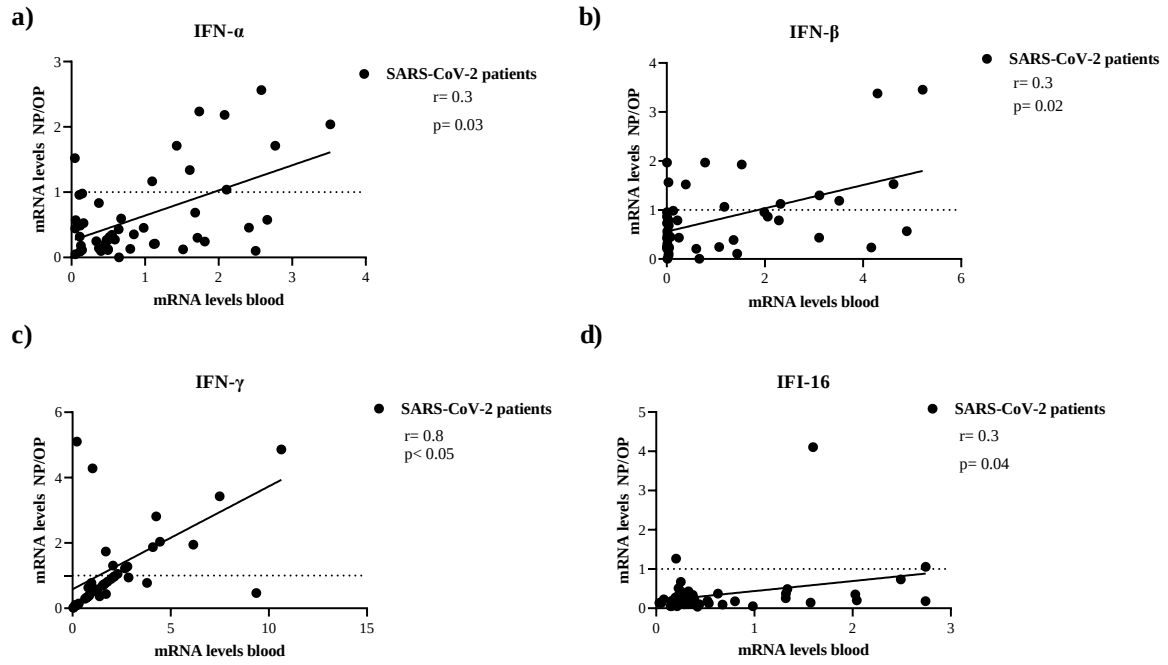
Supplementary Figures



Supplementary Figure 1: Correlation of Interferon and Interferon Stimulating gene responses to SARS-CoV-2 patients in blood and NP/OP. IFN-α **(a)**, IFN-β **(b)**, IFN-γ **(c)**, and IFI-16 **(d)**

Supplementary Tables

Supplementary Table 1: Characteristics of the study cohorts

Measures	**Asymptomatic COVID-19**		**Moderate COVID-19**		**Severe COVID-19**		***Statistic***	***p-value***
	Patients	**Matched controls**	**Patients**	**Matched Controls**	**Patients**	**Matched controls**		
	n = 16	***n* = 16**	***n = 16***	***n = 16***	***n =16***	***n = 16***		
Demographics								
Age—years; median (IQR)	46 (29-56.5)	45 (29.5-55)	39 (32.5-46)	37.5 (32-45.5)	32 (27-45.5)	42 (26.5-58)	0.4202***	0.17
Female; n (%)	7 (44)	7 (44)	8 (50)	8 (50)	5 (31)	5 (31)	1.2*	0.55
Vital signs								
Body temp (ºC); median (IQR)	36.4 (36.3-36.8)	36.35 (36.2-36.4)	36.5 (35.35-36.8)	36.5 (36.4-36.35)	36.65 (36.4-36.8)	36.8 (36.4-36.8)	0.0001***	0.71
O_2 saturation (%); mean± SD	94 31±2.86	94 56±2.52	92 51±4.37	93 34±4.37	79 11±11.59	94 58±2.26	22.91*** *	<0.0001

Key:

**: Chi-square,*

***: H-statistic,*

****: F-statistic.*

Supplementary Table 2: Anti-SARS-CoV-2 IgG titers in blood

Epitope	Study strata	Median	IQR	
			Lower	Upper
Spike	Asymptomatic	505.7	481.2	648.9
	Moderate	551.1	438.1	704.9
	Severe	815.2	510.6-	1356
RBD	Asymptomatic	739.0	568.0	839.5
	Moderate	541.7	426.6	752.3
	Severe	757.0	574.1	1158
Nucleocapsid	Asymptomatic	316.9	42.20	580.2
	Moderate	118.3	7.700	290.5
	Severe	748.8	585.6	1403

Supplementary Table 3: Anti-SARS-CoV-2 IgG titers in blood stratified by days post-symptom onset

Epitope	Days post-symptom onset	Median	IQR	
			Lower	Upper
Spike	0-3	508.4	481.2	648.9
	4-6	626.7	510.6	743.2
	7-9	931.5	306.0	3919
RBD	0-3	773.3	568.0	853.6
	4-6	597.0	428.7	753.3

	7-9	774.1	350.4	1521
Nucleocapsid	0-3	284.8	42.20	580.2
	4-6	290.5	49.10	683.3
	7-9	684.1	335.0	2497

Supplementary Table 4: Kinetics of anti-SARS-CoV-2 IgG responses in airway mucosa

Epitope	**Study strata**	**Median**	**IQR**	
			Lower	**Upper**
Spike	Asymptomatic	0.6800	0.0200	2.690
	Moderate	0.1650	0.0300	0.4400
	Severe	0.1200	0.0200	0.8200
RBD	Asymptomatic	0.5400	0.0400	2.560
	Moderate	0.3250	0.0800	0.9000
	Severe	0.1650	0.0300	1.270
Nucleocapsid	Asymptomatic	0.1000	0.000	0.3800
	Moderate	0.02500	0.0100	0.0400
	Severe	0.05000	0.0200	0.1200

Supplementary Table 5: Kinetics of anti-SARS-CoV-2 IgG responses in airway mucosa stratified by days post-symptom onset

Epitopes	**Days post-symptom onset**	**Median**	**IQR**	
			Lower	**Upper**
Spike	0-3	0.2700	0.02000	1.280
	4-6	0.1800	0.05000	0.4400

	7-9	0.1000	0.02000	0.7200
RBD	0-3	0.4700	0.04000	1.570
	4-6	0.1950	0.08000	0.9500
	7-9	0.1500	0.05000	1.070
Nucleocapsid	0-3	0.04000	0.000	0.2800
	4-6	0.03000	0.02000	0.05000
	7-9	0.02000	0.01000	0.08000