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Dysregulation of redox-inflammatory markers in COVID-19: A multi-parameter analysis with disease severity

Tehseen Fatima
tehseen.fatima@duhs.edu.pk
Dow University of Health Sciences
Sadaf Khan
Dow University of Health Sciences
Muhammad Wajih Uddin
Dow University of Health Sciences
Rafat Amin
Dow University of Health Sciences
Asif Ali
Dow University of Health Sciences
Naeem Solangi
Dow University of Health Sciences
Ayesha Khalid
Dow University of Health Sciences
Salman Ahmed Khan
Dow University of Health Sciences

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Abstract

Viral infections are one of the biggest threats ever known to humankind. Viral mutability against the human defense system makes them deadly. Virus manipulates host gene expressions, dictating the course of the disease using inflammation-induced oxidative stress. Characterized by uncontrolled Reactive oxygen species levels and weakened antioxidant defenses, which significantly impact viral disease severity. SARS-CoV-2, a constant threat due to its ability to mutate, showcases this interplay, emphasizing the need to understand host cellular responses. Unraveling these viral modulation patterns offers insight into predicting disease severity and identifying therapeutic targets.

The study used infected groups (Severe, Moderate, and Mild) and a control group. The samples were collected using a non-probability stratified sampling approach and were collected early after diagnosis to minimize treatment-related variability: at hospitalization for severe cases, within 12 hours of clinic visit for moderate cases, and within 24 hours of RT-PCR confirmation for mild cases. Disease severity classification was performed by clinicians based on clinical symptoms and oxygen saturation (SpO_2). This research delves into assessing the intricate interplay between the severity of the viral infection and inflammation-induced redox imbalance by expression profile of key antioxidant genes (GPx, NRF2, SOD2), pro-inflammatory genes (ACE2, NOS2), and RNA-binding gene (HuR), by comparing the total hydroperoxides as an indirect markeer for ROS, serum Ang II levels, and clinical data among the groups infected individuals.

Significant expressional dysregulation of proinflammatory, oxidative, and anti-oxidative markers is found among the study groups, especially in the severe group. ACE2, GPx, and SOD2 were downregulated, while NOS2, HuR, and Nrf2 were upregulated, which indicates a shift towards a pro-oxidant state, such as twice the amount of ROS in both severe and moderate groups, and Serum Ang II levels are halved in the severe group. Clinical biomarkers were also elevated. These findings establish a strong correlation between redox-inflammatory dysregulation and the severity of infections.

Introduction

Viruses, despite their minuscule size and lack of independent replication, pose a major threat to human health through their ability to establish chronic infections [1]. This phenomenon arises from their cunning ability to evade the watchful eyes of our immune system while mutating themselves and effectively turning the body's defenses against itself [2]. Mutations can alter proteins, allowing the virus to evade antibodies generated by vaccines or prior infection. The unpredictable nature of viral evolution makes it difficult to anticipate which mutations will arise next and what their functional consequences will be. At any point in time, a mutation in the genome of SARS-CoV-2 could cause another pandemic, because continuous viral evolution could theoretically lead to reduced efficacy of antiviral drugs over time, mirroring the challenges seen with drug resistance in other viruses [3]. According to “Our World in Data”, by the 11th of May 2025, COVID-19 still had 74,360 confirmed cases when 70.7% of the population was vaccinated by August 2024 [4]. Despite this high number of vaccinated individuals, viral infections still pose a serious threat.

Rather than targeting viral proteins, targeting host cellular mechanisms can enable us to resist viral infections more effectively without having the complete knowledge of any emerging virus, and can also enable us to design new strategies to combat different stages or severities of viral infections [5]. The Pattern Recognition Receptors (PRR) in the host system stand ready to identify specific “viral signatures”. The PRRs launch an aggressive counterattack upon detecting these signatures, activating signaling pathways that unleash pro-inflammatory cytokines and interferons to generate a response that can lead to cell senescence or Pyroptosis [6]. Increasing viral multiplication leads to an imbalanced redox-inflammatory homeostasis [7]. As a result, Oxidative Stress (OS) brought on by viral infections contributes to a variety of pathogenic processes. A recent study that focused on long-term OS-related effects of SARS-CoV-2 reported that even if the virus is eliminated from the body, the mitochondrial dysfunction and OS caused by the virus can start a feedback loop that stimulates chronic endothelial dysfunction, pyroptosis, and inflammasomic pathway [8].

SARS-CoV-2 envelope (E) protein induces phosphorylation of eIF2α, which ultimately limits host antiviral responses and promotes viral mRNA translation. SARS-CoV-2, instead of being harmed by cellular stress, takes control of these stressed-out cellular processes to make sure it can replicate as efficiently as possible. Inhibition of PKR-like ER kinase, which is responsible for

phosphorylating eIF2α, significantly reduces SARS-CoV-2 replication, showing that targeting host systems can be used as a promising therapeutic target [9].

To dissect the molecular foundations of this redox-inflammatory axis, this study focuses on selected genes and biomarkers that are involved in OS, inflammation, and host-viral interactions. The molecular targets include ACE2, the primary receptor involve in mediating viral entry and regulator of the renin-angiotensin system, Low levels of ACE2 are known for elevating NOS2-mediated oxidant formation [10]; NOS2, a pro-oxidant inflammatory gene, its expression is induced by cytokines [11]; Nrf2, a key transcription factor responsible for the Induction of Drug Metabolizing Enzymes, along with antioxidants and electrophiles [10,12,13]; GPx and SOD2, enzymatic antioxidants that combat ROS and HuR, an RNA-binding protein that stabilizes transcripts of stress-related genes [11]. Studies have reported that binding of HuR with the 3'-UTR of NOS-2 mRNA increases its stability and thus increases its expression [14], suggesting that HuR can be indirectly involved in increasing OS by promoting the oxidant gene [11]. Additionally, systemic indicators like Angiotensin II (Ang II) and total ROS are evaluated to quantify the infectious physiological burden and to give a more comprehensive view of the host's response. The study also analyzes clinical biomarkers like ferritin, lactate dehydrogenase (LDH), C-reactive protein (CRP), total leukocyte count (WBC), neutrophil and lymphocyte percentages, and hemoglobin levels. By correlating molecular and clinical data across varying severities of the disease, this study aims to discuss the complex interplay between oxidative stress, inflammation, and disease progression in COVID-19, and to identify potential host-targeted strategies for therapeutic intervention.

This study is a Multi-parameter Host-Centric Analysis where it focuses on host-centric interventions for future emerging pathogens where viral targets are unknown or rapidly modifying themselves. The goal is to unravel viral modulation patterns and the linkages of biomarkers with oxidative stress and inflammation. Previous studies have explored molecular imbalance, studied clinical findings, and analyzed redox imbalance, but this study offers detailed insights of these parameters in different stages of Covid-19 infection. Such a level of stratified analysis provides a nuanced understanding of disease progression [15,16].

Results

Interaction and Co-Expression of Target Genes

Study investigates six key genes that are directly involved in viral infection-mediated inflammatory redox responses. String interaction networks are used for both co-expression and interactions of protein products, with a confidence threshold of 0.150 (Fig. 1). ACE2 exhibited a potential functional interaction with the solute carrier family 6 member 19 (SLC6A19), supported by both co-expression (score: 0.109) and strong experimental/biochemical evidence (score: 0.981). While a weaker co-expression score (0.073) is observed between ACE2 and Nos2, although no direct functional link is identified. Interestingly, putative homologs of Nos2 and Gpx displayed co-expression in other organisms, hinting at a conserved evolutionary relationship that may need further investigation. Similarly, homologs of Gpx showed co-expression with SOD2-2 in other organisms, with a combined score of 0.902, suggesting a potential indirect interplay that needs further investigation. Also, Nrf2 demonstrated a robust interaction with GA-binding protein subunit beta-1 (GABPB1) across co-expression, experimental/biochemical data, and curated databases, with a combined score of 0.999. These results highlight the central role of Nrf2 signaling in regulating cellular stress responses in our system.

Angiotensin II Levels Vary with Disease Severity

Serum Ang II levels, assessed via ELISA assay (Fig. 3), the data weren't normally distributed; hence, the medians of the groups were compared using Kruskal-Wallis test, which displayed a significant decreasing trend from healthy controls to severe COVID-19 patients ($p < 0.0001$). Intra-group median variability was assessed using Dunn's multiple comparison test, revealing that the severe patients had significantly lower Ang II levels than moderate patients and Healthy individuals, aligning with ACE2 downregulation (Fig. 2) and inflammation-driven RAS disruption.

ROS Accumulation in COVID-19 Patients

The FOX assay is used in this study for the ROS estimation (Fig. 4). The ROS levels were significantly higher in the infected groups than control. The severe group showed the highest concentration of ROS, with consistently elevated values, but the moderate and mild groups showed lower mean ROS levels, still higher than healthy controls. The moderate group displayed considerable variability, and the mean ROS level in this group was statistically higher than that of the control group ($p < 0.05$), suggesting that oxidative stress begins early in disease onset. The statistical comparisons revealed a significant difference between the severe and the control group ($p < 0.001$), a moderate significance between the moderate and control group ($p < 0.05$), while mild vs. healthy and inter-infection group comparisons remained non-significant (ns). These findings highlight that the ROS levels progressively rise with disease severity, peaking in severe infections, and indicating a strong correlation between oxidative stress and COVID-19 pathophysiology.

Clinical Biomarker Trends and Inflammatory Load

To evaluate the systemic inflammatory status of COVID-19 patients, key clinical biomarkers were assessed. CRP and LDH levels were elevated in all infected groups, with severe cases showing values well above the healthy range, confirming their role as robust markers of acute inflammation and metabolic stress. Although overall WBC count varied, it was elevated in severe patients, while remaining relatively stable in mild and moderate groups. Neutrophil percentages increased, while lymphocyte percentages declined with severity, reflecting typical COVID-19 hematological shifts. Hb levels remained within normal range across all groups, with no significant variation, indicating no direct anemia in the situation of viral infection. Ferritin levels (Fig. 6) were markedly increased in all disease groups compared to healthy individuals ($p < 0.0001$), underscoring systemic inflammation and iron metabolism dysregulation.

Discussion

Inflammation and redox imbalance play a pivotal role in modulating molecular health, acting as double-edged swords in host defense. While acute inflammation and ROS are essential components of the innate immune response, chronic or dysregulated activation of these systems exerts harmful effects on cellular health. The oxidative burst generated during inflammation, if not counterbalanced by antioxidant mechanisms, leads to redox imbalance. Elevated ROS and impaired antioxidant capacity damage biomolecules, including DNA, lipids, and proteins, resulting in loss of cellular viability, mitochondrial dysfunction, and ultimately triggering pathways such as pyroptosis and apoptosis [17,18]. It is known in literature that enhanced viral infection risk, observed during neurodegeneration, is partly the result of increased ROS in brain cells. The molecular mechanisms of viral infection, occurring during the progression of neurodegeneration, remain unclear. However, it's clear that viral infections can contribute to neurodegeneration through various pathways, including neuroinflammation, oxidative stress, and the disruption of cellular processes [19–21].

OS has been suggested as an essential player in COVID-19 [15,22]. The intricate interplay between host genes and the SARS-CoV-2 virus plays a pivotal role in determining the course of infection, contributing to the diverse outcomes observed across mild, moderate, and severe stages. This study sheds light on the regulation of key biomarkers during different infection stages, unraveling their intricate linkages with OS and inflammation. The observed elevation in ROS levels suggests a potential link between heightened OS and disease severity in COVID-19 [23]. Previous studies have shown that a cascade of biological events related to imbalanced ROS drives the pathological response in the host. Evidence has suggested that elevated ROS levels, as reported in Fig. 4, play a role in the severity of SARS infection, and a high neutrophil-to-lymphocyte ratio (Fig. 5), which is associated with increased ROS production, in critically ill patients of COVID-19, ROS levels have been identified as predictors of in-hospital mortality [24–26].

The binding of SARS-CoV-2 and ACE2 drives the viral particles into the host cell, lowering the ACE2 bioavailability [15]. The ACE2 defensive function causes reduced levels of it, which is linked to adverse clinical phenotypes, and its key role in SARS-CoV-2 pathogenesis can actively induce the ‘cytokine storm’ by mediating worsened production and liberation of proinflammatory cytokines [27]. Studies have recently demonstrated that the affinity of SARS-CoV-2 and ACE2 is 5 to 10 folds higher than the forenamed SARS-CoV and ACE2 [28] also the co-expression analysis demonstrates potential operational interactions between ACE2, which is involved in the regulation of Ang II, likely contributes to dysregulation of the Renin-Angiotensin System (RAS) [29],

inflammation, and OS [30], and SLC6A19, which transports various neutral amino acids, essential building blocks for protein synthesis, across the cellular membrane [31]. This aligns with the hypothesis that Ang II levels correlate with disease severity. The observed variations in Ang II levels among disease severity groups further emphasize the intricate regulatory network in play during SARS-CoV-2 infection.

The upregulation of NOS2, which is a pro-oxidant enzyme [13], along with elevated HuR, which stabilizes NOS2 transcripts, supports the hypothesis that viral infection amplifies oxidative responses by modulating host gene expression [32]. This mechanism exacerbates tissue damage and inflammation [33]. On the other hand, antioxidant genes like SOD2 and GPx showed significant downregulation in severe cases, suggesting impaired ROS-scavenging capacity [34]. Nrf2, the master regulator of antioxidant defense, was upregulated in moderate and mild cases, but plateaued in severe disease, indicating possible feedback suppression or exhaustion of the antioxidant system (Fig. 1) [35]. Moreover, an ample amount of data is available showing a negative correlation of infectious severity and the expression pattern of these genes [26,36].

The levels of OS play a pivotal role in defining the progression of many disease conditions. Cardiovascular, neurodegenerative, metabolic, and autoimmune conditions are known to have a strong connection with the redox imbalance [37,38]. The FOX assay for ROS estimation highlights significantly elevated ROS levels in infected subjects compared to healthy individuals, showing a powerful correlation of OS in viral infections as well [39]. The observed variation in ROS levels across infection stages further substantiates the connection between OS and disease severity [40], aligning with the earlier findings.

Finally, the study also incorporates clinical markers, which are used for understanding the redox-inflammatory state of an individual during viral infection, to verify molecular findings. As shown in Fig. 5, the plots include green shaded regions and dotted lines, which represent the healthy reference ranges extracted from established literature [41–45]. Elevated CRP, LDH, WBC, and ferritin in severe patients confirm heightened systemic inflammation and metabolic stress (Fig. 5) [46]. CRP and LDH, both markers, are reliable for inflammation and tissue damage. CRP is produced in response to pro-inflammatory cytokines. The progressive increase in the levels of CRP indicates a poor prognosis in viral infection, while LDH, a marker of cellular damage and stress, further underlines the scope of tissue injuries and hypoxia in severely affected patients. Ferritin levels were also markedly increased in the patient group. As an iron-storage protein with pro-inflammatory properties, hyperferritinemia is linked to macrophage activation, cytokine storm, and increased oxidative burden, reinforcing the evidence of a hyperinflammatory state. Hematological shifts, particularly increased neutrophils and reduced lymphocytes, are consistent with established COVID-19 pathophysiology and reinforce the utility of these markers in monitoring disease progression [47].

The comprehensive analysis of this study provides valuable insights into the regulatory mechanisms of biomarkers during SARS-CoV-2 infection. The observed patterns in gene expression, Ang II levels, and ROS estimation contribute to the intricate host-virus interplay, paving the path for probable therapeutic targets and further exploration into the complex dynamics of viral infection's progression. Anti-inflammatory therapies have also been shown to be effective in treating other viral infections, and they may also be beneficial in treating SARS-CoV-2 infection [48]. However, a major limitation of this study is the relatively small sample size; increasing the participant numbers, particularly in the moderate category, would allow for more robust statistical power and a deeper understanding of host-specific variations in redox-inflammatory responses. A larger dataset potentially uncovers additional molecular patterns, host vulnerabilities, or adaptive responses that remain masked in smaller cohorts. Understanding all this is essential if we are to move towards prophylactic strategies that enhance host antioxidant responses as a first line of defense. Future research should focus on expanding population diversity and integrating longitudinal data to better capture both acute and long-term effects of viral-induced redox dysregulation.

Conclusion

This study investigated and highlighted the molecular markers of the mechanisms of inflammation and redox imbalance, as well as clinical findings in different disease severities caused by SARS-CoV-2 infection. The findings suggest a complex interplay between various oxidant and anti-oxidant biomarkers that influence disease severity, The study emphasizes the prominence of these molecular players in understanding the pathogenesis of SARS-CoV-2, while underscoring the significance of inflammation

and OS in COVID-19, providing insights that could inform future therapeutic approaches, as the number of active cases is still not zero [4] and there is still plausibility of mutation which can give rise to a new wave of epidemical situation.

Methodology

Subject recruitment:

This study was conducted using case control design. A non-probability stratified sampling approach was used to ensure proper representation, forming four distinct groups: healthy, severe, moderate, and mild. For the severe COVID-19 group, participants were selected through random sampling, while samples from the remaining groups were obtained using convenience sampling. Due to the dynamic nature of the COVID-19 pandemic, particularly the decline in active case numbers during the latter phase of the study period, the acquisition of samples became time-bound. This decline impacted the availability of moderate and mild COVID-19 cases, which in turn influenced the overall number of samples collected. While this approach allowed for timely data collection during a public health crisis, it resulted in a variable distribution of participants across the different disease severity categories.

Blood samples were collected shortly after clinical confirmation of COVID-19 to minimize variability due to treatment exposure. For the severe group, samples were obtained upon hospitalization at the Sindh Infectious Disease Hospital. For the moderate group, samples were collected within 12 hours of the patients' clinic visit. For mild cases, samples were collected within 24 hours following RT-PCR confirmation of SARS-CoV-2 infection. This sampling strategy ensured that specimens were obtained early during clinical evaluation and before prolonged therapeutic interventions.

Informed consent was obtained from all participants prior to their inclusion in the study. A total of 159 participants were included: 80 healthy controls and 79 qPCR-confirmed COVID-19 patients, categorized into severe (n=53)(samples were obtained upon hospitalization), moderate (n=6)(samples were collected within 12 hours of the patients' clinic visit), and mild (n=20)(samples were collected within 24 hours following RT-PCR confirmation of SARS-CoV-2 infection) groups, this sampling strategy ensured that specimens were obtained early during clinical evaluation and before prolonged therapeutic interventions. Classification of COVID-19 patients into different groups was done by clinicians based on their clinical symptoms and SpO2. Patients with mild illness had any of the different symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but didn't have abnormal chest imaging. Patients with moderate illness had lower respiratory disease during clinical assessments or imaging and had an oxygen saturation of SpO2 ≥94% on room air. Patients with severe illness had SpO2 <94% on room air and had lung infiltrates >50% [49,50]. Individuals meeting the study's inclusion and exclusion criteria were recruited after providing informed consent. For each group, samples were collected after the confirmation of the positive result of covid test. Patient demographics and available clinical history data were collected for each participant. The study was conducted in accordance with ethical guidelines and received approval from the DUHS Institutional Review Board (IRB-2256/DUHS/Approval/2021/655).

Sample Collection & Processing:

The collection site for the mild, moderate, and severe groups in this study was the Sindh Infectious Disease Hospital, Karachi. Blood samples were obtained from both genders with an age limit of 18-60 years, aiming for a diverse representation.

For healthy participants with any sort of chronic illness or infection, and in the case of virally infected participants, participants with any other respiratory disorder or any other viral infection were excluded from the study.

Collected blood samples underwent serum separation and RNA extraction for subsequent ELISA, Ferrous Oxidation Xylenol-Orange (FOX) assay, and RT-PCR. Blood collected in a yellow top vacutainer was centrifuged for 20 minutes at 2500rpm and 25°C to separate the serum. The serum was separated and aliquoted into microcentrifuge tubes. The isolated serum was stored in micro-centrifuge tubes at -40°C.

Molecular expression analysis of ACE2, Nrf2, GPx, NOS-2, SOD-2 and HuR:

To analyze the mRNA expression of ACE2, Nrf2, GPx, NOS-2, SOD-2, and HuR, total RNA was extracted using 250 µL of blood per 750 µL of Trizol LS Reagent (Thermo-Fisher – CAT no. 79852404) as per the protocol of the manufacturer. 500ng RNA sample

was treated with DNase and subsequently utilized for the synthesis of cDNA using the kit (Thermo Scientific™- CAT no. K1622). To estimate mRNA levels by RT-qPCR, cDNA was amplified for the expression profiling of target genes using respective primers (Table 1), QuantStudio7 Flex system, SYBER Green qPCR Master Mix & 2-ΔΔCT.

Protein's Expressional Analysis by Enzyme-Linked Immunosorbent Assay (ELISA):

Serum Ang II levels of each group were analyzed using ELISA. A commercially available ELISA kit (Cat # E1224Hu) was used, and ELISA was performed according to the instructions provided by the kit.

ROS Estimation:

The FOX assay was employed on serum samples to evaluate hydroperoxides as a proxy of oxidative stress (ROS) in all study groups[51,52]. Serum samples were diluted using deionized water by 1:10, then in the 96-well plate, 100 µL of diluted serum samples were mixed with 900 µL of FOX reagent and allowed to incubate for one hour in the dark. Standards of varying concentrations were made from 10µM and 50µM stock solutions of H_2O_2, and a standard calibration curve was constructed. The microplate reader was used for recording OD at 560nm.

Statistical analysis:

Data was analyzed using GraphPad Prism 8.0.1 and Microsoft Excel. Results are presented as mean + SD unless otherwise mentioned. Analysis among different sub-groups of COVID-19 patients is done by using ANOVA, followed by post-hoc comparison (Tukey's multiple comparison tests) if the data were normally distributed, and for data that were not normally distributed, the Kruskal-Wallis test is used, and Dunn's multiple comparison test was performed. The P-value, <0.05, is considered statistically significant. The standard curve and charts were prepared by using GraphPad Prism 8.0.1.

Study limitations

The most prominent limitation of this study is the unequal distribution of participants across disease severity groups, particularly the relatively small number of patients in the moderate category. This imbalance occurred due to the declining number of active COVID-19 cases during the later phase of the study period and the time-bound nature of sample collection at the clinical site. Additionally, strict inclusion criteria and reliance on clinically confirmed cases further limited the availability of eligible moderate cases. Despite this limitation, appropriate statistical methods were applied, including non-parametric tests where required, which are robust for datasets with unequal group sizes. While the findings provide meaningful insights into redox-inflammatory dysregulation across disease severity, future studies with larger and more balanced cohorts would further strengthen these observations.

Another limitation, which was actually a technical limitation, was not performing the formal PCR efficiency determination tests using the standard curve, and the melt curve, but the Primers were designed using Primer Express to select sequences with appropriate thermodynamic properties and minimal predicted primer–dimer formation.

Statements & Declarations

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

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Competing Interests

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Author Contributions

Rafat Amin: Conceptualization, Investigation, Methodology, Resources, Validation, Writing - original draft. **Sadaf Khan:** Conceptualization, Resources, Software, Supervision, Validation, Writing - review and editing. **Muhammad Wajih Uddin:** Data curation, Investigation, Software, Data analysis, Validation, Writing - original draft. **Asif Sheikh:** Provision of samples, clinical evaluation, and diagnosis of patients. **Naeem Solangi:** Data curation, Investigation. **Ayesha Khalid:** Data curation, Investigation. **Salman Ahmed Khan:** Investigation, Resources. **Tehseen Fatima:** Conceptualization, Investigation, Methodology, Project Administration, Resources, Supervision, Visualization, Writing - review and editing

Ethics approval

“This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the DUHS institutional review board, Dow University of Health Sciences (IRB-2256/DUHS/Approval/2021/655).”

Consent to participate

“Informed consent was obtained from all individual participants included in the study.”

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Tables

Table 1 - Primers used in this study

Gene	Forward Primer	Reverse Primer
GPx	5' CGGGACTACACCCAGATGAA 3'	5' TCTCTTCGTTCTTGGCGTTC 3'
NRF2	5' TCAGCATGCTACGTGATGAAG 3'	5' TTTGCTGCAGGGAGTATTCA 3'
ACE2	5'-ACAGTCCACACTTGCCCAAAT-3'	5'-TGAGAGCACTGAAGACCCATT-3'
GAPDH	5’-ACCCAGAAGACTGTGGATGG-3’	5’- TGACAAAGTGGTCGTTGAGG-3’
NOS-2	5’-ACAAGCCTACCCCTCCAGAT-3’	5’-TCCCGTCAGTTGGTAGGTTC-3’
SOD-2	5’-GCCATTGCTTTTGGTGTTTT-3’	5’-AAATGGTGCTGGGAAAACTG-3’
HuR	5’-GTGACATCGGGAGAACGAAT-3’	5’-GCGGTCACGTAGTTCACAAA-3’

Table 2 - Comparison of RQ values across different severity levels and healthy individuals.

Markers	ANOVA	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	P Value	
NRF2	<0.0001	Severe vs. Moderate	-0.7452	-1.149 to -0.3416	0.0004	***
		Severe vs. Mild	-0.8575	-1.300 to -0.4154	0.0003	***
		Severe vs. Healthy	0.08444	-0.2765 to 0.4454	0.9052	ns
		Moderate vs. Mild	-0.1124	-0.5899 to 0.3652	0.9037	ns
		Moderate vs. Healthy	0.8296	0.4260 to 1.233	0.0001	***
		Mild vs. Healthy	0.942	0.4999 to 1.384	0.0001	***
ACE2	<0.0001	Severe vs. Moderate	-1.066	-1.998 to -0.1333	0.0224	*
		Severe vs. Mild	-0.4109	-1.290 to 0.4683	0.554	ns
		Severe vs. Healthy	-2.009	-2.851 to -1.168	<0.0001	****
		Moderate vs. Mild	0.6549	-0.2776 to 1.587	0.2255	ns
		Moderate vs. Healthy	-0.9435	-1.841 to -0.04622	0.0376	*
		Mild vs. Healthy	-1.598	-2.440 to -0.7567	0.0003	***
SOD2	0.0015	Severe vs. Moderate	-0.89	-1.530 to -0.2501	0.0056	**
		Severe vs. Mild	-1.088	-1.762 to -0.4130	0.0016	**
		Severe vs. Healthy	-0.5222	-1.138 to 0.09359	0.111	ns
		Moderate vs. Mild	-0.1975	-0.8374 to 0.4424	0.8103	ns
		Moderate vs. Healthy	0.3679	-0.2098 to 0.9455	0.2959	ns
		Mild vs. Healthy	0.5654	-0.05038 to 1.181	0.0773	ns
NOS2	0.0047	Severe vs. Moderate	-0.4097	-0.9620 to 0.1427	0.1861	ns
		Severe vs. Mild	0.2927	-0.2597 to 0.8450	0.4468	ns
		Severe vs. Healthy	0.3502	-0.1484 to 0.8488	0.2229	ns
		Moderate vs. Mild	0.7023	0.1201 to 1.285	0.0159	*
		Moderate vs. Healthy	0.7598	0.2283 to 1.291	0.0045	**
		Mild vs. Healthy	0.0575	-0.4740 to 0.5890	0.9891	ns
HuR	0.0099	Severe vs. Moderate	-0.1948	-0.8302 to 0.4406	0.8095	ns
		Severe vs. Mild	-0.4332	-1.069 to 0.2022	0.2408	ns
		Severe vs. Healthy	0.3436	-0.2365 to 0.9236	0.3495	ns
		Moderate vs. Mild	-0.2384	-0.8737 to 0.3970	0.7009	ns
		Moderate vs. Healthy	0.5384	-0.04163 to 1.118	0.073	ns
		Mild vs. Healthy	0.7768	0.1967 to 1.357	0.0078	**
	Kruskal-Wallis test	**Dunn's multiple comparisons test**	**Mean rank diff.**		**Adjusted P Value**	
GPX	0.0016	Severe vs. Moderate	-1.179		0.0052	**
		Severe vs. Mild	-1.096		0.0129	*
		Severe vs. Healthy	-1.645		0.0002	***

	Moderate vs. Mild	0.08275	0.9925	ns
	Moderate vs. Healthy	-0.4659	0.3922	ns
	Mild vs. Healthy	-0.5487	0.2964	ns

Table 3 - Comparison of Angiotensin II protein expression among the healthy and SARS-CoV-2 patient groups.

Kruskal-Wallis test	Dunn's multiple comparisons test	Mean rank diff.	Adjusted P Value	
<0.0001	Severe vs. Moderate	-32.55	0.0004	***
	Severe vs. Mild	-15.55	0.0615	ns
	Severe vs. Healthy	-20.55	0.0042	**
	Moderate vs. Mild	17.00	0.3614	ns
	Moderate vs. Healthy	12.00	>0.9999	ns
	Mild vs. Healthy	-5.000	>0.9999	ns

Table 4 - Comparison of conc. of ROS values across different severity levels and healthy individuals.

Kruskal-Wallis test	Dunn's multiple comparisons test	Mean rank diff.	Adjusted P Value	
0.0004	Severe vs. Moderate	1.547	>0.9999	ns
	Severe vs. Mild	13.05	>0.9999	ns
	Severe vs. Healthy	36.85	0.0002	***
	Moderate vs. Mild	11.50	>0.9999	ns
	Moderate vs. Healthy	35.30	0.0135	*
	Mild vs. Healthy	23.80	0.5553	ns

Table 5 - Comparison of conc. of Ferritin values across different severity levels and healthy individuals, and other clinical markers among infected groups.

Marker	Kruskal-Wallis test	Dunn's multiple comparisons test	Mean rank diff.	Adjusted P Value	
Ferritin	<0.0001	Severe vs. Moderate	13.31	>0.9999	ns
		Severe vs. Mild	0.372	>0.9999	ns
		Severe vs. Healthy	37.62	<0.0001	****
		Moderate vs. Mild	-12.94	>0.9999	ns
		Moderate vs. Healthy	24.31	0.3066	ns
		Mild vs. Healthy	37.25	<0.0001	****
CRP	0.0009	Severe vs. Moderate	20.59	0.0912	ns
		Severe vs. Mild	20.7	0.0023	**
		Moderate vs. Mild	0.1078	>0.9999	ns
LDH	0.0006	Severe vs. Moderate	20.17	0.0985	ns
		Severe vs. Mild	18.2	0.0014	**
		Moderate vs. Mild	-1.963	>0.9999	ns
NEUTROPHILS (%)	<0.0001	Severe vs. Moderate	20.78	0.0926	ns
		Severe vs. Mild	26.73	<0.0001	****
		Moderate vs. Mild	5.944	>0.9999	ns
LYMPHOCYTES (%)	0.0001	Severe vs. Moderate	-19.67	0.1231	ns
		Severe vs. Mild	-24.11	0.0002	***
		Moderate vs. Mild	-4.444	>0.9999	ns
Marker	**ANOVA**	**Holm-Sidak's multiple comparisons test**	**Mean Diff.**	**Adjusted P Value**	
HB	0.7823	Severe vs. Moderate	-0.5648	0.8776	*
		Severe vs. Mild	0.04689	0.9274	ns
		Moderate vs. Mild	0.6117	0.8776	****
WBC	0.0005	Severe vs. Moderate	5.283	0.0528	ns
		Severe vs. Mild	5.365	0.001	***
		Moderate vs. Mild	0.08167	0.9743	ns

Figures

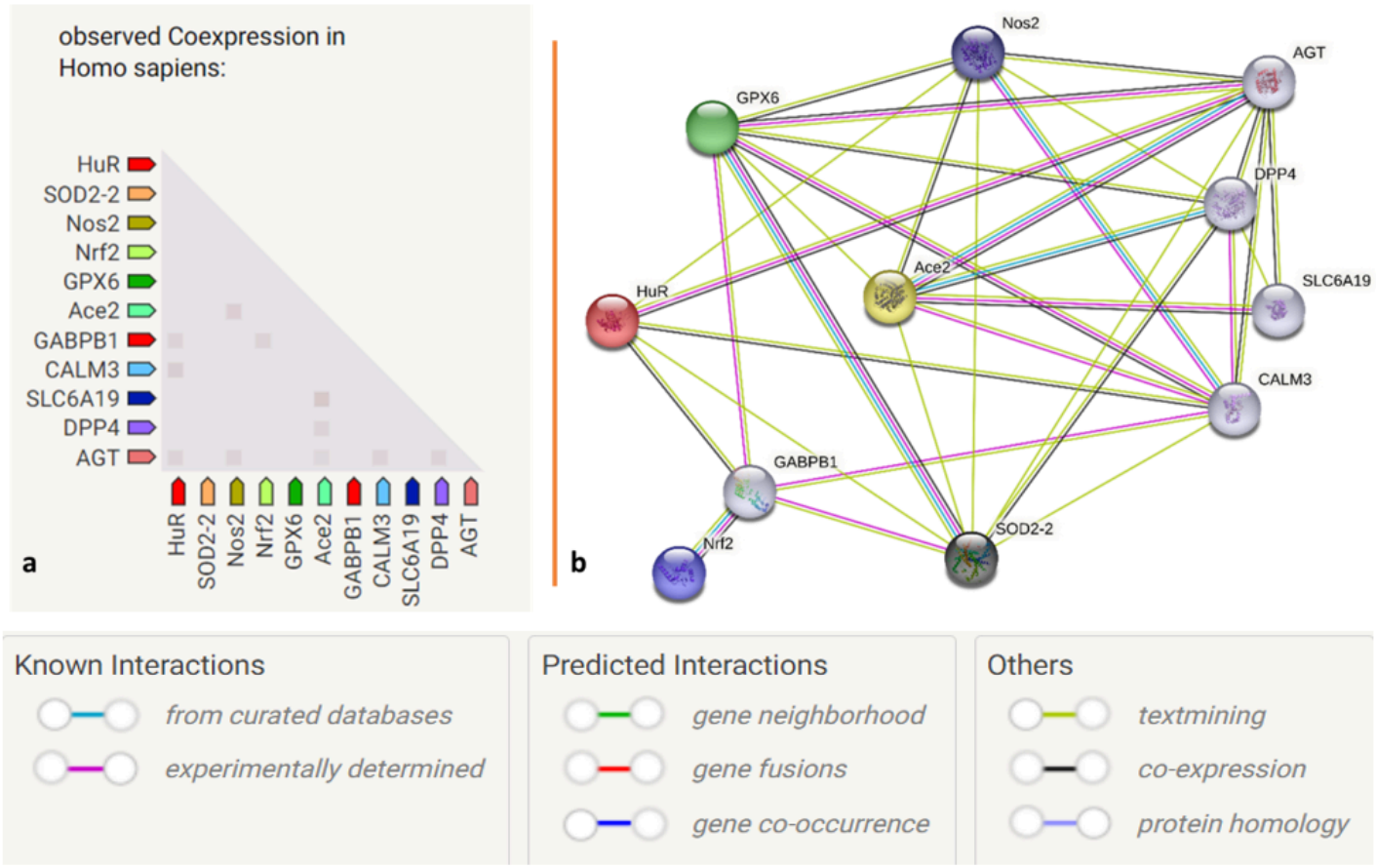


Figure 1

a, Co-expression scores based on RNA expression patterns, and on protein co-regulation provided by ProteomeHD. b, shows the interaction between SOD2-2, Nrf2, HuR, GPX6, Nos2, and Ace2 in Homo sapiens from string database, with the average clustering coefficient of 0.8 and PPI enrichment p-value of 0.000781.

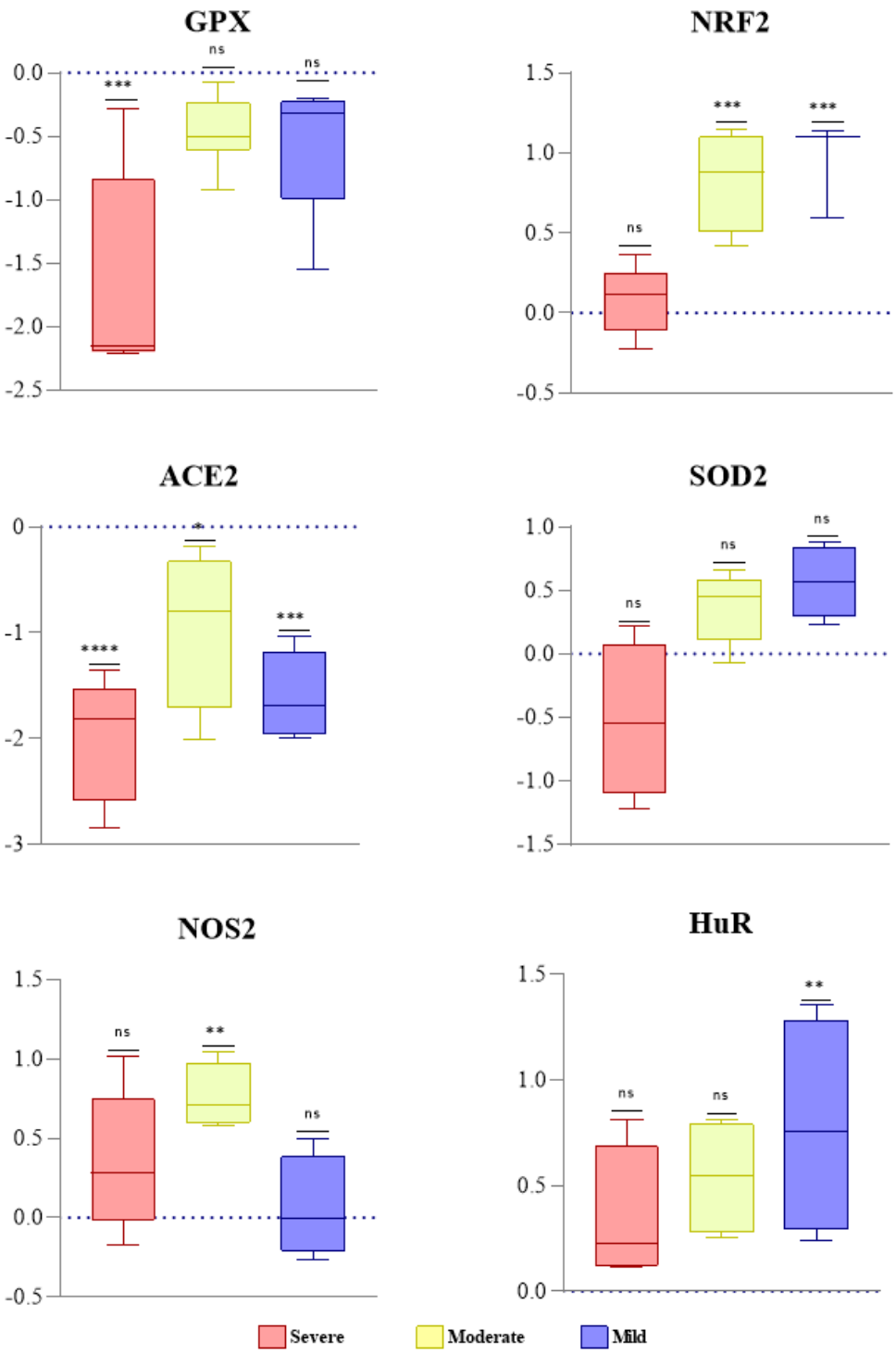


Figure 2

Expressional data of Ace2, HuR, Nrf2, SOD2, GPx, and NOS2 among healthy, mild, moderate, and severe.(***P<0.001, **p=0.01, ns=non-significant, p values represent the comparison of infected groups to the healthy group)

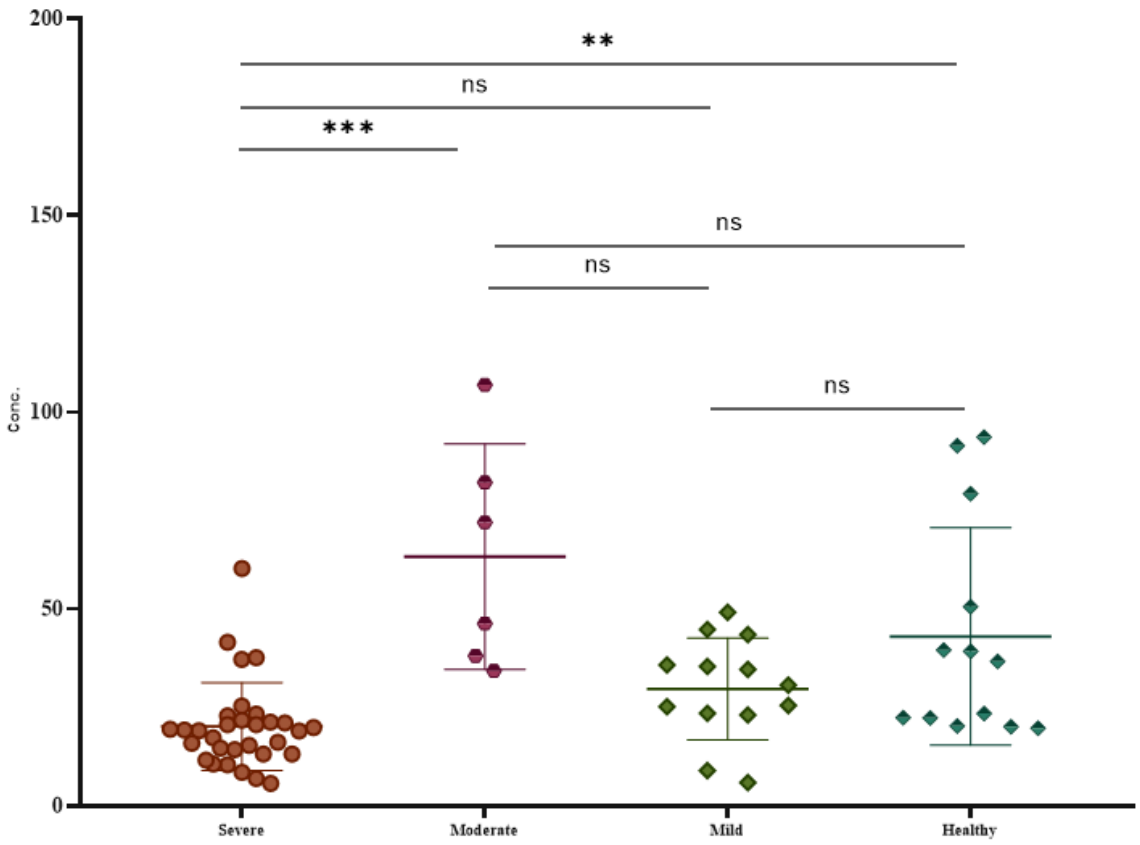


Figure 3

Serum Angiotensin II expression profiling using ELISA, among the healthy and SARS-CoV-2 patient groups.(***P<0.001, **p=0.01, ns= non-significant)

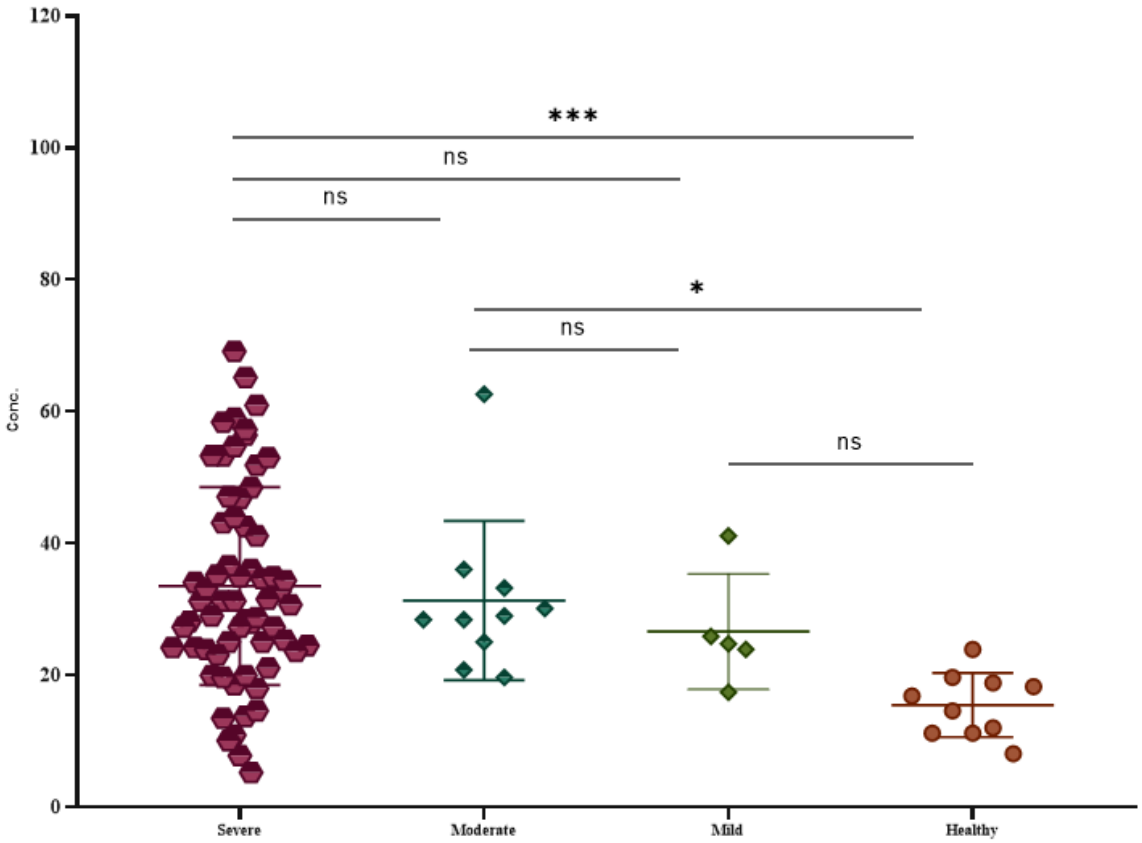


Figure 4

ROS levels in healthy, mild, moderate, and severe SARS-CoV-2 patients, quantified using FOX assay. (***P<0.001, *p=0.01, ns= non-significant)

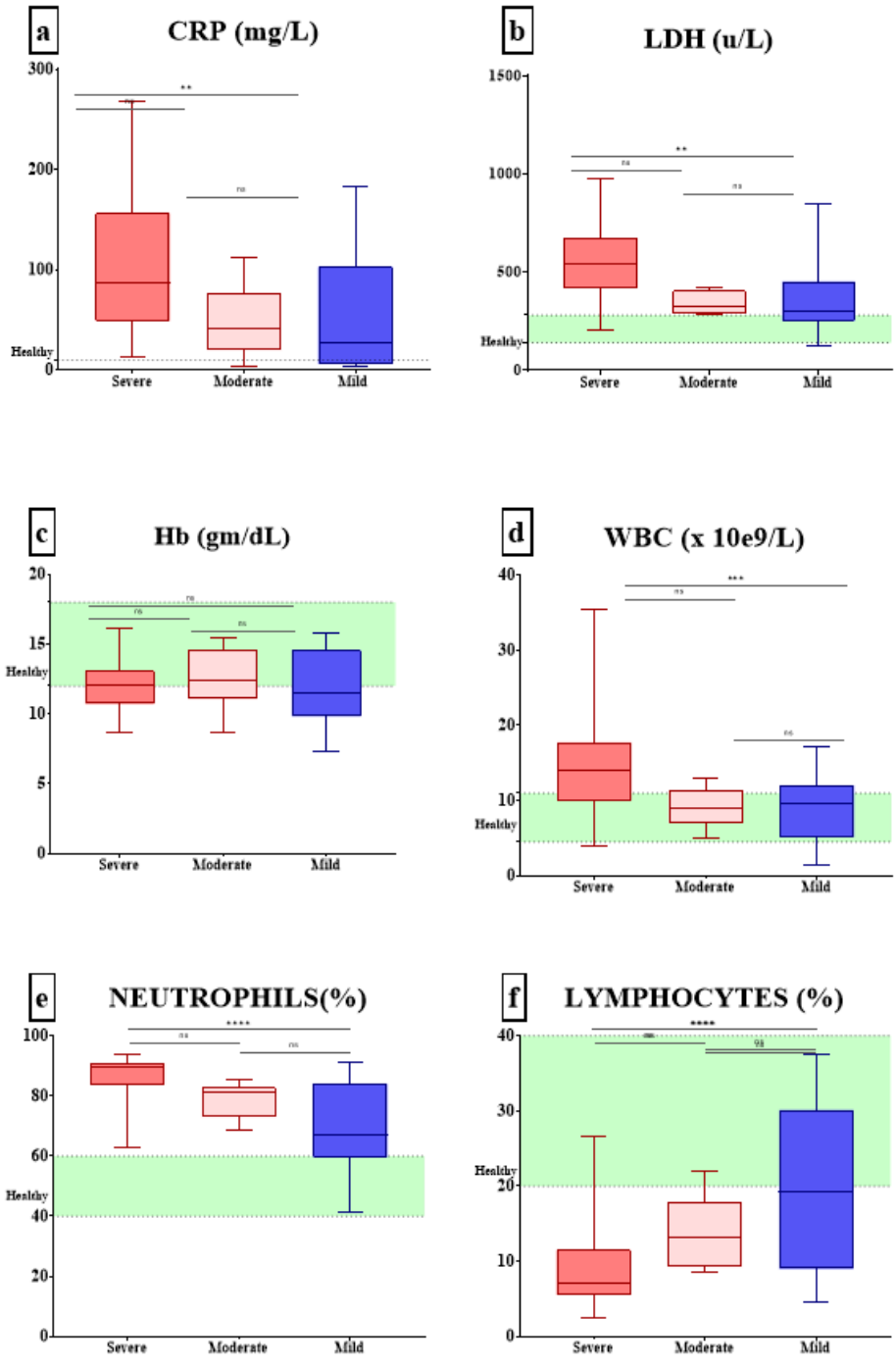


Figure 5

Clinical biomarker profiles of SARS-CoV-2 patients categorized by disease severity (Mild, Moderate, Severe). Green shaded regions and dotted lines indicate normal healthy reference ranges (*p < 0.05, **p < 0.01, ***p < 0.001).

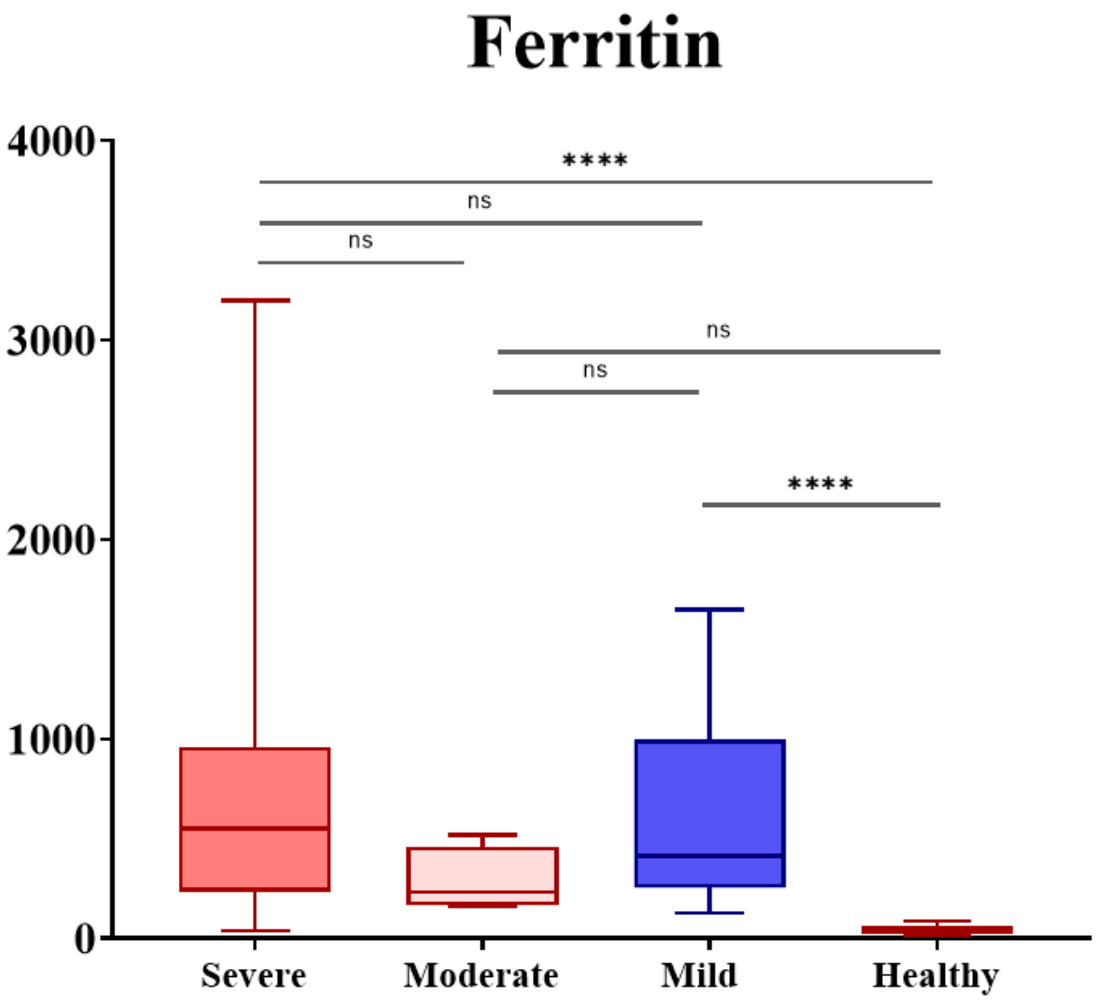


Figure 6

Serum ferritin levels in SARS-CoV-2 patients with varying disease severity (Severe, Moderate, Mild) compared to Healthy controls (****p < 0.0001; ns = not significant).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementrydata.xlsx