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Modulation of RAAS receptors and miRNAs in COVID-19: implications for disease severity, immune response, and potential therapeutic targets

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Abstract The SARS-CoV-2 spike protein interacts with ACE2, a key receptor within the renin-angiotensin-aldosterone system (RAAS), which plays a critical role in maintaining vascular homeostasis, regulating blood pressure, and modulating inflammation. An observational study analyzed the gene expression profiles of RAAS receptors and associated miRNAs in 88 hospitalized COVID-19 patients and 20 healthy controls, comparing the acute and post-acute phases to assess their impact on disease severity and recovery. Our findings revealed an association between reduced *MAS*1 expression in both advanced age ($P=0.03$) and the need for oxygen supplementation ($P=0.04$). Additionally, reduced *ACE* expression was associated with worse mortality outcomes ($P=0.01$). Notably, *ACE2* and *TMPRSS2* expression was significantly decreased ($P<0.0001$) in individuals requiring oxygen supplementation and in those with diabetes mellitus during both the acute and post-COVID-19 phases, further highlighting the impact of these conditions on RAAS. The miRNA analysis revealed significant downregulation of miR-200c ($P=0.005$), miR-let-7 ($P=0.01$), and miR-122 ($P=0.03$) in acute-phase COVID-19 patients. This dysregulation contributes to the inflammatory response and highlights the interaction between viral entry and immune regulation. These results underscore the significance of the ACE2/Ang-(1–7)/MAS1 axis in inflammation regulation and suggest that targeting this pathway may have therapeutic potential. Our study provides valuable insights into the molecular mechanisms of COVID-19 pathogenesis and identifies the modulation of RAAS receptors and miRNAs as promising biomarkers for disease severity and potential therapeutic interventions.

Clinical trial Not applicable

Keywords SARS-CoV-2, COVID-19, RAAS, ACE2, miRNA

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Background

Since its emergence in 2019, coronavirus disease (COVID-19) has affected over 770 million people globally and has led to more than 7 million deaths [1]. Like other respiratory infections, severe COVID-19 can develop in individuals with preexisting cardiovascular diseases and other risk factors, leading to the worsening of underlying chronic pathologies and the onset of new complications [2]. SARS-CoV-2 infection is triggered by the activation of the spike protein by transmembrane serine protease 2 (TMPRSS2). This activation allows the virus to attach to the host receptor angiotensin-converting enzyme 2 (ACE2), which is essential for viral replication [3].

ACE2 plays a crucial role in maintaining the homeostasis of the renin–angiotensin–aldosterone (RAA) system, which is responsible for homeostatic regulation of vascular function [4, 5]. Chronic activation of the RAAS can exacerbate pathological processes such as inflammation, fibrosis, apoptosis and aldosterone secretion [6]. ACE2 converts angiotensin II (Ang II) into angiotensin 1–7 (Ang 1–7), which exerts protective effects by activating the MAS1 receptor and counterbalancing the detrimental actions of Ang II [7, 8]. Ang II promotes sodium retention, oxidative stress, fibrosis, inflammation, and aldosterone release, contributing to severe complications such as acute respiratory distress syndrome (ARDS) and multi-organ damage [9, 10, 11].

*ACE*2 expression can be epigenetically modulated by various transcriptional and post-translational mechanisms, including microRNA (miRNA)-mediated regulation [12]. Recent studies have shown that certain miRNAs, including hsa-miR-125a-5p, hsa-let-7b-5p and members of the miR-200 family, share homology with the 3' untranslated region of *ACE*2 mRNA. This enables them to inhibit *ACE*2 expression [13, 14]. Since *ACE*2 acts as a counter regulator of the RAAS, repression of *ACE*2 may lead to pathological consequences, including myocardial fibrosis, inflammation, and cardiovascular dysfunction, in COVID-19 patients [15, 16].

The investigation of differential expression patterns of key receptors within the RAAS during acute infection and subsequent recovery stages is crucial for understanding the molecular and biological mechanisms underlying COVID-19 severity. Comprehensive studies utilizing patient-derived samples are vital to accurately assess the dynamic regulation of these receptors across diverse physiological and pathological contexts. This is particularly significant given the high prevalence of comorbidities such as cardiovascular diseases, hypertension, diabetes, and respiratory disorders, which are known to exacerbate COVID-19 outcomes. Given the critical role of RAAS in COVID-19 pathophysiology and the limited understanding of its dynamic regulation during disease progression, our study aims to elucidate the relationship between RAAS receptor expression in acute and post-COVID-19 phases. By examining its associations with clinical conditions observed throughout hospitalization, we seek to identify potential biomarkers that could aid in predicting disease severity and guiding therapeutic strategies.

Methods

A cohort of 88 individuals with a confirmed diagnosis of COVID-19 was enrolled, all of whom were admitted to the Centro Hospitalar da COVID-19, Instituto Nacional de Infectologia Evandro Chagas (INI-FIOCRUZ), Rio de Janeiro, Brazil, between June 2020 and December 2021. Participants were enrolled in the study based on their hospital admission sequence, with additional eligibility criteria including the absence of RAAS inhibitor use, age above 18 years, non-pregnancy, and provision of informed consent. The study involved two time points: an acute phase (D0) at the time of admission ($n = 88$), and a post-acute phase approximately 300 days after symptom onset, with a subset of 55 patients available for follow-up. Demographic and clinical information was collected during each patient's initial visit and no participant had received a COVID-19 vaccine. Blood samples from healthy individuals collected before the COVID-19 pandemic ($n = 20$) were used as controls. All samples were de-identified before analysis to protect participant confidentiality. The study was approved by the local Ethics Committee (CAAE: 32449420.4.1001.5262) and followed the Declaration of Helsinki, as well as all relevant guidelines and regulations.

Samples

Blood samples were centrifuged at room temperature, and the separated plasma was frozen at -80 °C. Peripheral blood mononuclear cells (PBMCs) were isolated via Histopaque 1077 density gradient centrifugation (Sigma–Aldrich, USA). The isolated cells were subsequently cryopreserved in fetal bovine serum with 10% DMSO and stored in liquid nitrogen until further use.

RNA and miRNA isolation

The cryopreserved PBMCs were thawed, and their viability was assessed via automated cell counting with trypan blue staining. Only vials with a cell viability above 90% were used for the subsequent steps. PureLink RNA Mini Kit columns (Invitrogen) were used for samples D0, D300, and the controls. The mirVana™ isolation kit (Thermo Fisher Scientific, USA) was used for miRNA extraction from the D0 and control samples following the manufacturer's instructions. The purity (260/280 ratio) and concentration of the RNA and miRNA samples were evaluated via a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Integrity was assessed via the RNA Integrity Number (Agilent).

Real-time quantitative RT–PCR

To quantify the expression of *ACE*, *ACE*2, *AT1R*, *AT2R*, *MAS*1, and *TMPRSS*2, cDNA was obtained from the extracted RNA via a high-capacity cDNA reverse transcription kit with an RNase inhibitor (Applied Biosystems). TaqMan Universal Master Mix II (Applied Biosystems™) was used, along with TaqMan® Gene Expression Assays (IDs: Hs01104600–Hs02786624), which included the targets of interest and endogenous controls β-actin or *ACTB* and glyceraldehyde-3-phosphate dehydrogenase or *GAPDH*.

For cDNA synthesis of miRNAs, the TaqMan® Advanced miRNA cDNA Synthesis Kit (Applied Biosystems™) was used as per the manufacturer's recommendations. The expression of miRNAs involved in *ACE2* regulation, including hsa-miR-200c-3p (ID: 002300), hsa-let-7-5p (ID: 002619), and hsa-miR-122-5p (ID: 002245), was quantified via TaqMan® Advanced miRNA Assays (Applied Biosystems™) in conjunction with TaqMan® Advanced Master Mix (Applied Biosystems™). hsa-miR-26-a (ID: Hs04231546_s1), located on chromosome 12:57824609, was used as an endogenous gene to normalize gene expression, as reported by Ragni et al. and Timoneda et al. [17, 18].

Amplifications were carried out via the 7500 Real-Time PCR System (Life Technologies). RQ Manager Software 1.2 was used to calculate the threshold cycle (CT) values. The expression levels of the genes of interest were calculated from the difference between the CT values of the gene and the endogenous housekeeping genes; $\Delta CT = (CT_{Target} - CT_{Endogenous})$. Higher ΔCT values indicate a greater number of cycles (CTs) required to quantify the target in the samples, which corresponds to lower levels of gene expression [19]. To calculate relative changes in gene expression between COVID-19 samples and the reference control group [20], we used the comparative CT method, also known as the fold change method, relative quantification (RQ), or its formula $2^{-\Delta\Delta Ct}$, via Expression Suite software version 1.3.

Statistical analysis

Absolute frequencies were calculated for qualitative variables, whereas arithmetic means and relative frequencies were calculated for quantitative variables. Spearman's rank correlation coefficient was used to correlate age with the expression level of each receptor. A generalized linear mixed model with a gamma distribution assumption was employed, adjusting for confounding variables (age and sex). Mann-Whitney U test was used to evaluate the ΔCT values and clinical, sociodemographic, and symptomatic data. To assess the likelihood that the observed differences in gene expression are not due to chance alone, the software conducts an unpaired, two-tailed Student's t-test. The Wilcoxon test was performed for the paired analyses of RAAS receptors in the acute COVID-19 and post-COVID-19 samples. Unpaired t-tests were used to compare the control and acute groups for miRNA analyses. The analyses were conducted via GraphPad Prism version 8.0 and R software (https://www.r-project.org/about.html), with a predetermined significance level of 0.05 (alpha).

Results

Demographic and clinical characteristics

The mean age of acute COVID-19 patients was 59.7 years (± 12.4), while that of the control group was 37.8 years (± 10.1); however, no statistically significant difference in age was observed between groups. Among the COVID-19 group, 57.5% (50) were male and 42.5% (38) were female, compared to 45% (9) male in the control group, with no statistical difference in gender distribution. Clinical characteristics on the first day of hospital admission showed that 41% of COVID-19 patients had systemic arterial hypertension (without RAAS inhibitors), 33% had diabetes mellitus, and 82% required oxygen supplementation or ventilatory support. Study outcomes showed a 24% mortality rate and a 76% discharge rate, as detailed in Supplementary Table 1.

Correlation between receptor expression levels and clinical/sociodemographic factors in COVID-19 Patients

After conducting a comprehensive analysis of the potential correlations between participants' age, gender, and receptor expression levels, we found a significant association between the average age of participants and *MAS*1 receptor expression ($P = 0.04$) (Fig. 1A). We observed a significant correlation between downregulation of the *MAS*1 receptor (indicated by higher ΔCT values) and individuals over 60 years of age ($P = 0.03$). Additionally, participants who required oxygen supplementation during hospitalization also showed lower expression for *MAS*1 ($P = 0.04$). Our analysis also revealed that individuals with oxygen saturation below 95% during hospitalization had significantly lower expression *TMPRSS*2 compared to those with normal oxygen saturation ($P < 0.01$). Furthermore, participants who died during hospitalization exhibited downregulation of *ACE* expression compared to those who were discharged ($P < 0.01$).

Differential gene expression

The analysis of receptor gene expression demonstrated significant upregulation of *ACE*, *ACE*2, and *MAS*1 in participants without comorbidities during both the acute COVID-19 and the post-COVID-19 phase compared to the healthy control group (Fig. 2A). In contrast, *TMPRSS*2 expression was downregulated, while no significant changes were observed in *AT1*R and *AT*2R expression levels.

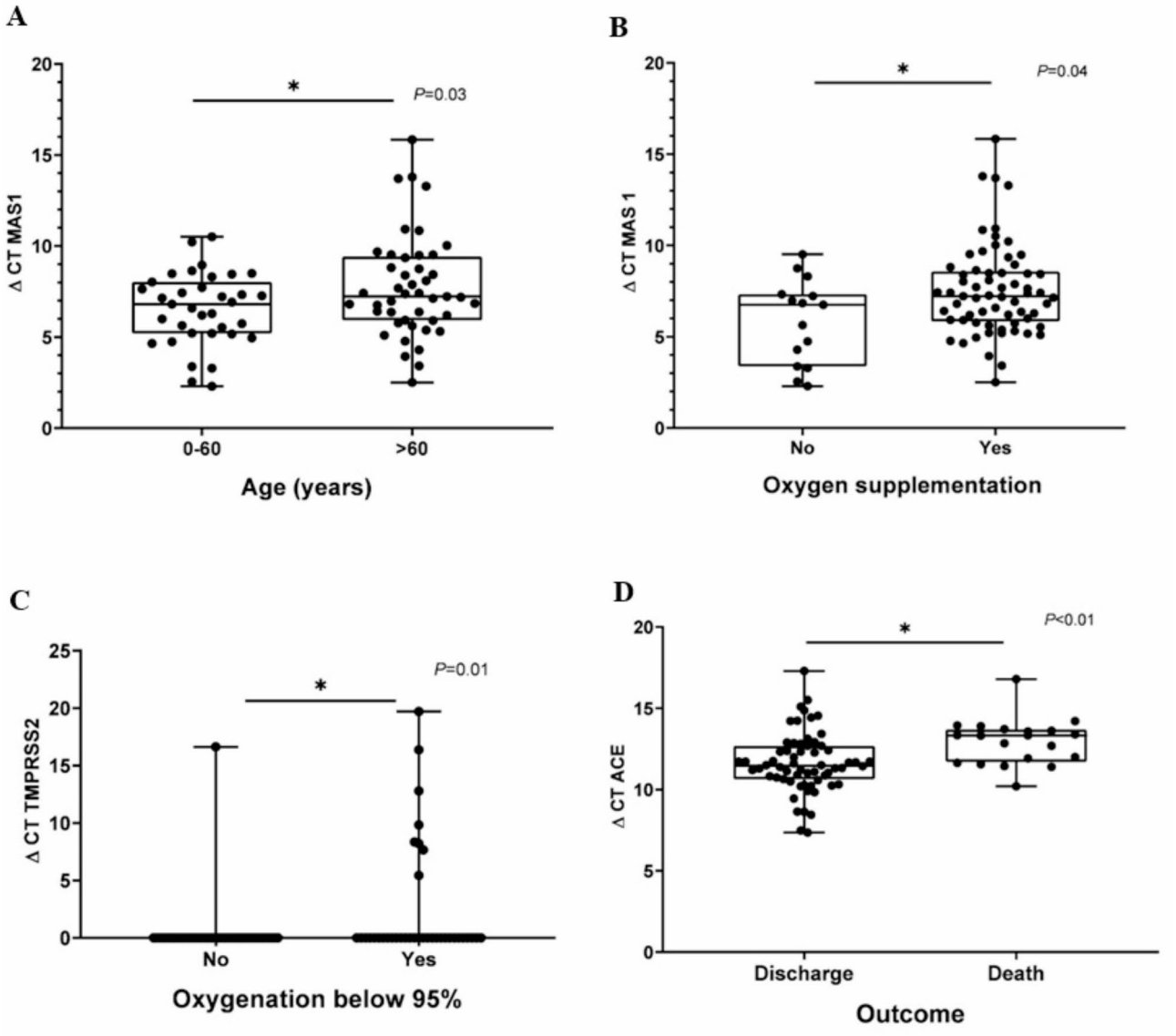

Fig. 1 Distribution of gene expression values in ΔCT of RAA system receptors in relation to clinical, sociodemographic and symptomatic data of participants in the acute phase of infection. **A**: *MAS*1 expression in ΔCT and age of participants; **B**: *MAS*1 expression in ΔCT and the need for oxygen supplementation; **C**: *TMPRSS*2 expression in ΔCT and oxygenation < 95%; **D**: ACE expression in ΔCT and participant outcome. **P* value from the Mann-Whitney non-parametric test

To gain deeper insights into the influence of health conditions such as diabetes mellitus (DM), hypertension, oxygen requirements during hospitalization, and RAAS modulation on disease progression, we analyzed relative expression data based on participants' health status. In COVID-19 patients with diabetes mellitus (DM), *ACE*2 expression was found to be downregulated, with a significant difference observed between the acute and post-COVID-19 phases (Fig. 2B). The group of participants with systemic arterial hypertension exhibited upregulation of *TMPRSS*2 compared to the healthy control group. Additionally, *MAS*1 upregulation showed a statistically significant difference between the acute and post-COVID-19 phases (Fig. 2C). Compared to healthy controls, participants requiring oxygen supplementation during hospitalization exhibited a gene expression profile akin to that of individuals with diabetes mellitus (DM), characterized by downregulation of *ACE*2. A significant difference in *TMPRSS*2 expression was observed between the acute and post-COVID-19 phases (Fig. 2D). These findings underscore the impact of underlying conditions on RAAS modulation during COVID-19, suggesting that *ACE*2 downregulation may serve as a potential biomarker for disease severity, long-term sequelae, in patients with DM or those requiring oxygen supplementation.

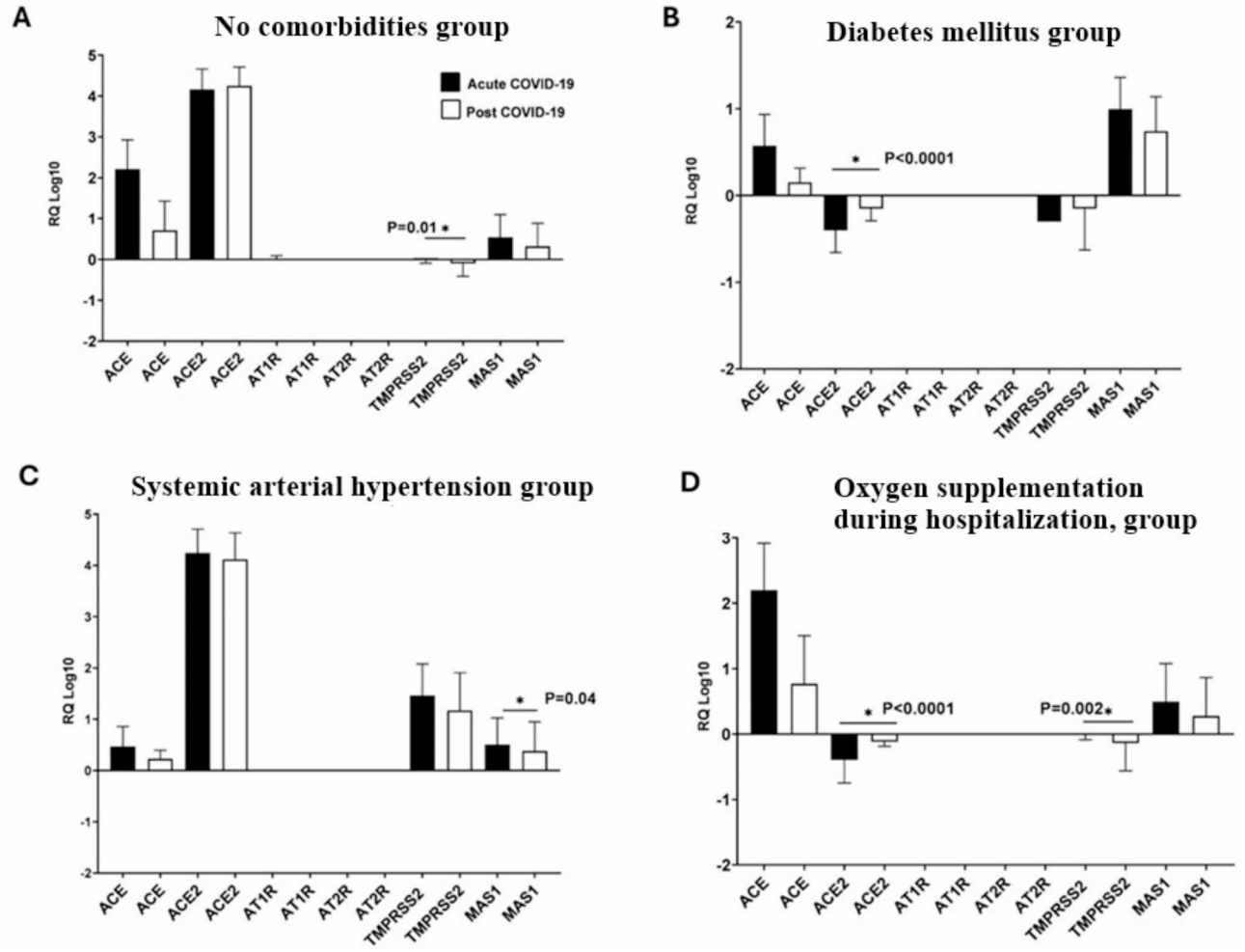

Fig. 2 Log10-transformed relative quantification data, represented as RQ ($2^{-\Delta\Delta CT}$), for the acute COVID-19 and post COVID-19 phases for *TMPRSS2*, *AT2R*, *ACE2*, *ACE*, *MAS*1, *AT1R* targets, with the healthy control group used as the baseline. * The *P*-value from the Student's t-test was statistically significant. The analysis was carried out using Expression Suite v1.3 software. Paired analysis of gene expression results for RAAS receptors in relative quantification (RQ) transformed into log10 for *ACE*. *ACE2*, *AT1*R *AT2*R, *TMPRSS2*, *MAS*1. **A**: All participants in the acute COVID-19 and post-COVID-19 phases. **B**: Acute COVID-19 and post-COVID-19 phases of individuals with diabetes mellitus. **C**: Acute COVID-19 and post-COVID-19 phases of individuals with systemic arterial hypertension. **D**: Acute COVID-19 and post-COVID-19 phases of individuals who required oxygen supplementation during hospitalization. *The *P*-value from the Wilcoxon test is statistically significant. The analysis was performed using GraphPad Prism v8.0

MicroRNA analysis

Compared to the control group, all the miRNAs studied were significantly downregulated in the acute infection group: miR-200c ($P=0.005$), miR-let-7b ($P=0.01$), and miR-122 ($P=0.03$) (Fig. 3A). The downregulation of these miRNAs may reflect underlying inflammatory and immune dysregulation mechanisms, with potential implications for disease severity, recovery, and the development of miRNA-based biomarkers for monitoring disease progression and therapeutic response.

Discussion

Extensive research has highlighted the pivotal role of the RAAS in blood pressure regulation and its involvement in the pathogenesis of COVID-19 [4, 7, 21, 22, 23, 24]. Our findings suggest that advanced age, comorbidities, and receptor expression are closely tied to COVID-19 severity, underscoring the intricate relationship between the virus-induced immunoinflammatory response and RAAS pathway disruption. Decreased expression of receptors such as *MAS*1 and *ACE* may be an early indicator of risk, while the relationship between *TMPRSS*2 and oxygenation highlights the direct impact of respiratory function on disease progression. Additionally, genetic variations within the RAAS have been explored as potential contributors to COVID-19 severity [25]. We compared the expression of RAAS receptors between COVID-19 severe patients and healthy controls, observing a significant reduction in *TMPRSS2* mRNA expression during the acute phase of infection in participants without comorbidities, which persisted up to 300 days post-COVID-19. These findings reveal a dynamic

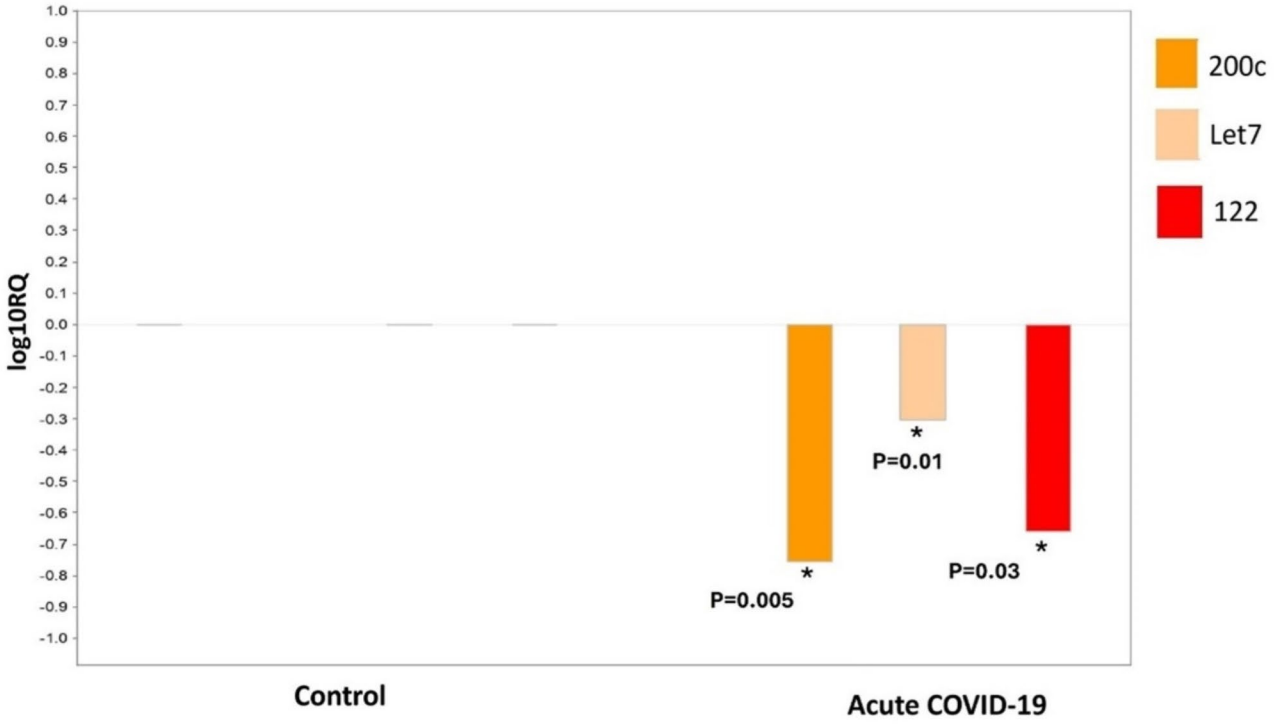

Fig. 3 Log10-transformed relative quantification (RQ) data for the miRNAs: miR-200c, miR-Let7 and miR-122. Healthy control and acute COVID-19 patients, with the control group used as the baseline. *The *P*-value from the Student's t-test is statistically significant. The analysis was conducted using Expression Suite v1.3 software

modulation of *TMPRSS*2 expression, which is crucial for viral activation [26]. This modulation may exacerbate disease progression by enhancing viral load, aligning with previous studies linking *TMPRSS*2 expression to COVID-19 severity [27]. Taking together, these findings suggest that *TMPRSS*2 holds potential as a valuable prognostic biomarker.

Although an increase in *MAS*1 expression was observed in individuals with COVID-19 compared to healthy controls, we conclude that the presence of this receptor should not be considered an optimal biomarker, as its expression is closely linked to age. However, a significant elevation in *MAS1* expression was observed in the COVID-19 group compared to the healthy control group, indicating persistent RAAS modulation even 300 days after acute infection. This finding aligns with existing evidence that RAAS function is regulated by a delicate balance between the classical vasoconstrictive pathway and the opposing vasorelaxant pathway [28, 29, 30]. Consequently, the observed dysregulation in the expression of *MAS*1, *ACE*2, and *ACE* indicates a prolonged disruption of RAAS activity extending beyond the acute phase of the disease. These findings suggest that the disruption of homeostasis may adversely affect the recovery of COVID-19 patients.

Notably, we identified a significant association between reduced *ACE* expression and mortality during the acute phase of infection, suggesting that diminished *ACE* gene expression may profoundly impair physiological RAAS balance. While therapeutic inhibition of ACE via medication has the potential to regulate RAAS, this raises the question of whether this pathway can be effectively targeted with ACE inhibitors in the context of severe COVID-19, without a comprehensive evaluation of RAAS receptor expression profiles in patients. The decreased expression of *ACE* has already been associated with severe COVID-19, as previously demonstrated by Garvin et al. in their research involving bronchoalveolar fluid cells from COVID-19 patients [31]. These findings suggest that this expression profile is responsible for the worsening of respiratory symptoms due to increased vascular permeability mediated by bradykinin, thereby promoting an inflammatory condition.

According to the literature, SARS-CoV-2 infection may cause downregulation of receptors due to internalization after binding to the spike protein [32], this could explain the decrease in *ACE2* and *TMPRSS*2. Complementary findings from other studies indicate that elevated blood glucose levels and glycation products in individuals with diabetes exacerbate RAAS activity [33], a fact that could synergistically aggravate the RAAS imbalance and contributes to impaired insulin secretion, accelerated pancreatic cell damage, and a heightened risk of diabetic

ketoacidosis, ultimately worsening clinical outcomes in patients with diabetes following SARS-CoV-2 infection.

The inclusion of a control group consisting of individuals with diabetes and/or hypertension, but without COVID-19, is crucial for accurately evaluating the true impact of SARS-CoV-2 on individuals with these comorbidities. This is particularly important given the limitation of our analysis, which only compared RAAS expression during infection to participants who were healthy prior to COVID-19 pandemic. Additionally, miRNA analysis was not performed post-COVID-19, leaving uncertainty regarding whether miRNA modulation reverted to baseline patterns, as seen in the control group. Nevertheless, the miRNA data from the acute COVID-19 phase provided valuable insights into *ACE*2 gene expression, contributing meaningfully to our understanding of the disease's molecular impact.

Studies have demonstrated that miRNAs exhibit diverse functions, even among members of the same family [13, 14, 22, 34, 35, 36]. In addition to regulating *TMPRSS*2 and *ACE*2 expressions, miRNA let-7-5p plays a role in amplifying the inflammatory response [37]. In line with our findings, Wang et al. reported that hsa-let-7-5p expression levels were downregulated in both mild and severe COVID-19 patients [38]. Notably, hsa-let-7-5p has also been shown to target IL6R [39], suggesting that the let-7 family may inhibit the translation of both IL6 and IL6R. According to the study by Khanal et al. hepatocytes transfected with let-7-5p exhibited a significant downregulation of *TMPRSS*2 mRNA and protein levels [40], which aligns with our findings and further supports the potential interplay between miRNA regulation, viral entry, and the severity of COVID-19.

In addition to its role in *ACE*2 regulation, miR-122 influences the progression of cardiovascular diseases such as myocardial infarction, heart failure, and atherosclerosis [41]. The suppression of this miRNA has been shown to mitigate aortic remodeling and fibrosis in rats via apelin, a molecule known to counteract adverse myocardial remodeling and dysfunction mediated by Ang II [42, 43]. This observation may provide a mechanistic basis for the downregulation of miR-122 during the acute phase of COVID-19, suggesting that this negative regulation could play a role in exacerbating the severity of the disease.

Our differential gene expression data were normalized with data from healthy individuals, revealing agreement with previous studies, which also found a significant increase in *ACE*2 expression [44, 45]. Our observations indicate that miRNAs involved in the regulation of *ACE2* expression exhibit consistent patterns in healthy individuals, which are markedly altered in individuals with COVID-19. In the latter, a negative regulatory shift was observed, with downregulation of miRNAs leading to the overexpression of *ACE*2. Specifically, we identified the downregulation of miR-200c, along with other miRNAs (hsa-let-7b-5p and hsa-miR-122-5p) during the acute phase, suggesting their modulation by SARS-CoV-2. These findings align with prior research, which reports a decrease in these miRNAs at disease onset [46].

Therefore, the impact of epigenetics on the regulation of RAAS receptors could improve screening for COVID-19 severity. Collectively, these findings offer valuable insights into the interactions between clinical presentations in the acute phase of the disease and the RAAS, thus enhancing our understanding of COVID-19 pathogenesis and supporting the development of more targeted and effective therapeutic strategies.

Conclusion

Based on our findings, we conclude that SARS-CoV-2 infection significantly modulates the RAAS and alters the expression of miRNAs that regulates *ACE*2, particularly in hospitalized patients. Our study highlights critical differences in the expression of RAAS receptors in individuals with underlying conditions such as diabetes mellitus or systemic arterial hypertension, as well as in those requiring oxygen supplementation during the acute phase of infection. These findings provide compelling evidence for the pivotal role of the ACE2/Ang- [1-7]/MAS1 pathway in the pathophysiology of COVID-19, suggesting that its activation is not only essential for recovery but could be a key determinant in patient survival. Furthermore, our results underscore the potential of targeting this pathway as a novel therapeutic strategy for improving outcomes in COVID-19 patients, particularly those in high-risk groups. This research enhances our understanding of the molecular mechanisms underlying disease severity and lays the groundwork for developing precise, targeted interventions to modulate the RAAS and its related pathways, aiming to reduce the impact of both acute and post-COVID-19 conditions.

Abbreviations

ACE	Angiotensin-converting enzyme
ACE2	Angiotensin-converting enzyme 2
ACTB	Beta-actin (endogenous control)
Ang II	Angiotensin II
ARDS	Acute respiratory distress syndrome
AT1R	Angiotensin II type 1 receptor
AT2R	Angiotensin II type 2 receptor
COVID-19	Coronavirus disease 2019
CT	Cycle threshold
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase (endogenous control)
IL6R	Interleukin 6 receptor
MAS1	Mitochondrial assembly
miRNA	MicroRNA
Ox.supple	Oxygen supplementation
PBMC	Peripheral blood mononuclear cell
RAAS	Renin-angiotensin-aldosterone system

RQ	Relative quantification
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SAH	Systemic arterial hypertension
TMPRSS2	Transmembrane serine protease 2

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10803-y.

Supplementary Material 1: **Supplementary Table 1**: Clinical characteristics of study participants at baseline.

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Author contributions

Conceptualization: DVA, BGData curation: MSBQFormal Analysis: MSBQ, TFBF, DVAFunding acquisition: DVA, MGM, FHC, BG, VGVInvestigation: FHC, CBWG, NBRDS, MSBQ, SWC, BG, VGV, JHP, DVAMethodology: TFBF, PAC, FHC, CBWG, NBRDS, AC, AFN, DVAProject administration: MPDR, SWC, BG, VGV, DVAResources: MGM, BG, VGVSupervision: MGM, BG, VGV, JHP, DVAValidation: TFBF, DVAVisualization: TFBF, DVAWriting – original draft: TFBF, DVAWriting – review & editing: DVA, JHP, FHC, MGM.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Instituto Nacional de Infectologia Evandro Chagas [INI]/FIOCRUZ, Rio de Janeiro, Brazil, under the approval number CAAE 32449420.4.1001.5262. All participants or their legal representatives signed an informed consent form prior to enrollment in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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