

Expression of *ACE2* and *TMPRSS2* and the severity of COVID-19

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Abstract. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for coronavirus disease 2019 (COVID-19), uses the angiotensin-converting enzyme 2 (*ACE2*) receptor and transmembrane serine protease 2 (*TMPRSS2*) to enter host cells. Variability in gene expression of these entry factors has been hypothesized to influence susceptibility and disease severity. The present study aimed to evaluate the expression of *ACE2* and *TMPRSS2* in nasopharyngeal cells and their association with COVID-19 infection and clinical outcomes. Nasopharyngeal samples from 491 individuals (aged 18-80 years) treated in public hospitals in Brazil during 2020 were analyzed. Patients were categorized based on SARS-CoV-2 reverse transcription-quantitative polymerase chain reaction (RT-qPCR) results and disease severity (including intensive care unit admission). Gene expression levels of *ACE2* and *TMPRSS2* were quantified using RT-qPCR, and statistical analyses assessed associations with infection status, age, sex, and disease severity. The findings revealed that infected individuals were significantly older ($P=0.0010$) and predominantly male (52.9%). *ACE2* expression was significantly reduced in SARS-CoV-2-positive individuals compared with negative individuals ($P<0.001$), but no association with severity or sex was observed. By contrast, high *ACE2* levels were found in moderately symptomatic SARS-CoV-2-negative individuals. *TMPRSS2* expression did not significantly differ by infection status or disease severity. In summary, *ACE2* downregulation appeared to be associated with SARS-CoV-2 infection, potentially reflecting viral evasion mechanisms.

However, neither *ACE2* nor *TMPRSS2* gene expression in the nasopharynx served as a reliable biomarker for predicting COVID-19 severity. These findings underscore the need for multifactorial models integrating host, viral, and clinical factors to improve our understanding disease progression.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), had a profound impact on global health and society. The World Health Organization (WHO) officially declared COVID-19 a pandemic on March 11, 2020, acknowledging the widespread and severe nature of the disease (1,2). Morbidity and mortality due to COVID-19 markedly increase with age and pre-existing health conditions, such as cancer and cardiovascular diseases. While most patients recover from the illness, even the youngest and healthiest can unexpectedly succumb to COVID-19 (3). These observations raise questions about how much these variations in disease severity are due to the genetic susceptibility of the patient: Genetic factors may contribute both to increased transmissibility of the virus and to the worsening of the disease, as observed in a small fraction of those affected (4).

The SARS-CoV-2 virus enters host cells through a specific mechanism involving the angiotensin-converting enzyme 2 (*ACE2*) receptor located on the host cell membrane and the viral spike (S) protein (5-7). The spike protein (S) is initially synthesized in an inactive precursor form and is converted by host cell proteases into its active form in a process known as activation (8). After the binding of the spike protein to the *ACE2* receptor, transmembrane serine protease 2 (*TMPRSS2*) cleaves the spike protein (9), inducing the fusion of the viral envelope's plasma membrane and the direct entry of SARS-CoV into the cells and its activation (10).

Research indicates that the expression of the *ACE2* receptor may increase with age, which accounts for the greater susceptibility of older individuals to COVID-19 (11,12). Furthermore, increased expression of *TMPRSS2* is associated with worse prognosis in infections by the H1N1 virus and susceptibility to infections by the H7N9 virus (13). Understanding these

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molecular interactions between the virus and host cell receptors is crucial for developing targeted therapies and interventions to disrupt viral entry and replication (14). In fact, identifying host factors involved in viral entry, such as *TMPRSS2*, has emerged as a potential therapeutic approach to prevent COVID-19 infection (15). By unraveling the mechanisms of viral entry and pathogenesis, effective antiviral strategies and treatments can be developed to combat the spread and impact of COVID-19.

The aim of the present study was to evaluate changes in the expression of *ACE2* and *TMPRSS2* genes in nasopharyngeal cells from patients with varying degrees of COVID-19, with the aim of determining their prognostic potential.

Patients and methods

Patient samples and classification. The present study collected nasopharyngeal cell samples from individuals attended at hospitals and emergency services within the public health network of the municipalities of Santo André, São Bernardo do Campo, São Caetano and São Mateus (São Paulo, Brazil) during 2020. The inclusion criteria were as follows: Participants of both sexes, aged between 18 and 80 years, with symptoms of respiratory diseases and who underwent SARS-CoV-2 detection tests, with or without the need for intensive care unit (ICU) admission, from April to December 2020. Samples were collected prior to the initiation of the National COVID-19 Vaccination Operationalization Plan.

The exclusion criteria were as follows: Patients under the age of 18 years and those who were hospitalized for reasons unrelated to respiratory diseases.

Disease severity classification in this study followed the clinical criteria established by the World Health Organization (WHO), which define mild cases as those with symptoms such as fever, cough, sore throat, malaise, headache, muscle pain, nausea, or anosmia, but without dyspnea, abnormal imaging, or the need for hospitalization (16). Moderate cases present with clinical or radiological signs of lower respiratory disease and maintain $\text{SpO}_2 \geq 90\%$ on room air. Severe cases are defined by respiratory distress, $\text{SpO}_2 < 90\%$, respiratory rate > 30 breaths per minute, or the need for intensive care. In the present study, these WHO clinical definitions were combined with the level of care required (such as outpatient, hospital ward, or ICU admission) to classify patients accordingly. In cases of discrepancy between the WHO-defined clinical severity and the level of care received, for example, a patient with $\text{SpO}_2 \geq 90\%$ admitted to the ICU due to comorbidities or precautionary monitoring, the clinical criteria were prioritized whenever sufficient data were available. However, if clinical data were insufficient to confidently determine severity, the level of care served as a surrogate marker for disease severity.

Although symptom information was documented during routine triage in 2020, a standardized and complete symptom survey was not systematically applied across all patients, as data collection occurred during routine clinical care under overwhelming operational constraints at the peak of the COVID-19 pandemic.

The study was approved by the Ethics Committee of FMABC University Center (protocol number 5.610.755; São Paulo, Brazil) on August 29, 2022. This is a retrospective

analysis of nasopharyngeal samples that were obtained during the COVID-19 pandemic as part of routine diagnostics. The samples were promptly processed and stored under appropriate conditions according to established guidelines, including storage at -80°C for long-term storage, ensuring the integrity and stability of the specimens for subsequent research analysis. Patients registered at public health units in the region, or their families, were subsequently approached by the research team to obtain written informed consent for the use of their samples and participation in the study.

Total RNA isolation. Total RNA was isolated from the nasal swab samples using the PureLink™ Total RNA Blood Kit (cat no. K156001), following the manufacturer's protocol, and quantified using a Qubit 4 fluorometer (cat no. Q33238) with the Qubit RNA HS Assay Kit (cat no. Q32852; all from ThermoFisher Scientific, Inc.). RNA samples (100 ng) were converted into cDNA using the QuantiTect Reverse Transcription Kit (cat no. 205311; QIAGEN), following the manufacturer's instructions.

Gene expression. The expression of the *ACE2* and *TMPRSS2* genes in nasopharyngeal cells was evaluated by quantitative polymerase chain reaction (qPCR). Specific primers were designed using the Primer3 Input 0.4.0 software (<https://bioinfo.ut.ee/primer3-0.4.0/>) and validated for specificity using the Primer-BLAST program (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). The sequences of the primers and characteristics of the amplicons are described in Table I.

The relative expression of the target genes was normalized by the reference *RPL13a* gene expression (17-19). *RPL13a* was selected as the reference gene because its Ct values consistently remained below 35 and its amplification showed greater stability compared with other candidate reference genes tested (including *GUSB*, *TFRC*, *GAPDH*, and β -actin). Amplifications were performed in an ABI 7500 thermocycler (Applied Biosystems; ThermoFisher Scientific, Inc.) in a final volume of 15 μl containing 1X SYBR Green (QuantiFast® SYBR Green PCR kit; cat. no. 204054; QIAGEN), 10 pmol of each specific primer, and 2 μl of cDNA. The amplification parameters consisted of an initial hot start at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 25 sec. Differential expression was determined using the formula $2^{-\Delta\Delta\text{Ct}}$ (20).

Statistical analysis. An initial descriptive statistical analysis was performed, expressing categorical variables as absolute and relative frequencies. Quantitative variables were summarized using medians, interquartile ranges (IQR), minimum, and maximum values. The assumption of normality for quantitative variables was formally assessed through the Shapiro-Wilk test. Based on the distribution, comparisons between two groups were performed using the Mann-Whitney U test for non-normal data, and comparisons among more than two groups were performed using the Kruskal-Wallis test. Graphical representations were standardized according to data distribution: Variables with a normal distribution are presented as the means $\pm$ standard deviation, whereas non-normally distributed variables are shown as medians and interquartile ranges (IQR).

Table I. Characteristics of specific primers and their amplicons.

Gene	Sequence (5'-3')	Amplicon
<i>ACE2</i>	F: AGGGCGACTTCAGGATCCTT R: TGTGGCTGCAGAAAGTGACA	185 bp
<i>TMPRSS2</i>	F: GAACACAAGTGCCGGAATG R: CTGGACGTGCAGGCTGAC	193 bp
<i>RPL13a</i>	F: TTGAGGACCTCTGTGTATTTGTCAA R: CCTGGAGGAGAAGAGGAAAGAGA	126 bp

Specific primer sequences and expected amplicon sizes for target genes used in the present study are presented. Forward and reverse primer sequences are listed in the 5' to 3' direction. Amplicon size is given in base pairs (bp). *ACE2*, angiotensin-converting enzyme 2; *TMPRSS2*, transmembrane serine protease 2; *RPL13a*, ribosomal protein L13a; F, forward; R, reverse.

To evaluate the expression levels of the *ACE2* and *TMPRSS2* genes across different COVID-19 severity groups, the non-parametric Kruskal-Wallis test was used (version 10.4.1 GraphPad Prism; Dotmatics). When significant differences were detected, Dunn's post hoc test was applied for pairwise comparisons. Correlation analyses were performed using Spearman's rank correlation. All statistical tests were two-tailed, and $P < 0.05$ was considered to indicate a statistically significant difference.

Results

The study included samples from 491 individuals, of whom 158 tested negative and 333 tested positive for SARS-CoV-2 via molecular testing. The demographic and clinical characteristics of the patients are summarized in Table II. Among the 158 individuals with a negative test, only 3 required ICU admission for reasons unrelated to COVID-19. By contrast, among the 333 individuals who tested positive, 208 experienced mild to moderate symptoms and did not require ICU admission, while 125 developed severe symptoms and required intensive care. Although a standardized symptom survey was not employed in the present study, the symptoms most frequently documented in patients classified as mild cases included fever, cough, sore throat, and fatigue. For moderate cases, in addition to these symptoms, patients often presented dyspnea and tachypnea, consistent with clinical signs of pneumonia. Severe cases predominantly exhibited respiratory distress, hypoxia (oxygen saturation $< 90\%$), and altered mental status, which aligned with the WHO criteria for severe COVID-19 and frequently justified ICU admission (16). While exact frequencies could not be uniformly quantified due to variability in record completeness, these symptom patterns were consistent with the clinical stratification applied throughout the cohort.

Men represented the majority of participants infected with the virus: 176 (52.9%) compared with 157 (47.1%) women; among participants without SARS-CoV-2 infection, 93 (58.9%) were female and 65 (41.1%) were male. Additionally, infected participants had a higher mean age (47.4 ± 19) than those without the virus (41.8 ± 18) ($P = 0.001$), as shown in Table II. Regarding severity, as expected, there was a higher proportion of severe cases (37.5%) and a lower proportion of

Table II. Clinical characteristics of the patients.

Characteristics	n (%)
Sex	
Female	250 (50.9)
Male	241 (49.1)
Age (years)	
18 to 40	227 (46.2)
41 to 60	154 (31.4)
61 to 80	110 (22.4)
SARS-CoV-2-negative (n=158)	
No ICU admission	155 (98.1)
With ICU admission	3 (1.9)
SARS-CoV-2-positive (n=333)	
No ICU admission	208 (62.5)
With ICU admission	125 (37.5)
Sex distribution (SARS CoV-2-positive) (n=333)	
Male	176 (52.9)
Female	157 (47.1)
Sex distribution (SARS CoV-2-negative) (n=158)	
Male	65 (41.1)
Female	93 (58.9)
Mean age ($\pm$ SD) (n=491)	
SARS CoV-2-positive	47.4 ± 19
SARS CoV-2-negative	41.8 ± 18

Clinical characteristics of the study population. Data are presented as absolute numbers and percentages. Age groups are categorized in years. ICU admission status is shown separately for SARS-CoV-2-negative and -positive patients. ICU, intensive care unit; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

moderate cases (14.4%) among those with SARS-CoV-2 infection compared with the group without the infection (Table III).

The analysis of gene expression data for *ACE2* and *TMPRSS2* among patients diagnosed with COVID-19 was

Table III. Contingency by severity compared with SARS-CoV-2 diagnosis.

Severity			
Without SARS-CoV-2 infection, n (%)		With SARS-CoV-2 infection, n (%)	
Mild	88 (55.7)	Mild	160 (48.0)
Moderate	67 (42.4)	Moderate	48 (14.4)
Severe	3 (1.9)	Severe	125 (37.5)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

conducted to investigate possible associations with sex, age and disease severity.

Both *ACE2* [men (n=125), 0.3021 ± 0.5056 vs. women (n=104), 0.3686 ± 0.6313] and *TMPRSS2* [men (n=102), 0.0463 ± 0.0896 vs. women (n=89) 0.0745 ± 0.1192] expression levels were slightly higher in women than in men; however, the difference was not statistically significant for *ACE2* ($P=0.24$), while *TMPRSS2* expression showed a trend toward significance ($P=0.07$) (Table IV). These values refer only to the subset of samples in which the respective gene expression was detected.

The analysis of the age of patients revealed no significant difference between sexes ($P=0.42$), with a mean age of 48 ± 19 years for men and 46 ± 18 years for women, as shown on Table IV. Similarly, no significant correlation was found between age and the expression of *ACE2* or *TMPRSS2* in either SARS-CoV-2-positive or -negative groups (Fig. 1). These findings indicated that gene expression in the nasal epithelium is not directly associated with age or SARS-CoV-2 infection status.

ACE2 expression was revealed to be significantly higher in uninfected individuals (2.750 ± 1.775) compared with those with SARS-CoV-2 infection (0.122 ± 0.528) (Fig. 2A). While reduced *ACE2* expression in SARS-CoV-2-infected individuals may initially appear counterintuitive given the receptor's role in viral entry, an alternative explanation is that this reduction reflects a host-mediated defense mechanism aimed at limiting viral spread. By downregulating *ACE2*, host tissues may decrease the number of entry points available to the virus, thereby restricting replication and dissemination (21).

Analysis of *TMPRSS2* expression revealed greater variability among individuals, with no significant difference between SARS-CoV-2-infected and uninfected groups (Fig. 2B). This suggests that *TMPRSS2* levels in the nasopharyngeal epithelium are not directly affected by infection, although the presence of *TMPRSS2* may still facilitate viral entry when *ACE2* is available.

The present study analyzed the expression dynamics of the *ACE2* and *TMPRSS2* receptors in the nasopharyngeal epithelium and their relationship with COVID-19 severity. While no direct association was observed between *ACE2* expression and disease severity, the *TMPRSS2/ACE2* ratio more effectively distinguished individuals with SARS-CoV-2 infection from those without it (with SARS-CoV-2, 3.094 ± 10.920 vs. without SARS-CoV-2, 0.024 ± 0.047 ; $P < 0.0001$), indicating a possible synergistic interaction between these receptors in

viral pathogenesis (Fig. 3). On the other hand, when evaluating whether this ratio varied according to disease severity within the COVID-19-positive group, it appeared to be independent of severity ($r = -0.033$; $P = 0.67$) (Fig. 4).

To further explore the association between *ACE2* and *TMPRSS2* expression levels and COVID-19 severity, patients were categorized according to clinical presentation. Among individuals with respiratory symptoms caused by agents other than SARS-CoV-2, *ACE2* expression was significantly increased in nasopharyngeal cells from patients with moderate symptoms (Fig. 5A). By contrast, this pattern was not observed among SARS-CoV-2-infected individuals: *ACE2* expression remained downregulated following infection, regardless of clinical severity (Fig. 5B).

Gene expression patterns differed between COVID-19 patients and those with other respiratory infections. In non-COVID infections, *ACE2* expression increased during moderate symptoms, whereas in COVID-19 patients, *ACE2* remained suppressed even after infection, suggesting a potential viral strategy to evade host defenses. Additionally, both *ACE2* and *TMPRSS2* showed age-related increases, with males over 65 having significantly higher *ACE2* levels, which may help explain the higher mortality in this group.

In SARS-CoV-2-infected individuals, opposing patterns were observed: Symptomatic patients had lower baseline *ACE2*, which increased during active infection, while *TMPRSS2* levels, initially elevated, decreased after infection. As previously shown in Fig. 4, the *TMPRSS2/ACE2* ratio did not show a significant correlation with clinical severity in COVID-19 cases (Spearman's $r_s = -0.033$; $P = 0.67$), suggesting that this parameter is not associated with disease progression. Despite limitations, such as the small number of severe cases in the non-COVID cohort, sensitivity analyses confirmed the robustness of these findings.

Similarly, an evaluation of *TMPRSS2* gene expression was conducted according to symptom severity in individuals without and with SARS-CoV-2 infection (Fig. 6). In both scenarios, no difference in the expression of this gene in the nasopharyngeal cells was associated with disease severity. There was no significant correlation between *TMPRSS2* expression and disease severity in patients infected with COVID-19 (Spearman's $r_s = -0.103$; $P = 0.154$; $n = 191$), indicating that *TMPRSS2* expression does not appear to be associated with disease severity in this cohort. Likewise, no significant correlation was observed between *ACE* expression and disease severity in COVID-19 patients (Spearman's $r_s = -0.030$;

Table IV. Comparison of *ACE2* and *TMPRSS2* levels and age between men and women.

Variable	Group	P-value	Statistical significance
<i>ACE2</i> expression			
Men (n=125)	0.3021±0.5056	0.24	NS
Women (n=104)	0.3686±0.6313		
<i>TMPRSS2</i> expression			
Men (n=102)	0.0463±0.0896	0.07	Trend towards significance
Women (n=89)	0.0745±0.1192		
Age (years)			
Men	48±19	0.42	
Women	46±18	NS	

ACE2 and *TMPRSS2* expression levels and age distribution of patients by sex. Data are presented as the mean ± standard deviation. *ACE2* expression was slightly higher in women than in men, but the difference was not statistically significant ($P=0.24$). *TMPRSS2* expression showed a trend toward significance ($P=0.07$). No significant difference in age was observed between sexes (men, 48±19 years; women, 46±18 years; $P=0.42$). These values refer only to the subset of samples in which the respective gene expression was detected. *ACE2*, angiotensin-converting enzyme 2; *TMPRSS2*, transmembrane serine protease 2; NS, not significant.

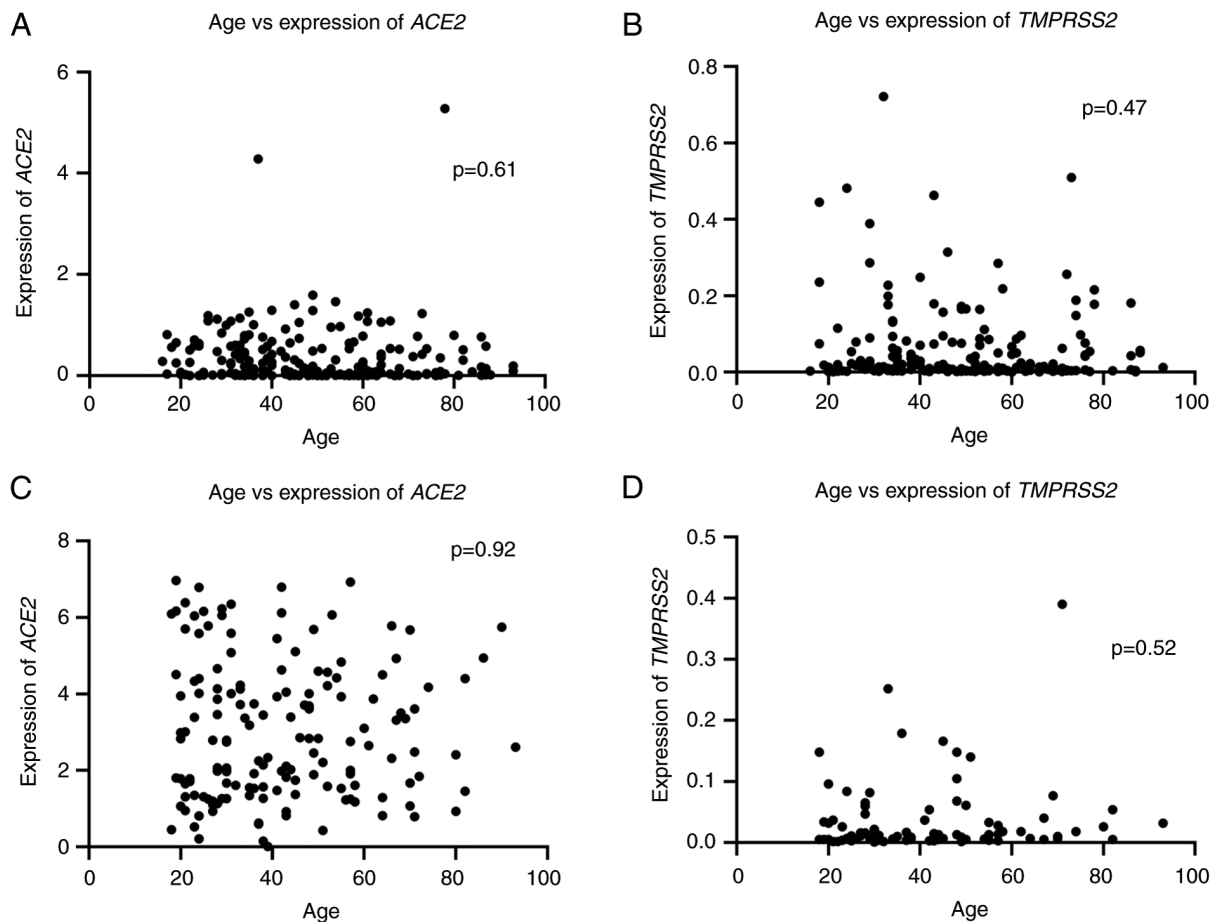


Figure 1. (A-D) Correlation between age and *ACE2* expression in nasopharyngeal cells from individuals (A) with SARS-CoV-2 infection and (C) without SARS-CoV-2 infection, and *TMPRSS2* expression in individuals (B) with SARS-CoV-2 infection and (D) without the virus. Spearman's correlation test was used to analyze the data. *ACE2*, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; *TMPRSS2*, transmembrane serine protease 2.

$P=0.649$; $n=229$), as shown in Table V, suggesting that *ACE* expression is not associated with disease severity in this cohort. However, the decrease in expression observed among

infected individuals may indicate a condition of infection that could potentially render the patient more vulnerable, but does not directly determine the severity of the disease.

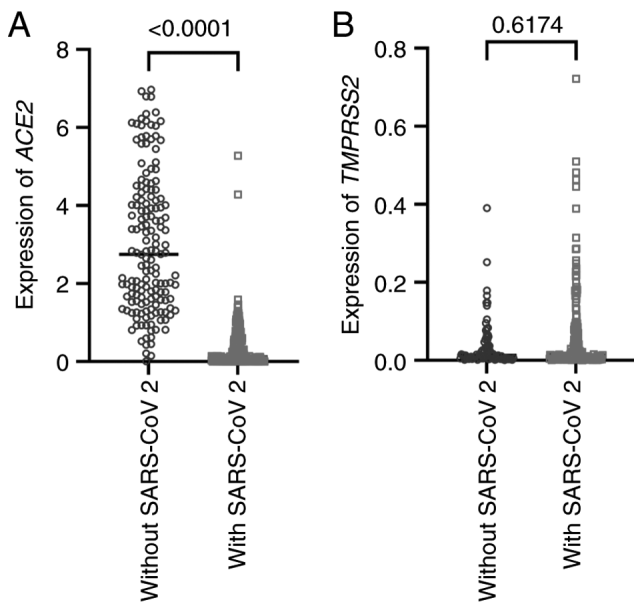


Figure 2. Expression of (A) *ACE2* and (B) *TMPRSS2* in the nasopharyngeal cells of individuals with and without SARS-CoV-2 infection. Expression values were obtained using the formula $2^{-\Delta\Delta C_q}$. *ACE2*, angiotensin-converting enzyme 2; *TMPRSS2*, transmembrane serine protease 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

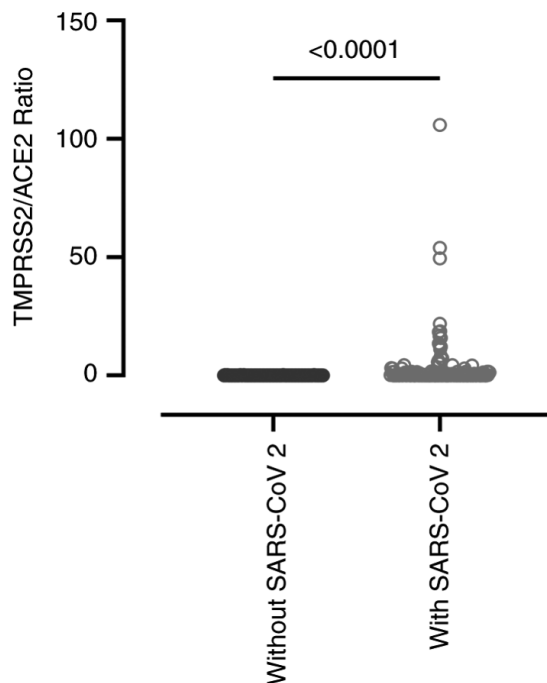


Figure 3. Comparison of the *TMPRSS2/ACE2* ratio between individuals with and without SARS-CoV-2 infection. While *ACE2* expression alone was not associated with disease severity, the *TMPRSS2/ACE2* ratio clearly differentiated infected from non-infected individuals (with SARS-CoV-2, 3.094 ± 10.920 vs. without SARS-CoV-2, 0.024 ± 0.047 ; $P < 0.0001$), suggesting a potential synergistic role of these receptors in viral pathogenesis.

This observation supports the notion that receptor expression dynamics in COVID-19 may be more reflective of viral evasion mechanisms and host-pathogen interactions, rather than directly predicting disease outcomes. Further investigation into how these changes in receptor expression interact

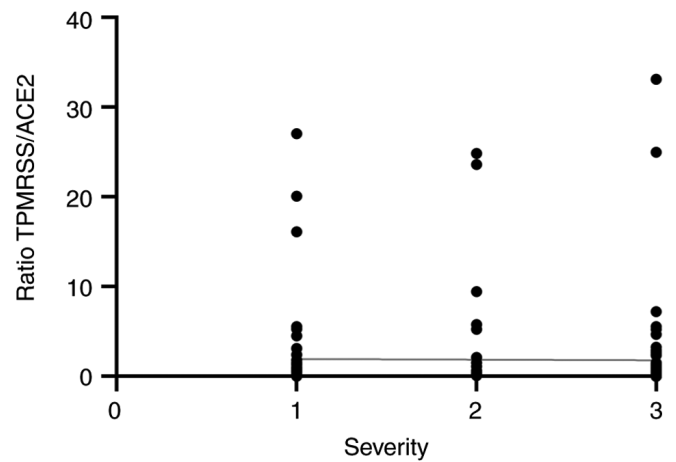


Figure 4. *TMPRSS2/ACE2* ratio across COVID-19-positive individuals stratified by disease severity (1, mild; 2, moderate; 3, severe). No significant correlation was observed between the ratio and disease severity ($r = -0.033$; $P = 0.67$), indicating that this ratio is independent of disease severity.

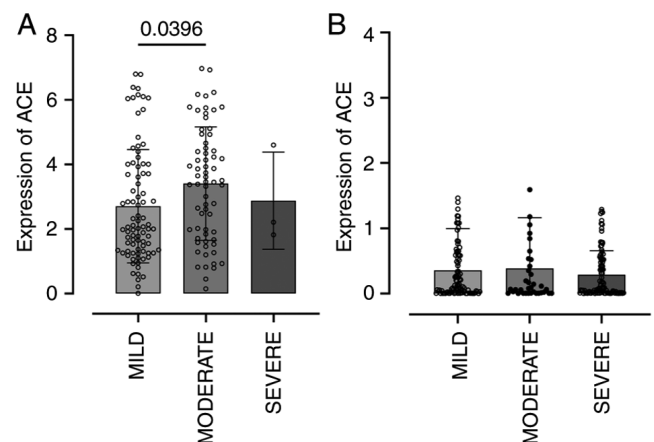


Figure 5. *ACE2* gene expression in the nasopharyngeal cells of individuals (A) without SARS-CoV-2 and (B) with the virus, according to their disease status. The Kruskal-Wallis test was used to analyze the data. *ACE2*, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

with other factors, such as immune response or co-morbidities, will be essential to fully understand the complex determinants of COVID-19 severity.

Discussion

The presence of COVID-19 is associated with greater severity in patient hospitalizations. Patients diagnosed with COVID-19 are more likely to present severe cases, which can influence clinical decisions and treatment strategies. These findings may support the need for more stringent measures for the prevention and treatment of COVID-19, aiming to reduce the severity of hospitalizations. Therefore, there is strong evidence that the detection of COVID-19 is significantly associated with greater severity in patient hospitalizations. This study aimed to understand how the *ACE2* and *TMPRSS2* receptors vary in patients with different levels of COVID-19, seeking to determine their potential as prognostic markers.

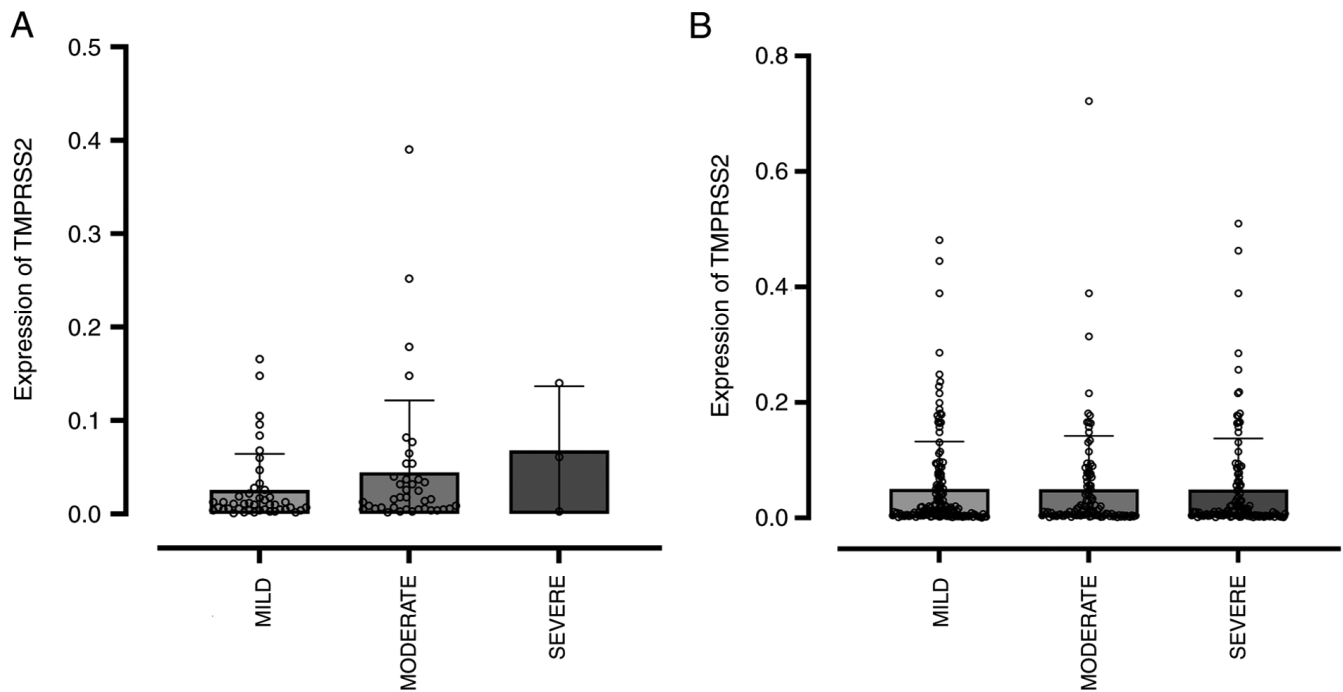


Figure 6. *TMPRSS2* gene expression in the nasopharyngeal cells of individuals (A) without SARS-CoV-2 and (B) with the virus, according to their disease status. The Kruskal-Wallis test was used to analyze the data. *TMPRSS2*, transmembrane serine protease 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

By comparing their expression in respiratory cells of patients with COVID-19 and individuals without the virus, the goal was to identify which receptor is more useful for predicting clinical outcomes. *ACE2* and *TMPRSS2* are essential proteins for the entry of the SARS-CoV-2 virus into cells and play a crucial role in the pathogenesis of COVID-19 (22). The expression of these genes can be influenced by factors such as genetic polymorphisms, cytokines associated with asthma, hormones including testosterone, and even obesity (23,24). Additionally, the expression of these genes can vary across different tissues, such as the lungs, upper respiratory tract, and intestine, which are potential sites of SARS-CoV-2 replication (25).

It is known that *ACE2* expression in the airways is high in the nasal epithelium but progressively decreases in the bronchial and alveolar regions, correlating with the levels of SARS-CoV-2 infection in different compartments of the airways (26). However, the results of the present study indicated that *ACE2* expression was significantly lower in the nasal epithelial cells of patients infected with SARS-CoV-2 compared with individuals without the virus, suggesting a possible negative regulation of this gene during infection. Research has indicated that SARS-CoV-2 infection can modulate *ACE2* function and subsequent inflammatory responses, leading to a decrease in the expression of this gene overtime (22). In fact, there is evidence suggesting that the virus leads to a reduction in *ACE2* expression, occurring through various mechanisms, including the release of *ACE2* from tissues, the reduction of *ACE2* levels in infected cells, the induction of clathrin- and AP2-dependent endocytosis, and subsequent lysosomal degradation (27-29). This downregulation of *ACE2* by the virus has been associated with lung injury and inflammation, further exacerbating

pathological processes in infected individuals (29). In the intestines, on the other hand, the absence of a positive correlation between susceptibility to infection and *ACE2* expression has been described in a subpopulation of enterocytes considered the primary target of the virus. According to the aforementioned study, infected cells activated strong pro-inflammatory programs and produced interferon, while the expression of interferon-stimulated genes was limited to uninfected cells due to the virus's suppression of interferon (30).

The decrease in *ACE2* expression by SARS-CoV-2 may play a role in disease progression and its severity, including the development of acute respiratory distress syndrome (31), as it disrupts the physiological balance between *ACE/ACE2* and *Ang-II/angiotensin-(1-7)*, potentially causing severe organ damage (32). Understanding the mechanisms underlying *ACE2* downregulation by the virus is essential for the development of targeted therapeutic interventions to mitigate the effects of SARS-CoV-2 infection.

In order to assess whether there is an association between *ACE2* expression in the nasal epithelium of individuals with various clinical manifestations of COVID-19, the patients were grouped according to the severity of the disease (mild, moderate, or severe). No significant changes in *ACE2* expression associated with COVID-19 severity were observed. In fact, studies indicate that the presence of *ACE2* in nasal epithelial cells does not necessarily correlate with an increase in the severity of SARS-CoV-2 infection (33,34). It appears that greater vulnerability to severe outcomes in infected individuals may be attributed to other factors, such as the immune response and the presence of different *ACE2* variants, which could influence the severity of the infection (35,36). Therefore, while *ACE2* expression is crucial for

Table V. Correlation between gene expression and COVID-19 severity.

Gene	No. of patients	r_s	P-value
<i>TMPRSS2</i>	191	-0.103	0.154
<i>ACE2</i>	229	-0.030	0.649

Spearman's correlation coefficients (r_s) between the expression of *TMPRSS2* and *ACE* genes and COVID-19 severity. None of the correlations were statistically significant ($P>0.05$).

viral entry into cells, it may not serve as a reliable predictor of disease severity (33).

TMPRSS2, a transmembrane protease, is crucial for the activation of SARS-CoV-2 within human airway cells (35). This protease is expressed in various tissues, including nasal epithelial cells, where it cleaves and activates the viral S protein, facilitating viral entry and infection (36). The co-expression of *TMPRSS2* and *ACE2* in nasal epithelial cells has been associated with increased SARS-CoV-2 infectivity and transmissibility, suggesting a potential role of these cells in the early stages of infection (37). However, despite the decreased *ACE2* expression in infected individuals, the results did not reveal any alteration in *TMPRSS2* gene expression between individuals with and without the virus, indicating that the regulation of this gene may not be influenced by its presence. Research conducted on SARS-CoV, a member of the coronavirus family, has revealed that *TMPRSS2* expression is indeed not affected during viral production (23). According to this study, *TMPRSS2* influences viral entry but not other phases of viral replication, suggesting that the spatial orientation of *TMPRSS2* in relation to the S protein is a key mechanism underlying this phenomenon (23).

In the present study, it was observed that the expression of the *ACE2* and *TMPRSS2* genes does not vary between men and women with COVID-19, nor with the age of infected patients. A previous study suggests that *ACE2* expression may vary with age (38), while another study did not find a consistent correlation (39).

Although a relationship between age, the expression of these genes, and susceptibility or severity of SARS-CoV-2 infection was anticipated, other factors beyond this variable appear to have a greater influence on the modulation of the expression of these genes (23,40). Factors such as genetic variations, pre-existing health conditions, environmental exposures, and other biological and social determinants may play a significant role in regulating *ACE2* and *TMPRSS2* expression, thus influencing SARS-CoV-2 susceptibility and severity.

Additionally, the expression of these genes may be modulated by factors such as inflammation, smoking exposure, and diseases such as asthma and chronic obstructive pulmonary disease (40). Therefore, it is crucial to consider a broad range of factors beyond age when investigating the expression of these genes and their relationship with COVID-19. A potential limitation of the study is that it

did not account for pre-infection factors, such as those aforementioned, which may influence gene expression. However, it is important to emphasize that the present study was conducted during the peak of the pandemic in Brazil, a time when strict social isolation rules and regulations imposed significant limitations on data collection and analysis. These constraints may have impacted the ability to fully explore the role of these additional factors in the context of COVID-19 severity. Furthermore, it is important to highlight that, as this classification relied exclusively on RT-qPCR results, the possibility of false-negative results cannot be ruled out. Therefore, some individuals classified as SARS-CoV-2-negative might have actually been infected, especially in cases of improper sample collection or low viral load at the time of testing.

The downregulation of *ACE2* observed during SARS-CoV-2 infection, in contrast to the stable expression of *TMPRSS2*, highlights the importance of considering multiple influences on gene modulation, such as environmental exposures and other external factors. These insights underscore the need for multifactorial approaches in COVID-19 research and treatment to better understand and mitigate susceptibility and disease severity.

In conclusion, the present study demonstrated that *ACE2* gene expression is significantly reduced in individuals infected with SARS-CoV-2, regardless of symptom severity, age, or sex, suggesting that this downregulation may be a direct consequence of viral infection. By contrast, *TMPRSS2* expression exhibited no significant variation between infected and non-infected individuals, nor across different levels of disease severity. Although both proteins are essential for viral entry, their expression levels in nasopharyngeal cells do not serve as reliable biomarkers for predicting COVID-19 severity. These findings highlight the complexity of COVID-19 pathogenesis and underscore the need for multifactorial approaches, integrating genetic, immunological, and clinical factors, for improved understanding and management of disease progression.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

All authors (SSC, ACBCS, MYSG, MCP, RSS, IDRT, GLV, FLAF, BCBA) contributed equally to the conception, design, data acquisition, analysis, and writing of the manuscript. SSC and BCBA confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of FMABC University Center (protocol number 5.610.755; São Paulo, Brazil) and adheres to Resolution 466/12 of the National Health Council, and all patients received information concerning their participation in the study and provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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