The Journal of Infectious Diseases

MAJOR ARTICLE

TNFRSF1B and *TNF* Variants Are Associated With Differences in Levels of Soluble Tumor Necrosis Factor Receptors in Patients With Severe COVID-19

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Background. The impact of genetic variants in the expression of tumor necrosis factor-α (TNF-α) and its receptors in coronavirus disease 2019 (COVID-19) severity has not been previously explored. We evaluated the association of *TNF* (rs1800629 and rs361525), *TNFRSF1A* (rs767455 and rs1800693), and *TNFRSF1B* (rs1061622 and rs3397) variants with COVID-19 severity, assessed as invasive mechanical ventilation (IMV) requirement, and the plasma levels of soluble TNF-α, TNFR1, and TNFR2 in patients with severe COVID-19.

Methods. The genetic study included 1353 patients. Taqman assays were used to assess the genetic variants. ELISA was used to determine soluble TNF-α, TNFR1, and TNFR2 in plasma samples from 334 patients.

Results. Patients carrying TT (*TNFRSF1B* rs3397) exhibited lower PaO_2/FiO_2 levels than those with CT + CC genotypes. Differences in plasma levels of TNFR1 and TNFR2 were observed according to the genotype of *TNFRSF1B* rs1061622, *TNF* rs1800629, and rs361525. According to the studied genetic variants, there were no differences in the soluble TNF-α levels. Higher soluble TNFR1 and TNFR2 levels were detected in patients with COVID-19 requiring IMV.

Conclusions. Genetic variants in *TNF* and *TNFRSFB1* influence the plasma levels of soluble TNFR1 and TNFR2, implicated in COVID-19 severity.

Keywords. COVID-19; genetics; *TNF*; *TNFRSF1A*; *TNFRSF1B*; TNFR2.

Coronavirus disease 2019 (COVID-19) presents a broad spectrum of clinical manifestations. Although most patients are expected to present a mild or moderate form of the disease, almost 15% progress to severe COVID-19 and 5% to a critical form characterized by acute respiratory distress syndrome (ARDS), septic shock, and/or multiple organ failure [1]. The most severe stage of COVID-19 is related to an extrapulmonary systemic hyperinflammation syndrome, in which the enhancement in levels of cytokines promotes lung inflammation and ARDS development, multiple organ failure, and even death [2–4].

Plasma cytokine levels have been evaluated in patients with COVID-19 and related to the worst disease outcome [5]. For instance, patients admitted to the intensive care unit (ICU) had higher plasma levels of interleukin 2 (IL-2), IL-7, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein 1-α (MIP1-α), tumor necrosis factor-α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) [1]. Also, TNF-α was found to be elevated in critical COVID-19 patients [6] and patients with ARDS and acute kidney injury [7]. In addition, a recent meta-analysis reported higher levels of soluble TNF-α (sTNF) in nonsurvivors compared to survivors of COVID-19 [8].

Moreover, the TNF-α receptors (TNFR1 and TNFR2) play an essential role in cellular mechanisms mediated by this cytokine [9], which could also be implicated in COVID-19 severity and mortality. In this regard, the serum levels of soluble TNFR1 (sTNFR1) have been reported to be significantly higher in ICU patients when compared to non-ICU patients with COVID-19

Received 10 January 2022; editorial decision 10 March 2022; accepted 14 March 2022; published online 16 March 2022.

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The Journal of Infectious Diseases® **2022;226:778–87**

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https://doi.org/10.1093/infdis/jiac101

[10]. Recently, our research group reported an increase of sTNFR1 and ADAM17 related to severity and mortality of COVID-19, as well as higher levels of sTNFR1 and sTNFR2 in patients compared to healthy controls [11].

Variants in the genes encoding TNF-α and its receptors have been widely studied and associated with autoimmune [12–14], chronic [15–17], and infectious diseases [12, 18], as well as with cancer [19]. *TNF* rs1800629 and rs361525 single-nucleotide polymorphisms are considered the most critical variants in human disease susceptibility as these might influence the transcription of the cytokine gene [20]. The former has been associated with severe sepsis [21], while the rs361525 was recently related to influenza A (H1N1) susceptibility [22].

In COVID-19, only the *TNF* G-308 (rs1800629) promoter variant has been evaluated and associated with susceptibility and a more aggressive pattern of the disease [23]. Nevertheless, there is a lack of studies investigating other genetic variants in *TNF*, and its receptors' genes (*TNFRSF1A* and *TNFRSF1B*), on the course and outcome of the disease. We aimed to evaluate the association of single-nucleotide variants in *TNF* (rs1800629, rs361525), *TNFRSF1A* (rs767455, rs1800693), and *TNFRSF1B* (rs1061622, rs3397) with COVID-19 severity, and with sTNF, sTNFR1, and sTNFR2 plasma levels in patients with severe COVID-19.

METHODS

We evaluated 1353 patients diagnosed with COVID-19 and admitted to the Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas (Mexico City, Mexico). Only patients with a positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reverse transcription-polymerase chain reaction (RT-PCR) test and ≥18 years old were consecutively enrolled. The patient or responsible family member signed informed consent. The study protocol was approved by the local Research Ethics Committee (C53-20), and it complies with the Helsinki Declaration criteria. All patients were residents from Mexico City and the metropolitan area, and they had at least 3 prior generations born in Mexico (parents and grandparents) and were considered Mexican mestizo. We have previously demonstrated that this criterion is a good proxy of Mexican ancestry evaluated by ancestry-informative markers [24]. All the included patients were diagnosed with severe COVID-19 as they presented dyspnea, a respiratory rate of ≥30 breaths per minute, blood oxygen saturation ≤90%, and/or $PaO_2/FiO_2 \leq 300$ at the hospital admission [25].

Blood samples for genetic analysis and protein determination were collected in tubes with EDTA. The sampling was performed during the patients' hospital stay. Available clinical data from the electronic medical records were registered in the database and included in the statistical analyses (demographic data, comorbidities, signs and symptoms, length of hospital stay, clinical outcome, and severity of COVID-19). The invasive mechanical ventilation (IMV) requirement (IMV or non-IMV) was considered a severity indicator and dependent variable for the association study.

Genetic Analysis

All patients included in the study were genotyped. Genomic DNA was isolated from peripheral blood mononuclear cells using the commercial BDtract Genomic DNA isolation kit (Maxim Biotech), and stored at 4°C until processed. The *TNF* (rs1800629, rs361525), *TNFRSF1A* (rs767455, rs1800693), and *TNFRSF1B* (rs1061622, rs3397) variants were determined by TaqMan SNP Genotyping Assays (C___7514879_10, C___2215707_10, C___2298465_20, C___2645714_10, C___8861232_20, C___8861228_20, respectively), according to the supplier instructions, in a 7300 Real-Time PCR System (Applied Biosystems/ThermoFisher Scientific).

Quantification of Soluble TNF and TNF Receptors in Plasma Samples

Of the 1353 patients with COVID-19, 334 (selected considering *TNF*, *TNFRSF1A*, and *TNFRSF1B* genotypes) were included in a study to determine soluble TNF and TNF receptors (sTNF, sTNFR1, and sTNFR2) in plasma obtained 4.2 (SD 4.5) days since their admission. A second sampling was performed in 115 patients from the 334 subgroup 10.4 (SD 3.0) days after the first sampling (Supplementary Figure 1). Due to the clinical heterogeneity of the disease, we could not perform the plasma sampling at the same time interval for all patients in relation to the days since symptoms onset, diagnostic test, IMV start, hospitalization days, and IMV days. Nevertheless, we performed a correlation analysis and the levels of sTNF, sTNFR1, and sTNFR2 were not influenced by the time points of sampling (Supplementary Figure 2).

Blood samples were centrifuged at 4500 rpm for 5 minutes, and plasma was separated using micropipettes and stored at −80°C until assayed. Soluble TNF (catalog No. DY210; R&D Systems), TNFR1/*TNFRSF1A* (catalog No. DY225; R&D Systems), and TNFR2/*TNFRSF1B* (catalog No. DY726; R&D Systems) were measured in the plasma samples by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's protocol. The absorbance was read at 450 nm. All samples were assessed in duplicate, reporting in pg/mL the mean value of the wells.

Statistical Analysis

Categorical data are presented as frequencies and continuous values as mean ± standard deviation [SD] or median and interquartile range (IQR). Normal distribution was assessed using the Kolmogorov-Smirnov test. The differences in continuous variables among groups were evaluated using the Mann-Whitney *U* test and Fisher exact test for categorical variables. Association studies and regression analysis were performed using PLINK version 1.07 [26]. We employed Haploview [27] for the linkage disequilibrium analysis of the studied variants.

The differences in the plasma levels of sTNF, sTNFR1, and sTNFR2 among the evaluated genotypes were assessed using the Kruskal-Wallis test; when a significant difference between groups was found, a pairwise comparison was performed using a Wilcoxon rank-sum test with continuity correction. The post hoc power analysis for Wilcoxon-Mann-Whitney tests (2 groups) was determined with G*Power version 3.1.9.7 [28]. We used the Spearman rank test for the correlation analyses. The statistical significance was set at a $P < .05$, and the tests were performed using RStudio version 1.3.1073 [29].

RESULTS

Patient Demographics and Clinical Features

The comparison of the clinical and demographical data among IMV and non-IMV groups is shown in Table 1. Most of the patients included in the study (72.2%) required IMV during their hospitalization. Older patients were present in the IMV group compared to non-IMV patients, and a high frequency of men in the IMV group was observed (odds ratio [OR] = 1.76; 95% confidence interval [CI], 1.38–2.26). In addition, lower PaO_2/FiO_2, more extended hospitalization, and a higher frequency of moderate and severe ARDS were found among the IMV group than non-IMV. We observed mortality of 35.2% among the whole group of the studied patients; however, there was a higher frequency of nonsurvivors in the IMV group than non-IMV (45.7% vs 8.9%; $P < .0001$; OR = 8.63; 95% CI, 5.83–12.78).

Dyspnea, fever, cough, myalgia, and arthralgia were the most common symptoms among patients included in the study. Dyspnea and cough were most frequent in the IMV group, while myalgia and anosmia were common among non-IMV patients (Table 1).

Table 1. Clinical and Demographic Data of Patients with Severe COVID-19 Included in the Study

Characteristic	All (n = 1353)	IMV (n = 977)	Non-IMV (n = 376)	P Value[a]
Age, y	59 (49–67)	60 (50–68)	56 (48–65)	**.0007**
Sex, %, male/female	66.8/33.2	70.4/29.6	57.4/42.5	**<.0001**
BMI, kg/m^2	29.3 (26.1–33.2)	29.4 (26.1–33.2)	28.7 (25.7–32.9)	.0635
Tobacco smoking, %	28.8	29.3	27.5	.4605
Smoking index, pack-years smoked	4.2 (1.5–13.3)	4.2 (1.5–14.1)	4.2 (1.5–11.6)	.9418
DM2, %	28.0	28.1	27.8	.5991
SAH, %	35	35.1	34.8	.9736
Respiratory disease, %	7.8	7.6	8.3	.5755
Heart disease, %	3.9	4.2	3.2	.4952
PaO_2/FiO_2	147 (101–197)	134 (90.9–178)	201 (141–240.2)	**<.0001**
Hospitalization, d	18 (11–28)	22 (15–33)	10 (7–15)	**<.0001**
IMV, d	NA	17 (11–27.3)	NA	NA
Mortality, %				
Survivors	64.8	54.3	91.1	**<.0001**
Nonsurvivors	35.2	45.7	8.9	
ARDS level, %				
Mild	22.8	14.7	48.7	**<.0001**
Moderate	52.6	56.7	40.0	
Severe	24.6	28.6	11.3	
Symptoms, %				
Dyspnea	83.6	85.1	79.8	**.0345**
Fever	70.4	68.9	74.4	.0886
Cough	66.4	68.4	61.4	**.0343**
Myalgia	64.2	62.2	69.4	**.0258**
Arthralgia	61.4	59.7	65.9	.0683
Cephalea	44.5	43.5	47.2	.3874
Odynophagia	24.5	24.8	23.7	.8393
Rhinorrhea	16.0	16.5	14.7	.5984
Ageusia	12.2	12.4	11.7	.6017
Diarrhea	9.7	8.7	12.5	.0857
Chest pain	9.6	9.3	10.4	.2208
Anosmia	6.7	5.4	9.9	**.0058**
Vomit	2.7	2.1	4.2	.0673

Continuous values are presented as median (interquartile range) and categorical data as frequencies in percentage.

Abbreviations: ARDS, acute respiratory distress syndrome; BMI, body mass index; COVID-19, coronavirus disease 2019; DM2, diabetes mellitus type 2; IMV, invasive mechanical ventilation; NA, not apply SAH, systemic arterial hypertension.

[a]Mann-Whitney *U* test and Fisher exact test. Significant differences are highlighted in bold.

PaO_2/FiO_2 Value at Hospital Admission Was Different Among the *TNFRSF1B* rs3397 Genotypes

The allele and genotype frequencies of the variants included in the study are shown in Supplementary Table 1. The frequencies of *TNFRSF1A* variants were similar, probably due to the linkage disequilibrium observed (D′ = 0.94, $r^2 = 0.87$). However, the *TNF* and *TNFRSF1B* variants were not observed in linkage disequilibrium (D′ < 0.1, $r^2 = 0$).

We found a lack of association of the *TNF, TNFRSF1A*, and *TNFRSF1B* genetic variants with IMV requirement (Supplementary Table 1). Only a marginal association was observed for the *TNFRS1B* rs1061622 ($P = .0702$). Likewise, the genetic variants were not found to be predictors variables of the IMV condition in the logistic regression model, even when controlling for age and sex as covariates (Supplementary Table 2).

We also investigated the impact of *TNF*, *TNFRSF1A*, and *TNFRSF1B* genetic variants on the frequency of symptoms observed among IMV and non-IMV groups (Table 1). The *TNF* rs361525 GA + AA genotypes were more frequently found in patients presenting cough when compared to those that did not have this symptom (8.81% vs 4.42%; $P < .01$; OR = 2.09; 95% CI, 1.26–3.46; Supplementary Table 3). In addition, the PaO_2/FiO_2 values determined at hospital admission were higher in patients with *TNFRSF1B* rs3397 TT genotype in comparison to those carrying TC + CC (TT = 141 [IQR, 96.60–194]; TC + CC = 155 [IQR, 111–203]; $P < .01$; Supplementary Figure 3). The remaining variants were not found to be associated with PaO_2/FiO_2, cough, or other COVID-19 symptoms (Supplementary Figure 3 and Supplementary Table 3).

sTNFR1 and sTNFR2 Plasma Levels Are Different According to *TNFRSF1B* rs1061622, *TNF* rs1800629, and rs361525 Genotypes

The plasma levels of sTNFR1 were significantly different according to the *TNFRSF1B* rs1061622 genotype ($P < .05$). Patients carrying TT or GT genotype exhibited higher sTNFR1 (1580 pg/mL [IQR, 1017–2485 pg/mL] and 1499 pg/mL [IQR, 1102–2507 pg/mL], respectively) than those with GG genotype (1031 pg/mL [IQR, 730–1304 pg/mL]) (Figure 1A). Likewise, we observed a trend of the sTNFR2 levels according to the same variant ($P = .06$; Figure 1B). However, the plasma levels of sTNF

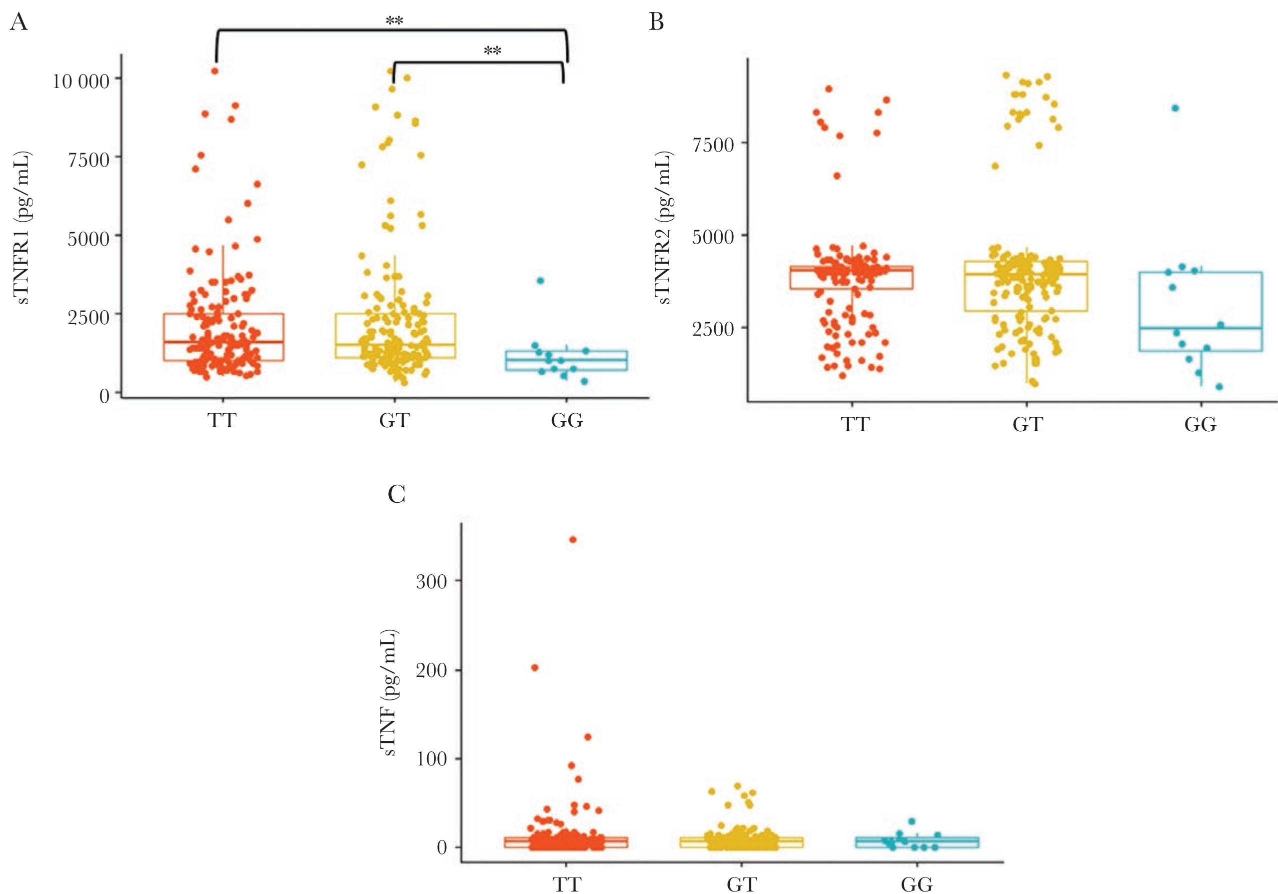

Figure 1. Soluble TNFR1 (*A*), TNFR2 (*B*), and TNF (*C*) plasma levels according to *TNFRSF1B* rs1061622 genotypes. Only significant *P* values are shown. $^{**}P < .01$, Kruskal-Wallis test with Benjamini-Hochberg correction for multiple comparisons. Post hoc statistical power = 80%. The x-axes represent the rs1061622 genotypes, dots correspond to the plasma levels of each individual included in the study, and the boxes contain the median and interquartile range. Abbreviations: sTNF, soluble tumor necrosis factor; sTNFR, soluble tumor necrosis factor receptor.

were similar among all rs1061622 genotypes ($P > .05$; Figure 1C).

The sTNFR2 plasma levels were different among the *TNF* rs1800629 and rs361525 genotypes, ($P < .05$ in both cases; Figure 2B and Figure 3B). For both variants, the higher receptor levels were observed among patients homozygous for the common allele (rs1800629 GG = 3993 pg/mL [IQR, 3329–4220 pg/mL], AG + AA = 2881 pg/mL [IQR, 2221–4023 pg/mL]; rs361525 GG = 3996 pg/mL [IQR, 3277–4161 pg/mL], AG = 3919 pg/mL [IQR, 3055–4338 pg/mL], AA = 1935 pg/mL [IQR, 1482–2372 pg/mL]). However, sTNF and sTNFR1 were not different among the *TNF* rs1800629 and rs361525 genotypes (Figures 2A and 2C and Figure 3A and 3C). Likewise, the plasma levels of sTNF, sTNFR1, and sTNFR2 were not significantly different among the genotypes of the other studied variants (*TNFRSF1A* rs767455, rs1800693, and *TNFRSF1B* rs3397; Supplementary Figures 4–6).

In addition, we evaluated the correlation among the levels of sTNF, sTNFR1, sTNFR2, age, body mass index (BMI), PaO_2/FiO_2, IMV days, and hospitalization stay. A significant correlation was only observed among sTNFR1 and sTNFR2 levels ($\rho = 0.59$, $P < .0001$, Spearman rank correlation test; Supplementary Figure 7), suggesting that both receptors respond in parallel in the immune response.

We also investigated if the increased levels of sTNF, sTNFR1, and sTNFR2 are implicated in patients with severe COVID-19, according to IMV requirement and the severity of ARDS. There were significantly higher levels of sTNFR1 (1701 pg/mL [IQR, 1187–2879 pg/mL] vs 1101 pg/mL [IQR, 872–1654 pg/mL]); sTNFR2 (4020 pg/mL [IQR, 3385–4296 pg/mL] vs 3743 pg/mL [IQR, 2726–4007 pg/mL]) in the patients that required IMV compared to those that did not (Figure 4A and 4B); however, sTNF level did not show differences between IMV and non-IMV (6 pg/mL [IQR, 0.0–11 pg/mL] vs 6 pg/mL [IQR, 0.0–9 pg/mL]; Figure 4C). Interestingly, sTNFR1, and sTNFR2 levels were also observed to be different when the severity of ARDS was considered ($P < .05$ in all cases; Figure 5A–5C and Supplementary Table 4).

We also wanted to know if the sTNF, sTNFR1, and sTNFR2 plasma levels could vary in the stages described for COVID-19 and if this variability was related to the genetic variants. Therefore, considering that patients in this study mainly

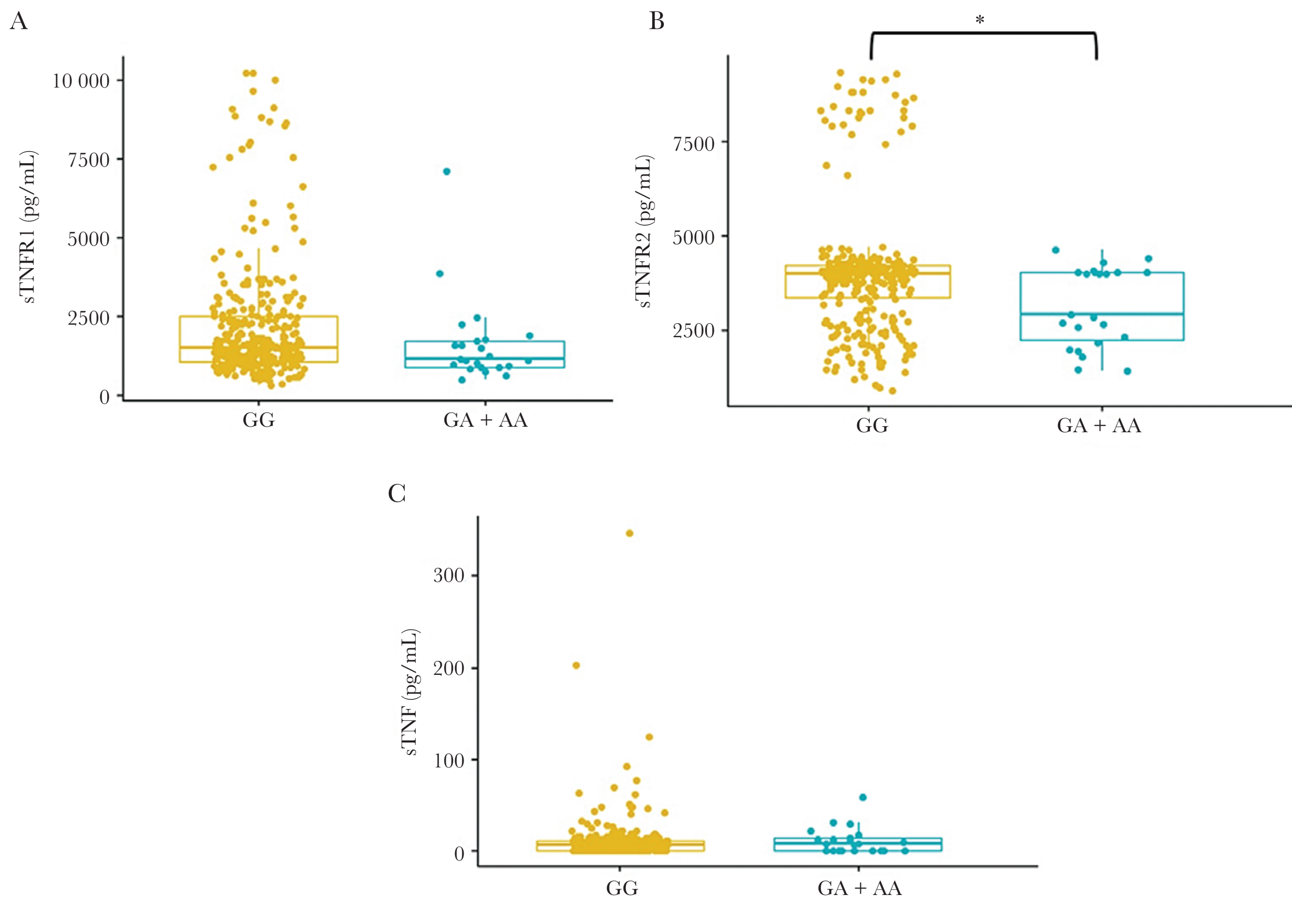

Figure 2. Differences in soluble TNFR1 (*A*), sTNFR2 (*B*), and sTNF (*C*) plasma levels among *TNF* rs1800629 genotypes. The only significant *P* value is shown, $^*P < .05$; Mann-Whitney *U* test. Post hoc statistical power = 88.7%. The x-axes represent the rs1800629 genotypes, dots correspond to the plasma levels of each individual included in the study, and the boxes contain the median and interquartile range. Abbreviations: sTNF, soluble tumor necrosis factor; sTNFR, soluble tumor necrosis factor receptor.

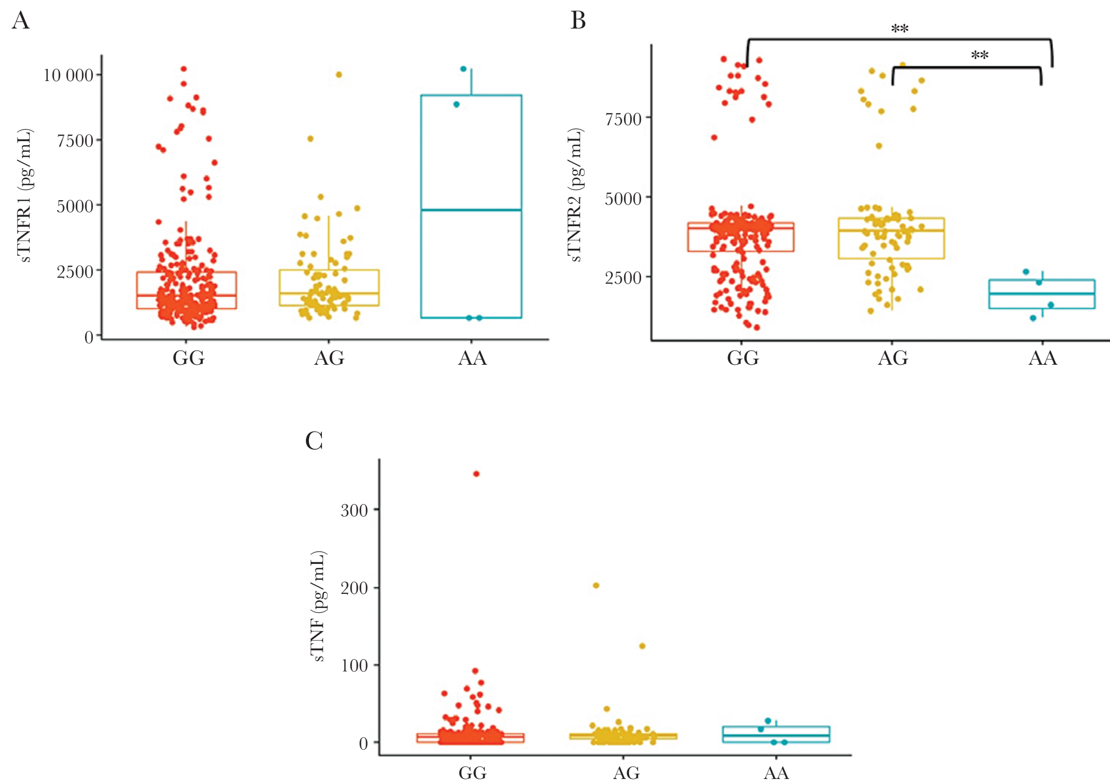

Figure 3. Differences in soluble TNFR1 (*A*), sTNFR2 (*B*), and sTNF (*C*) plasma levels among *TNF* rs361525 genotypes. Only significant *P* values are shown. ** $P < .01$, Kruskal-Wallis test with Benjamini-Hochberg correction for multiple comparisons. Post hoc statistical power >95%. The x-axes represent the rs361525 genotypes, dots correspond to the plasma levels of each individual included in the study, and the boxes contain the median and interquartile range. Abbreviations: sTNF, soluble tumor necrosis factor; sTNFR, soluble tumor necrosis factor receptor.

presented severe COVID-19, we focused on the pulmonary condition, when patients are admitted to hospital and require IMV, and the hyperinflammation stage characterized by an extrapulmonary systemic hyperinflammation syndrome and poor prognosis [3]. Thus, the plasma determination was performed in 115 patients, from the initial subgroup of 334, at 2 time points during their hospitalization. Most of these patients (80.0%) required IMV during hospitalization. The median time of the first determination (pulmonary phase) was 2 days (IQR, 1–4 days), while the second determination (hyperinflammation phase) was obtained 8 days (7–11 days) after the date of patients' admission.

sTNF and sTNFR2 plasma levels were similar at the 2 measurements. However, an increase of sTNFR1 was observed in the second determination compared to the first (Table 2), suggesting some relevance of sTNFR1 in the hyperinflammatory phase. The variations in sTNF, sTNFR1, and sTNFR2 in the 2 stages of severe COVID-19 were independent of the genetic variants included in the study (Supplementary Figures 8–10). In addition, we wondered if this variation could impact the clinical outcome of patients, but we did not find any differences in the plasma levels of sTNF, sTNFR1, and sTNFR2 of survivors and nonsurvivors of COVID-19 (Supplementary Figure 11). Finally, the Δ value was assessed (Table 2) and, in agreement with this last finding, the Δ value of sTNFR1 was the highest, and it was also not influenced by the *TNF*, *TNFRSF1A*, and *TNFRSF1B* genetic variants included in the study (Supplementary Figures 12–14).

DISCUSSION

The increase of sTNF, sTNFR1, and sTNFR2 levels has been previously linked to COVID-19 severity and mortality. Herein, we report, for the first time, that genetic variants in the genes encoding these proteins (mainly *TNF* and *TNFRSFB1*) are associated with the plasma levels of the receptors sTNFR1 and sTNFR2 in patients with severe COVID-19. Although we were not able to find a difference between the studied phenotype (IMV and non-IMV groups), we found a relationship of the genetic variants with differences in the levels of the TNF receptors, and this could be linked to the severity of the disease because higher levels of these proteins were observed among patients with IMV and a severe ARDS.

Increased plasma levels of sTNFR1 were observed among patients with TT and GT genotypes of *TNFRSF1B* rs1061622 and a correlation between the levels of the 2 receptors. This

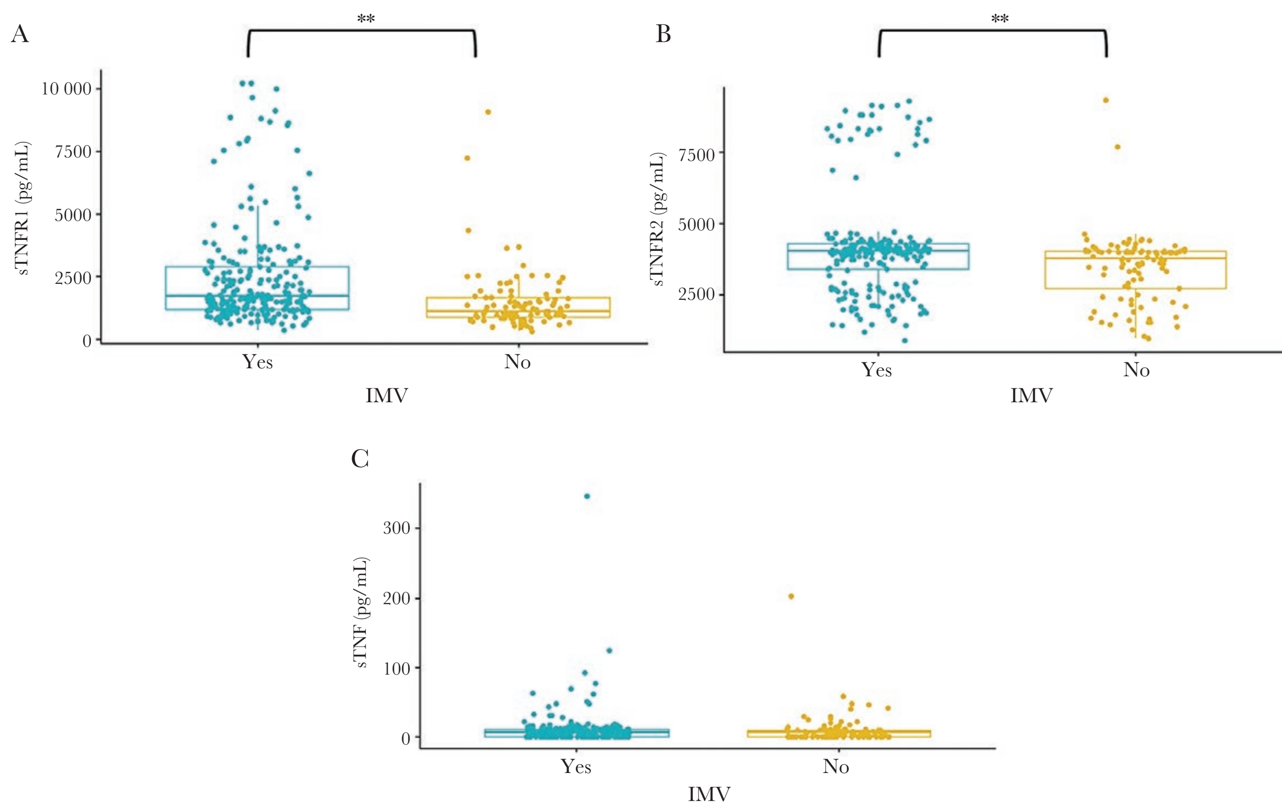

Figure 4. Soluble TNFR1 (*A*), sTNFR2 (*B*), and sTNF (*C*) plasma levels in IMV and non-IMV patients. Only significant *P* values are shown, $^{**}P < .01$; Mann-Whitney *U* test. Post hoc statistical power >95%. The x-axes represent the IMV requirement, dots correspond to the plasma levels of each individual included in the study, and the boxes contain the median and interquartile range. Abbreviations: IMV, invasive mechanical ventilation; sTNF, soluble tumor necrosis factor; sTNFR, soluble tumor necrosis factor receptor.

observation agrees with a previous study performed in patients with rheumatoid arthritis in which the serum levels of both receptors were higher in carriers of the TT genotype for this variant. The rs1061622 consists of a substitution of methionine to arginine, which results in a significantly lower capability to induce TNFR2-mediated nuclear factor-κB (NF-κB) activation [30]. It has also been associated with distinct chronic inflammatory diseases, including cystic fibrosis and severe pulmonary disorder [31].

Differences in sTNFR2 were also observed when the plasma levels of proteins were evaluated considering the *TNF* rs1800629 and rs361525 genotypes. In both cases, the variants are located in the gene promoter region and have been related to differences in TNF-α expression and several disorders, including chronic obstructive pulmonary disease [32]. We did not observe differences in the sTNF plasma levels between the *TNF* genotypes. However, investigations have reported the influence of *TNF* genotypes in the expression of inflammatory pathway components, including the TNFR1 and TNFR2 [33–35].

It has been reported that TNFR2 mediates the stimulatory activity of TNF on $CD4^+Foxp3^+$ regulatory T cells (Tregs) and $CD8^+Foxp3^+$ Tregs, and is involved in the phenotypic stability, proliferation, activation, and suppressive activity of Tregs. This receptor can also be expressed on $CD8^+$ effector T cells (Teffs), which delivers an activation signal and cytotoxic ability to $CD8^+$ Teffs during the early immune response. An apoptosis signal terminates the immune response, which is uncontrolled during the cytokine release syndrome. Due to TNFR2 distribution and its pleiotropic effects, the receptor appears to be crucial for keeping the balance between Tregs and Teffs. It has been proposed as an efficient therapeutic target for impaired immune responses, such as cancer and autoimmune diseases [36]. Furthermore, this study confirms the previous hypothesis of targeting the TNF receptors for COVID-19 treatment [11].

A study from Egypt identified the *TNF* rs1800629 AA genotype associated with an aggressive COVID-19 pattern [23]. The severity of the disease was determined using several variables, including mechanical ventilation. Accordingly, we observed a higher frequency of AA and GA genotypes among the IMV group when compared to non-IMV; however, this was not statistically significant. Only marginal associations were observed for the genotype frequency of *TNFRSF1B* rs1061622.

However, the GA and AA genotypes of *TNF* rs361525 were associated with cough, a frequent symptom in COVID-19, particularly in the IMV group. This variant has been previously

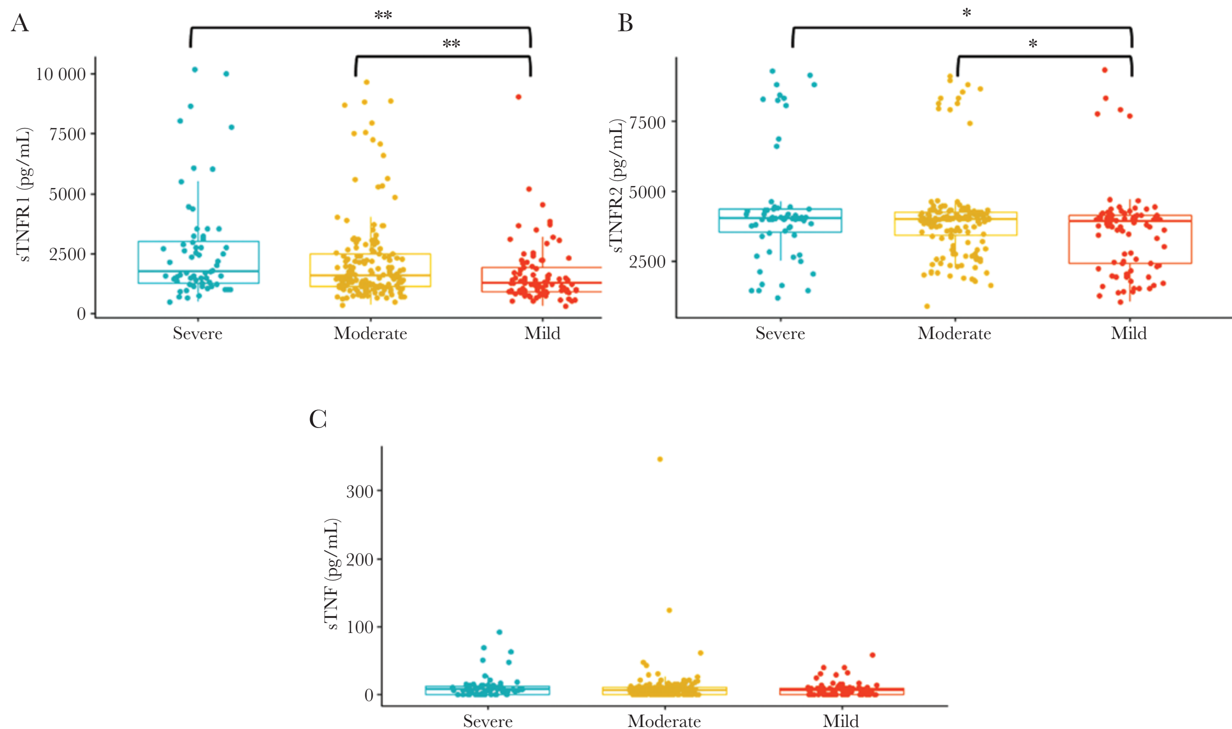

Figure 5. Plasma levels of soluble TNFR1 (*A*), TNFR2 (*B*), and TNF (*C*), according to the severity of the acute respiratory distress syndrome in patients with COVID-19. Only significant *P* values are shown. *$P < .05$, **$P < .01$; Kruskal-Wallis test with Benjamini-Hochberg correction for multiple comparisons. Post hoc statistical power >80%. The x-axes represent the level of acute respiratory distress syndrome, dots correspond to the plasma levels of each individual included in the study, and the boxes contain the median and interquartile range. Abbreviations: sTNF, soluble tumor necrosis factor; sTNFR, soluble tumor necrosis factor receptor.

associated with increased local production and downstream inflammation in chronic obstructive pulmonary disease [37]; primarily, the A allele was related to increased transcriptional activation of the TNF promoter and susceptibility to several arthritic conditions [38]. We also evaluated the PaO_2/FiO_2 values at the hospital admission as an indicator of COVID-19 severity, and these were different among the *TNFRSF1B* rs3397 genotypes. This variant has been related to an impaired *TNFRSF1B* expression [35, 36], worsening inflammation process, susceptibility to *Mycobacterium avium paratuberculosis* infection, and osteoporosis in rheumatoid arthritis [39]. Thus, in both cases, the variants *TNF* rs361525 and *TNFRSF1B* rs3397 have been previously related to inflammation process and immunologic diseases, which could explain the associations found with clinical variables of severe COVID-19.

In a different set of patients, our research group previously reported an increase of sTNFR1 related to the severity and mortality of COVID-19 [11]. In the present study, we found that the increase of both TNF receptors is present in patients with severe COVID-19 (IMV requirement and severest form of ARDS) and that sTNFR1 is even higher after 8 days since hospital admission. Although this last difference could not be associated with the genetic variants included in the study, it supports the previous finding that the increase of sTNFR1 in the COVID-19 evolution is related to the clinical outcome of the disease. The clinical and pharmacological management and other factors can probably affect the plasma levels of sTNF and the receptor; therefore, further studies are required to clarify the clinical implication of the sTNFR1 levels in the hyperinflammatory phase of severe COVID-19.

Table 2. Determination at 2 Time Points of Soluble TNF, TNFR1, and TNFR2 in Patients With Severe COVID-19 (n = 115)

Molecule	Sample 1, pg/mL	Sample 2, pg/mL	P Value[a]	Δ Time 2 − Time 1
sTNF	8 (0–12)	7 (4–11)	.6887	0.0 (−4 to 4)
sTNFR1	1494 (1137–2488)	1782 (1167–2911)	**.0288**	295 (−385 to 774)
sTNFR2	3839 (3090–4253)	4000 (2866–4422)	.4692	28 (−561 to 435)

Plasma levels of the molecules are presented as median (interquartile range).

Abbreviations: sTNF, soluble tumor necrosis factor; sTNFR, soluble tumor necrosis factor receptor.

[a]Mann-Whitney *U* test.

This study presents some limitations. We only included patients with severe COVID-19 because the recruitment center is a third-level reference hospital, so we could not compare with less severe forms of the disease and/or a control group. Likewise, we could not perform the determination of the cytokines' plasma levels at both the time points in all patients, but the results provide valuable information for further studies. In addition, the study of the relationship between the genetic variants and the clinical outcome of IMV and non-IMV patients and PaO_2/FiO_2 during the course of the disease may provide important information. Despite these limitations, we report the relevance of *TNF* and *TNFRSF1B* genetic variants in the severity of COVID-19 mediated by the plasma levels of TNF receptors, contributing to the knowledge of the severe COVID-19 at the inflammatory stage.

Conflict of Interest

The authors do not have any commercial or other association that might pose a conflict of interest.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgment. The authors acknowledge the support received from physicians and technicians from the clinical services at Instituto Nacional de Enfermedades Respiratorias to confirm the study participants' diagnosis and clinical care.

Financial support. This work was supported by Consejo Nacional de Ciencia y Tecnología through the program Estancias Posdoctorales por México-COVID19 (to I. F. G. and Y. P.).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Presented in part: e-ZOOMMIT Respiratorio 2021, El Pandemónium: COVID-19 y la Neumología. Mexican Society of Pneumology and Thoracic Surgery, online, June 2021; and 14º Congress ALAT, Latin American Thoracic Association, online, December 2021.

Data availability. The datasets for this study can be found in the ClinVar database: SCV001792244, SCV001792245, SCV001792246, SCV001792247, SCV001792248, and SCV001792249.

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