


# The relationship of serum testosterone levels with the clinical course and prognosis of COVID-19 disease in male patients: A prospective study

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## Abstract

**Background:** A potential role of testosterone among sex hormones has been hypothesized in identifying sex-related differences in the clinical consequences of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. Due to the high global prevalence of hypogonadism, the relationship between hypogonadism and SARS-CoV-2 infection outcomes deserves an in-depth study.

**Objective:** The present study aimed to investigate the relationship of serum testosterone with other laboratory parameters on the prognosis of coronavirus disease-19 (COVID-19) in male patients with COVID-19 diagnosis.

**Materials and methods:** This prospective cohort study included 358 male patients diagnosed with COVID-19 and 92 COVID-19 negative patients admitted to the urology outpatient clinics as a control group. The COVID-19 patients were divided into groups according to prognosis (mild-moderate and severe group), lung involvement in chest computed tomography (<50% and >50%), intensive care unit needs, and survival.

**Results:** The measured serum total testosterone level of the COVID-19 patients group was found to be significantly lower than that of the control group (median, 140 ng/dl; range, 0.21–328, 322 ng/dl; range, median, 125–674,  $p < 0.001$ , respectively). The serum TT levels were statistically significantly lower in severe COVID-19 patients compared to mild-moderate COVID-19 patients (median, 85.1 ng/dl; range, 0.21–532, median, 315 ng/dl; range, 0.88–486,  $p < 0.001$ , respectively), in COVID-19 patients in need of intensive care compared to COVID-19 patients who did not need intensive care (median, 64.0 ng/dl; range, 0.21–337, median, 286 ng/dl; range, 0.88–532  $p < 0.001$ , respectively), and in COVID-19 patients who died compared to survivors (median, 82.9 ng/dl; range, 2.63–165, median, 166 ng/dl; range, 0.21–532,  $p < 0.001$ , respectively).

**Discussion and conclusion:** Our data are compatible with low TT levels playing a role on the pathogenesis of the disease in Covid-19 patients with poor prognosis and a mortal course and may guide clinicians in determining the clinical course of the disease.

**KEYWORDS**

COVID-19, hypogonadism, mortality, prognosis, SARS-CoV-2, testosterone

## 1 | INTRODUCTION

In December 2019, a series of pneumonia cases of unknown etiology were observed in Wuhan, a city in China's Hubei province.<sup>1</sup> It was later stated that this pneumonia was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease was identified as coronavirus disease-2019 (COVID-19).<sup>2</sup> The virus has spread around the world very fast, and in March 2020, the World Health Organization (WHO) declared the COVID-19 outbreak as a global pandemic.<sup>3</sup>

Gender-related COVID-19 mortality is one of the most frequently reported epidemiological data, and it has been shown in post-COVID-19 outbreak studies that the disease is more severe and fatal in men, potentially due to gender-related immunological response and additional factors.<sup>4</sup> Various social factors, genetic, immunological, hormonal differences, and lifestyle habits (i.e., smoking and alcohol consumption) are thought to play a role in this gender disparity in the prognosis of the disease.<sup>5</sup> A potential role of testosterone among sex hormones has been hypothesized in identifying sex-related differences in the clinical consequences of SARS-CoV-2 infection.<sup>6</sup> Studies have shown that the serum total testosterone (TT) levels are decreased due to aging and in the presence of comorbidities such as obesity, diabetes mellitus, and cardiovascular diseases, which are quite common in SARS-CoV-2 patients.<sup>7</sup> Due to the high global prevalence of hypogonadism (estimated to be 15%–20% among middle-aged/elderly men), the relationship between hypogonadism and SARS-CoV-2 infection outcomes deserves an in-depth study.<sup>6,8,9</sup>

In line with all these scientific studies, in this cohort study, we aimed to investigate the serum testosterone level and its relationship with other laboratory parameters on COVID-19 prognosis in male patients diagnosed with COVID-19.

## 2 | MATERIALS AND METHODS

### 2.1 | Patient selection

Ethics committee approval was obtained from the Ataturk University prior to the study (meeting number: 06, meeting date: 28.05.2020, decision number: 72). A total of 358 male patients who were diagnosed with COVID-19 and followed-up on outpatient and inpatient bases and treated in the Health Sciences University Erzurum Regional Training and Research Hospital and Health Sciences University Trabzon Kanuni Training and Research Hospital between June 2020 and January 2021 were included in the study. The control group consisted of male

patients with no diagnosis of COVID-19, who had been admitted to the urology outpatient clinics of the same centers between the same dates. The control group included 92 male patients in total, who were compatible with the patient group in terms of age and gender. Informed consent was obtained from all patients included in the study. The study was begun with 402 patients who fulfilled inclusion criteria, 44 patients did not accept the drawing of blood, and hence, the study was completed with 358 COVID-19 patients. A detailed anamnesis was obtained from all male patients diagnosed with COVID-19. After the physical examinations of the COVID-19 patients were performed, their laboratory results and radiological images were examined and recorded. COVID-19 patients who were under 18 years of age, women, and those with real-time reverse transcription polymerase chain reaction nucleic acid amplification test (RT-PCR) negativity in nasopharyngeal and/or nasal swab samples and patients receiving testosterone replacement therapy (TRT) were excluded from the study.

### 2.2 | Study groups

The patients were divided into two groups as mild/moderate and severe patient groups according to the prognosis criteria published in the guide titled "COVID-19 Adult Patient Treatment" on October 9, 2020 by the Scientific Advisory Board Study of the Ministry of Health of the Republic of Turkey.

In accordance with this guide:

- Patients with symptoms such as fever, muscle/joint pain, cough, and sore throat, respiratory rate < 30/min, SpO<sub>2</sub> level > 90% at room air,
- Patients with signs of mild to moderate pneumonia on chest radiography or tomography (<50% involvement),
- Patients who do not have poor prognostic criteria in blood tests performed on admission (blood lymphocyte count 10 x Upper limit of normal value, or ferritin > 500 ng/ml, or D-Dimer (DD) > 1000 ng/ml, etc.)

constituted the *Mild-moderate patient group*:

- Patients with symptoms such as fever, muscle/joint pain, cough and sore throat, tachypnea (30/min), and SpO<sub>2</sub> level ≤ 90% at room air,
- Patients with bilateral diffuse pneumonia on chest radiography or tomography,

- c. Patients with poor prognostic criteria (blood lymphocyte count  $10 \times$  Upper limit of normal value or ferritin  $> 500$  ng/ml or DD  $> 1000$  ng/ml, etc.) in blood tests carried out on admission

constituted the *Severe patient group*.

## 2.3 | COVID-19 diagnosis

SARS-CoV-2 infection was confirmed using pharyngeal and/or nasal swab positivity (RT-PCR). Positive radiological findings of SARS-CoV-2 infection (unilateral or bilateral ground glass images, parenchymal consolidation) in the thorax computed tomography (CT) (Toshiba Aquilion 64 CT Scanners) were recorded.

## 2.4 | Statistical method

Categorical data were presented as numbers and percentages. Descriptive statistics were used to define continuous variables (mean, standard deviation, minimum, median, maximum). The Shapiro–Wilk test was used to determine whether the distributions of continuous variables were normal. The difference between the demographic data and the mean hormone levels of the control and COVID-19 patients was compared using the independent sample *t* test in normally distributed data. In non-normaly distributed data, Mann–Whitney *U* test was used. The preoperative comorbidity distribution of the control group and COVID-19 patients was compared using the Pearson chi-square test. Patients were grouped according to clinical features, lung involvement severity, and intensive care admission. Mean differences of TT, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and TLR levels were compared using ANOVA in normally distributed data and Kruskal–Wallis test in non-normally distributed data. Statistical significance was considered when *p* value was considered when *p* value was  $< 0.05$ . Sperman's correlation coefficient was used to evaluate the factors that affect the length of hospital stay and length of intensive care unit stay. Sperman's correlation coefficient was used to evaluate the correlation of testosterone/TLR with laboratory parameters. All variables that were found to be statistically significant in univariable binary logistic regression were executed in multivariable analysis to determine predictive factors of need for ICU, exitus, and bad prognosis. Receiver operating characteristic (ROC) analysis was used to assess the cut-off value and the predictive ability of testosterone level on ICU and exitus. Statistical analysis was performed using Statistical Package of Social Sciences version 21 (IBM SPSS Statistics; IBM Corp., Armonk, NY).

## 2.5 | Evaluation of serum TT, FSH, and LH levels

The blood samples of the patients were collected on a voluntary basis, and the volunteer information form was read and signed by the patient or their relative as consent form. Venous serum samples were obtained from each patient diagnosed with COVID-19 in a tube of at least 5 ml on the first day of hospitalization. Sera were collected between 7 and 11

o'clock in the morning to measure the TT, FSH, and the LH levels considering the circadian rhythm. These hormones were measured using the chemiluminescence immunoassay method (Siemens Atellica Reagent) in both centers (Erzurum Regional Training and Research Hospital and Trabzon Kanuni Training and Research Hospital). Hypogonadism was accepted as a serum TT level of  $< 300$  ng/dl, which is the cut-off value of the American Urological Association (AUA).<sup>10</sup>

## 3 | RESULTS

The study included 358 male patients diagnosed with COVID-19 and 92 COVID-19 negative patients admitted to the urology outpatient clinics as a control group. The mean age of the control group was 67.2 (median, 69; range, 25–91) years, the mean age of the patients was 64.9 (median, 69; range, 25–91) years, and there was no statistically significant difference in age between the patient and the control groups ( $p = 0.10$ ). The mean BMI of the control group was 26.4 (median, 26.6; range, 20.1–34.4)  $\text{kg}/\text{m}^2$ , and the mean BMI of the patient group was 25.9 (median, 27.7; range, 16.6–59.1)  $\text{kg}/\text{m}^2$ , and no statistically significant difference was observed ( $p = 0.28$ ).

The most frequently observed symptoms in patients were fever (62%), cough (60%), dyspnea (48.6%), tachypnea (48.3%), weakness-fatigue (48%), headache (43%), myalgia (39.1%), sore throat (36.9%), GIS symptoms (31%), and loss of smell (21%), respectively.

The measured serum TT level of the patient group was found to be significantly lower than that of the control group (median, 140 ng/dl; range, 0.21–328, median, 322 ng/dl; range, 125–674,  $p < 0.001$ , respectively). It was observed that the measured serum FSH level of the patients was significantly higher than the control group (median, 6.37 mIU/ml; range, 0.4–56.4, median, 4.52 mIU/ml; range, 1.23–14.6, respectively,  $p < 0.001$ ). The measured serum LH levels of the patients were found to be significantly higher than the control group (median, 6.59 mIU/ml; range, 0.03–35.1, median, 3.64 mIU/ml; range, 1.05–8.76, respectively,  $p < 0.001$ ). In addition, it was observed that the ratio of serum TT to serum LH (TT:LH) determined in patients was significantly lower than that of the control group (median, 21.4; range, 0.01–328, median, 86.3; range, 27.9–401, respectively,  $p < 0.001$ ). The demographic characteristics and the comparative results of hormone values of the patients and the control group have been displayed in Table 1.

Of the 358 patients, 153 (42.7%) had a good prognosis and 205 (57.2%) had a poor prognosis. It was determined that 152 of the patients (42.4%) needed intensive care. Forty-two of the patients (11.2%) died. The serum TT levels were statistically significantly lower in severe COVID-19 patients compared to mild-moderate COVID-19 patients (median, 85.1 ng/dl; range, 0.21–532, median, 315 ng/dl; range, 0.88–486,  $p < 0.001$ , respectively), in COVID-19 patients in need of intensive care compared to COVID-19 patients who did not need intensive care (median, 64.0 ng/dl; range, 0.21–337, median, 286 ng/dl; range, 0.88–532  $p < 0.001$ , respectively), and in COVID-19 patients who died compared to survivors (median, 82.9 ng/dl; range, 2.63–165, median, 166 ng/dl; range, 0.21–532,  $p < 0.001$ , respectively).

**TABLE 1** Comparison of demographic findings and hormone values between control group and patient groups diagnosed with COVID-19

Variables	COVID-19 <i>n</i> = 358		Controls <i>n</i> = 92		<i>p</i> -value
	Mean (SD)	Median (range)	Mean (SD)	Median (range)	
Age (years)	64.9 (11.6)	69 (25–91)	67.2 (13.6)	69 (25–91)	0.10*
BMI (kg/m <sup>2</sup> )	25.9 (3.8)	27.7 (16.6–59.1)	26.4 (3.1)	26.6 (20.1–34.4)	0.28*
TT (ng/dl)	179 (136)	140 (0.21–328)	344 (115)	322 (125–674)	<0.001**
FSH (mIU/ml)	7.52 (6.69)	6.37 (0.4–56.4)	4.64 (2.05)	4.52 (1.23–14.6)	<0.001**
LH (mIU/ml)	7.30 (4.35)	6.59 (0.03–35.1)	3.75 (1.72)	3.64 (1.05–8.76)	<0.001**
TLR	34.8 (47.1)	21.4 (0.01–328)	116 (74)	86.3 (27.9–401)	<0.001**
	COVID-19 <i>n</i> (%)		Controls ( <i>n</i> %)		
Smoking	263 (73.5)		66 (71.7)		0.52 <sup>‡</sup>
DM	83 (23.2)		15 (16.3)		0.15 <sup>‡</sup>
COPD	102 (28.5)		8 (8.7)		<0.001 <sup>‡</sup>
CAD	142 (39.7)		35 (38.0)		0.77 <sup>‡</sup>
HT	212 (59.2)		32 (34.8)		<0.001 <sup>‡</sup>
CRF	23 (11.2)		8 (5.2)		0.046 <sup>‡</sup>

Abbreviations: BMI, body mass index; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CRF, chronic renal failure; DM, diabetes mellitus; FSH, follicle stimulating hormone; HA, hospital admission; ICU, intensive care unit; LH, luteinizing hormone; SD, standard deviation; TLR, testosterone to luteinizing hormone ratio; TT, total testosterone.

\*Independent *T* test.

\*\*Mann–Whitney *U* test.

<sup>‡</sup>Pearson chi-square.

When the patients were evaluated in terms of thorax CT, no involvement was observed in the lungs in 78 of the 358 patients, while 161 had less than 50% involvement in both lungs, and 119 had an involvement of more than 50% in both lungs. The median serum TT levels were 87.7 ng/dl (range, 0.21–433) in patients with severe CT involvement, 166 ng/dl (range, 0.88–532) in patients with mild CT involvement and 300 ng/dl (range, 15.6–486) in patients without lung involvement. The comparative results of laboratory parameters of the control group and the patients have been demonstrated in Table 2.

While the median duration of hospitalization of patients diagnosed with COVID-19 was 6 (range, 2–18) days, it was found to be 13 (range, 3–45) days in patients who needed an intensive care unit. A significant negative correlation was observed between the serum TT level and the length of stay in the hospital. In addition, a significant negative correlation was found between the serum TT level of the patients followed in the intensive care unit and the length of stay in the intensive care unit (Table 3).

While a statistically significant negative correlation was observed between the serum TT level and serum DD, lactate dehydrogenase (LDH), C-reactive protein (CRP), and ferritin levels, a significant positive correlation was determined with the lymphocyte levels. Furthermore, while a significant negative correlation was observed between the TT:LH ratio measured in patients and the serum DD, LDH, CRP and ferritin levels, a significant positive correlation was found with the lymphocyte levels (Table 4).

We found that serum TT level was statistically significant in the univariate and the multivariate binary logistic regression analysis performed to estimate the intensive care unit need and mortality of

patients (OR: 0.985, %CI: 0.985–0.993; OR: 0.989, %CI: 0.989–0.998 respectively). The results of the univariate and the multivariate binary logistic regression analysis performed to estimate the intensive care unit needs and the mortality of patients have been presented in Table 5.

ROC analysis was performed to determine whether the serum TT levels could be used to determine poor prognosis, intensive care unit needs, and mortality in patients with COVID-19 diagnosis. The area under the curve calculated for the serum TT level (AUC = 0.873) was sufficient to be used in determining a poor prognosis (AUC > 0.5). The serum TT level had 90.7% sensitivity and 76.5% specificity in determining the poor prognosis of the patients when using a cut-off of 211.7 ng/dl (Figure 1). In addition, the area under the curve calculated for the serum TT level (AUC = 0.858) was sufficient to be used in determining the need for intensive care (AUC > 0.5). The serum TT level had 96.1% sensitivity and 68% specificity in determining the intensive care needs of the patients when using a cut-off of 185.2 ng/dl (Figure 2).

The area under the curve calculated for the serum TT level (AUC = 0.707) was sufficient to be used in determining the mortality (AUC > 0.5). The serum TT level had 97.6% sensitivity and 49.7% specificity in determining mortality by taking the cut-off point as 165.6 ng/dl (Figure 3).

## 4 | DISCUSSION

Since the report of the first cases of pneumonia of unknown cause by the WHO at the close of 2019, the Sars-CoV-2 and its related disease COVID-19 have spread rapidly all over the globe. According to

**TABLE 2** Comparative results of laboratory parameters of patients with COVID-19 according to prognosis

Groups	N	TT (ng/dl)		FSH (mIU/ml)		LH (mIU/ml)		TLR	
		Mean (SD)	Median (range)	Mean (SD)	Median (range)	Mean (SD)	Median (range)	Mean (SD)	Median (range)
Controls (1)	92	344 (1.15)	322 (125–674)	4.64 (2.05)	4.52 (1.23–14.65)	3.75 (1.72)	3.64 (1.05–8.76)	1.16 (74.7)	86.3 (27.9–401)
Mild-moderate (2)	153	283 (1.14)	315 (0.88–486)	8.51 (6.87)	6.85 (0–32.9)	8.06 (3.74)	7.77 (1.17–18.3)	44.7 (43.1)	36.9 (0.24–278)
Severe (3)	205	101 (92)	85.1 (0.21–532)	6.78 (6.47)	5.63 (0–56.4)	6.73 (4.69)	5.78 (0.03–35.1)	27.4 (48.7)	14.0 (0.01–328)
p-value		<0.001 <sup>*</sup>		<0.001 <sup>*</sup>		<0.001 <sup>*</sup>		<0.001 <sup>*</sup>	
		1 vs. 2 < 0.001		1 vs. 2 < 0.001		1 vs. 2 < 0.001		1 vs. 2 < 0.001	
		2 vs. 3 < 0.001		2 vs. 3 0.048		2 vs. 3 < 0.001		2 vs. 3 < 0.001	
Lung involvement									
Absent (1)	78	279 (112)	300 (15.6–486)	6.77 (4.67)	6.85 (0–17.1)	8.36 (3.90)	7.44 (4.22–18.2)	38.3 (19.5)	33.6 (1.28–77.1)
Mild <sup>†</sup> (2)	161	191 (145)	166 (0.88–532)	8.51 (7.60)	6.73 (0–56.4)	6.87 (4.21)	6.59 (0.03–23.9)	44.6 (62.0)	30.6 (0.23–328)
Severity <sup>††</sup> (3)	119	96.2 (74.5)	87.7 (0.21–433)	6.68 (6.35)	5.36 (0.26–32.9)	6.73 (4.69)	6.35 (0.61–35.1)	19.4 (29.5)	12.6 (0.01–217)
p-value		<0.001 <sup>*</sup>		0.041 <sup>††,‡</sup>		0.043 <sup>††</sup>		<0.001 <sup>*</sup>	
		1 vs. 2 < 0.001		1 vs. 2 0.14		1 vs. 2 0.035		1 vs. 2 0.56	
		1 vs. 3 < 0.001		1 vs. 3 0.99		1 vs. 3 0.15		1 vs. 3 < 0.001	
		2 vs. 3 < 0.001		2 vs. 3 0.06		2 vs. 3 0.81		2 vs. 3 < 0.001	
ICU (-)	206	252 (130)	286 (0.88–532)	8.34 (6.63)	6.89 (0–32.9)	7.92 (3.80)	7.63 (0.45–18.3)	40.7 (43.8)	32.3 (0.21–291)
ICU (+)	152	78 (58)	64.0 (0.21–337)	6.41 (6.63)	5.21 (0.23–56.4)	6.46 (4.90)	5.54 (0.03–35.1)	26.9 (50.3)	12.4 (0.01–328)
p-value		<0.001 <sup>††</sup>		0.007 <sup>†††</sup>		0.002 <sup>†††</sup>		0.006 <sup>†††</sup>	
Alive	316	191 (138)	166 (0.21–532)	7.64 (6.98)	6.56 (0–56.4)	7.35 (4.27)	6.61 (0.03–35.1)	38.0 (47.3)	23.9 (0.01–295)
Exitus	42	84 (59)	82.9 (2.63–165)	6.61 (3.83)	5.89 (0.28–12.2)	6.94 (4.97)	5.18 (0.13–23.9)	25.7 (44.9)	13.3 (0.72–328)
p-value		<0.001 <sup>†††</sup>		0.34 <sup>†††</sup>		0.56 <sup>†††</sup>		0.18 <sup>†††</sup>	
Surviving COVID-19 patients									
Mild-moderate	153	283 (114)	315 (0.88–486)	8.51 (6.87)	6.85 (0–32.9)	8.06 (3.74)	7.77 (1.17–18.3)	44.7 (43.1)	36.9 (0.24–278)
Severe	163	107 (101)	86.5 (0.21–532)	7.13 (7.03)	5.60 (0–56.4)	6.98 (4.74)	5.84 (0.03–35.1)	25.2 (43.5)	14.0 (0.01–295)
p-value		<0.001 <sup>†††</sup>		0.07 <sup>†††</sup>		0.025 <sup>†††</sup>		<0.001 <sup>†††</sup>	

Abbreviations: FSH, follicle stimulating hormone; ICU, intensive care unit; LH, luteinizing hormone; SD, standard deviation; TLR, testosterone to luteinizing hormone ratio; TT, total testosterone.

<sup>\*</sup>Kruskal wallis.<sup>††</sup>One-Way ANOVA.<sup>†††</sup>There was no statistically significant differences between group after post hoc analysis.<sup>††††</sup>Mann–Whitney U test.<sup>†††††</sup>Independent T test.<sup>†</sup>Patients with signs of mild pneumonia on chest tomography (<50% involvement).<sup>††</sup>Patients with signs of severity pneumonia on chest tomography (> 50% involvement).

**TABLE 3** The association between length of hospital stay, length of ICU stay, and laboratory characteristics

Sperman'srho*		TT	FSH	LH	TLR	DD	LDH	CRP	Ferritin	Lymphocytes
LOS	<i>r</i>	−0.316	−0.088	0.086	−0.244	0.248	0.278	0.284	0.255	−0.248
	<i>p</i>	<0.001	0.09	0.10	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LOIS	<i>r</i>	−0.599	−0.183	−0.225	−0.383	0.347	0.598	0.621	0.542	0.514
	<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations: CRP, C reactive protein; DD, d-dimer; FLR, follicle stimulating hormone to luteinizing hormone ratio; FSH, follicle stimulating hormone; LDH, lactate dehydronegase; LH, luteinizing hormone; LOIS, length of ICU stay; LOS, length of hospital stay; TLR, testosterone to luteinizing hormone ratio; TT, total testosterone.

**TABLE 4** The association between total testosterone, TLR, and other laboratory characteristics

Sperman'srho*		DD	LDH	CRP	Ferritin	Lymphocytes	Monocyte	Monocyte ratio
TT	<i>r</i>	−0.278	−0.510	−0.527	−0.466	0.515	0.023	0.020
	<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	0.67	0.70
TLR	<i>r</i>	−0.247	−0.357	−0.349	−0.348	0.437	0.057	0.017
	<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	0.28	0.74

Abbreviations: CRP, C reactive protein; DD, d-dimer; LDH, lactate dehydronegase; TLR, testosterone to luteinizing hormone ratio; TT, total testosterone.

the online database of the worldometers by January 3rd, 99,405,023 infections, 2,132,033 deaths, and 71,477,926 recoveries had been confirmed worldwide.<sup>11</sup>

The ACE2 enzyme has been shown to be a potential receptor for SARS-CoV-2, and independent studies have shown that one of the tissues in which the ACE2 enzyme is expressed most is the testicles.<sup>12–14</sup> Similar to the results of this study, we found that the serum FSH and LH levels of male patients diagnosed with COVID-19 were higher than the control group. In addition, we determined that the TT:LH ratio, which is an indicator of testicular function, was significantly higher in men with COVID-19, compared to the control group. In general, when testosterone decreases in seriously ill men, it is caused by decreased hypothalamic-pituitary stimulation; that is, a secondary testosterone deficiency; however, ACE2, which has been shown to be expressed in adult Leydig and Sertoli cells, plays an important role in the entry of the virus into the cell, suggesting a possible testicular involvement in patients with COVID-19 infection.

In the study conducted by Çayan et al on 232 male patients with COVID-19 without a control group, it was found that as the initial serum TT levels decreased, the patients' need for intensive care and mortality rates increased significantly.<sup>15</sup> In our study with a group of 358 patients and 92 healthy individuals as the control group, we found that the baseline serum TT levels were significantly lower in male patients diagnosed with COVID-19 compared to the control group. In this study, which we conducted in accordance with the results of the study conducted by Çayan et al. We found that low serum TT levels were significantly associated with the severity and mortality of the disease in men with COVID-19. Considering the presence of the control group in our study and the volume of our study, we think that our results are statistically more significant than that study. In the study of Baldassarri et al conducted with 638 males stating the variability in COVID-19 severity with the differences in host genome, the low

serum testosterone levels observed in the carriers of long polyQ alleles in their androgen receptors were determined to predict the intensive care need in COVID-19-infected males. In addition, consistent with the known anti-inflammatory effect of testosterone, the CRP levels were shown to increase in male patients with long polyQ and above 60 years of age. A tendency toward hypogonadism was reported in males with repetitive long polyQ. In that study, which explained the variability in COVID-19 severity with the differences in the host genome, males with polyQ alleles were reported to have a tendency toward hypogonadism; these patients had a poorer prognosis, and the CRP levels were higher in these patients. The results of that study are consistent with those of ours, which indicate that the serum TT levels and the disease prognosis are associated, and this may explain the pathophysiology of this association at genomic level.<sup>16</sup>

The plasma testosterone concentration is known to decrease with comorbidities such as age, obesity, diabetes, hypertension, coronary artery disease (CAD), and chronic obstructive pulmonary disease (COPD), and these comorbidities are known to be quite common in COVID-19 patients.<sup>17,18</sup> In the meta-analysis conducted by Balasubramanian et al, it was reported that the prevalence of hypogonadism in men with COPD ranged between 22% and 69% and that hypogonadism was associated with many other systemic diseases, including osteoporosis, depression, and muscle weakness.<sup>19</sup> In the study by Montaña et al, the testosterone levels were shown to be associated with higher FEV1 (volume of air exhaled in the 1st second of forced expiration) and forced vital capacity in men, and high testosterone levels contributed to better lung function in men, and low testosterone levels were found to cause a decrease in respiratory muscle activity and exercise capacity.<sup>20</sup> In addition, in the randomized controlled study conducted by Caminiti et al, an improvement in peak oxygen consumption was reported in men who received TRT.<sup>21</sup>

**TABLE 5** Univariable and multivariable binary logistic regression analysis to predict ICU and exitus in patients with COVID-19

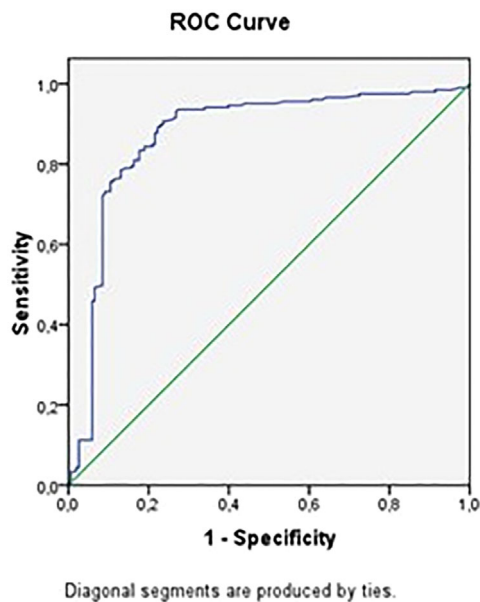
n		Univariable			Multivariable		
		OR	95% CI	p-value	OR	95% CI	p-value
206	ICU (+)						
	Age (year)	1.032	1.013–1.052	<0.001	1.015	0.983–1.048	0.35
	BMI (kg/m <sup>2</sup> )	1.008	0.955–1.065	0.76			
	Smoking	0.762	0.475–1.222	0.25			
	DM	1.982	1.207–3.256	0.007	0.909	0.403–2.051	0.81
	COPD	2.029	1.275–3.230	0.003	1.382	0.607–3.151	0.44
	CAD	1.252	0.816–1.920	0.30			
	HT	3.306	2.092–5.225	<0.001	5.606	2.482–12.669	<0.001
	CRF	1.994	0.945–4.208	0.07			
	TT	0.985	0.982–0.988	<0.001	0.989	0.985–0.993	<0.001
	LDH	1.008	1.006–1.009	<0.001	1.002	1.000–1.004	0.049
	CRP	1.021	1.016–1.025	<0.001	1.011	1.005–1.017	<0.001
	DD	1.001	1.000–1.001	<0.001	1.000	1.000–1.000	0.28
	Ferritin	1.003	1.002–1.004	<0.001	1.001	1.000–1.002	0.032
	Lymphocytes	0.999	0.999–0.999	<0.001			
Monocyte	1.175	0.677–2.039	0.56	1.000	1.000–1.000	0.70	
42	Exitus (+)						
	Age (year)	1.043	1.011–1.076	0.008	1.054	1.014–1.043	0.008
	BMI (kg/m <sup>2</sup> )	0.970	0.887–1.061	0.50			
	Smoking	0.342	0.177–0.661	0.001	0.437	0.195–0.977	0.044
	DM	1.575	0.778–3.192	0.20			
	COPD	3.632	1.879–7.018	<0.001	0.616	0.251–1.513	0.29
	CAD	0.734	0.372–1.449	0.37			
	HT	3.927	1.693–9.108	<0.001	0.513	0.228–1.154	0.10
	CRF	1.940	0.746–5.046	0.17			
	TT	0.990	0.988–0.993	<0.001	0.993	0.989–0.998	0.006
	LDH	1.003	1.002–1.004	<0.001	1.003	1.001–1.005	0.001
	CRP	1.009	1.005–1.013	<0.001	0.999	0.995–1.002	0.46
	DD	1.000	1.000–1.000	0.43			
	Ferritin	1.001	1.000–1.001	0.011	1.000	0.999–1.000	0.05
	Lymphocytes	0.999	0.998–0.999				
Monocyte	0.556	0.205–1.505	<0.001	0.999	0.999–1.000	0.05	

Abbreviations: BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CRF, chronic renal failure; CRP, C reactive protein; DD, d-dimer; DM, diabetes mellitus; HT, hypertension; ICU, intensive care unit; LDH, lactate dehydrogenase; OR, odds ratio; TT, total testosterone.

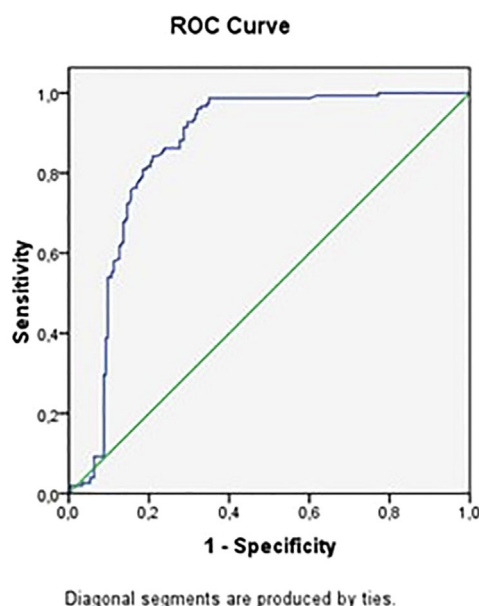
Pro-inflammatory cytokines (IL-1, IL-6, and TNF- $\alpha$ ) play a central role in the progression of COVID-19 infection. Studies have reported that these cytokines increase significantly in patients with COVID-19 who have severe pulmonary involvement or require long-term intensive care.<sup>22</sup> Various studies in both animals and humans have shown that hypogonadism is associated with increased pro-inflammatory cytokines, and TRT decreases the IL-1, IL-6, and TNF- $\alpha$ .<sup>23</sup> In the study conducted by Mohamad et al, testosterone was reported to have anti-inflammatory effects *in vivo*, and this was linked to mainly two observations; the first being that testosterone deficiency is associated with increased inflammatory cytokine levels, and the second observation being that TRT reduces the inflammatory cytokine levels.<sup>24</sup> In our

study, we compared men diagnosed with COVID-19 according to the severity of their lung involvement, and we found that the serum TT levels and the TT:LH ratios of patients with severe lung involvement were significantly lower than those of patients with mild involvement. Considering the studies conducted on the effects of low testosterone levels on the lungs in the literature, it does not seem surprising that patients with low TT levels had extensive lung involvement in our study.

Hypogonadism can make older men more prone to progression of atherosclerosis.<sup>25</sup> For these reasons, it is thought that low total and free testosterone levels, especially in elderly male patients, may be responsible for deaths related to CAD.<sup>26,27</sup> Studies have shown that



**FIGURE 1** ROC curve for decreasing testosterone level in bad prognosis. (AUC value: 873, Cutoff value: 211.7 [Sensitivity: %90.7; specificity: %76.5], CI: 0.831–0.915)  
Abbreviation: AUC, area under the curve.



**FIGURE 2** ROC curve for decreasing testosterone level in predicting ICU. (AUC value: 858, Cutoff value: 185.2 [Sensitivity: %96.1; specificity: %68.0], CI: 0.817–0.899)  
Abbreviation: AUC, area under the curve.

CAD may have an important role in the severity and mortality of the disease in COVID-19 patients.<sup>28</sup> Given these scientific data, it is not surprising that men with initially low circulating serum TT levels have poor cardiovascular health that may contribute to an increased risk of CAD in COVID-19.

In the light of all these scientific studies in the literature, we started our study with the hypothesis that serum testosterone levels in men diagnosed with COVID-19 may be associated with the prognosis of the disease, and we found that low serum TT levels were significantly associated with disease severity and mortality.

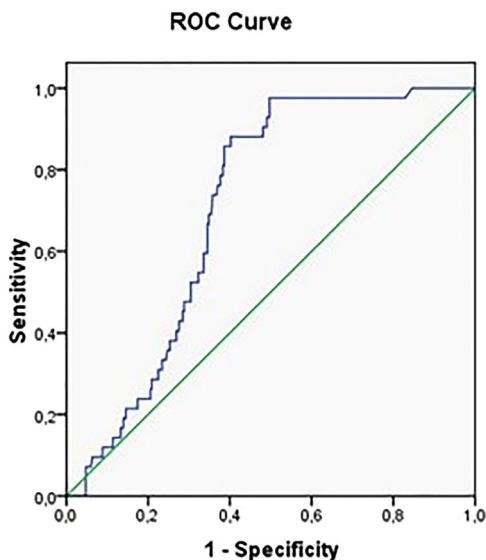
In a retrospective study by Schroeder et al, it was shown that 68% of male patients with COVID-19 who had been followed up in the intensive care unit had low testosterone levels, and 54% had serum testosterone levels below 141 ng/dl. Furthermore, the same study reported that 32% of all men and 17% of men with low testosterone level had elevated LH levels consistent with testicular dysfunction.<sup>29</sup> In the study of Rastrelli et al, it was found that the TT levels were negatively correlated with LDH and ferritin levels in patients diagnosed with COVID-19.<sup>6</sup> Our results were consistent with the results of these studies, and we found that there was a significant negative correlation between the serum TT level and TT:LH ratio and the serum DD, LDH, CRP, and ferritin levels in male patients with COVID-19 diagnosis. In addition, in our analysis, we found that as the serum TT levels and TT:LH ratio of the patients decreased, the duration of hospitalization and the duration of stay in the intensive care unit increased significantly. Moreover, we determined that the serum TT level was statistically significant in the univariate and multivariate binary logistic regression analysis performed to estimate the patients' intensive care unit needs and mortality. The results of our study are consistent with the results

of the study performed by Rastrelli et al, and we think that our results are more significant when the number of patients is taken into consideration.

The results of our study suggests that decreased TT levels may play an important role in the pathogenesis of the disease in men diagnosed with COVID-19 who have a poor prognosis, who need intensive care, and who have a mortal course. Although there are studies reporting that men with prostate cancer with COVID-19 who received androgen deprivation therapy had a milder disease than those who did not, our study does not support these findings.<sup>30,31</sup> However, we believe that the results of our study support the pathophysiological mechanisms between COVID-19 and low testosterone levels. However, COVID-19 may affect the testicles as well as other organs. For this reason, the primary affected Leydig cell function and reduced testosterone level may also be merely a marker of the disease. Despite the presence of studies on the relationship between the serum testosterone level and the prognosis of the disease in male patients with COVID-19 diagnosis in the literature review, this study will take its place in the literature as a prospective study with the largest volume, including a control group.

The limitations of the study are that it did not include men diagnosed with COVID-19 who did not have determined testosterone levels prior to the diagnosis, absence of long-term results of testosterone levels after the diagnosis, absence of patients in need of intensive care unit without COVID-19 diagnosis in the same period, and not measuring the ACE2 receptor concentration levels of the patients. In addition, in this study, TRT could not be given to patients due to ethical and scientific concerns, and whether or TRT was effective in





Diagonal segments are produced by ties.

**FIGURE 3** ROC curve for decreasing testosterone level in predicting exitus. (AUC value: 0.707, Cutoff value: 165.6 [Sensitivity: %97.6; specificity: %49.7], CI: 0.647–0.768)  
Abbreviation: AUC, area under the curve.

the prognosis of the disease, could not be evaluated at this stage. Although these limitations make it difficult to answer the question of whether COVID-19 itself worsens the disease by causing hypogonadism or hypogonadism makes men more susceptible to COVID-19, our clinic continues to study the long-term testosterone results of male patients with COVID-19 and plans to contribute to the literature in this direction.

## 5 | CONCLUSION

Our data are compatible with low TT levels playing a role on the pathogenesis of the disease in Covid-19 patients with poor prognosis and a mortal course and may guide clinicians in determining the clinical course of the disease. More controlled studies are needed to confirm the place of serum TT levels in pathophysiological assumptions in male patients with COVID-19 and to determine the therapeutic strategies such as TRT.

## CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

## AUTHOR CONTRIBUTIONS

Ahmet Emre Cinislioglu performed the evidence synthesis, wrote the original draft, and revised and edited the manuscript. Nazan Cinislioglu, Saban Oguz Demirdogen, and Emre Sam wrote the paper and edited the manuscript. Fatih Akkas analyzed the data. Mehmet Sefa Altay, Mustafa Utlu, Irem Akin Sen, Fatih Yildirim,

and Seyfi Kartal performed data acquisition. Hasan Riza Aydin, Ibrahim Karabulut, and Isa Ozbey critically reviewed the article. All authors qualify for authorship by contributing substantially to this article.

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## REFERENCES

- Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382:727–733.
- Li H, Liu SM, Yu XH, Tang SL, Tang CK. Coronavirus disease 2019 (COVID-19): current status and future perspectives. *Int J Antimicrob Agents*. 2020;55:105951.
- Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature*. 2020;579:265–269.
- Porcheddu R, Serra C, Kelvin D, Kelvin N, Rubino S. Similarity in case fatality rates (CFR) of COVID-19/SARS-COV-2 in Italy and China. *J Infect Dev Ctries*. 2020;14:125–128.
- Wenham C, Smith J, Morgan R. COVID-19: the gendered impacts of the outbreak. *Lancet*. 2020;395:846–848.
- Rastrelli G, Di Stasi V, Inglese F, et al. Low testosterone levels predict clinical adverse outcomes in SARS-CoV-2 pneumonia patients. *Andrology*. 2021;9:88–98.
- Mehra MR, Desai SS, Kuy S, Henry TD, Patel AN. Cardiovascular disease, drug therapy, and mortality in COVID-19. *N Engl J Med*. 2020;382:e102.
- Tajar A, Forti G, O'Neill TW, et al. Characteristics of secondary, primary, and compensated hypogonadism in aging men: evidence from the European Male Ageing Study. *J Clin Endocrinol Metab*. 2010;95:1810–1818.
- Araujo AB, Esche GR, Kupelian V, et al. Prevalence of symptomatic androgen deficiency in men. *J Clin Endocrinol Metab*. 2007;92:4241–4247.
- Mulhall JP, Trost LW, Brannigan RE, et al. Evaluation and management of testosterone deficiency: aUA guideline. *J Urol*. 2018;200:423–432.
- [Worldometers.info](https://www.worldometers.info/coronavirus/). COVID-19CORONAVIRUS PANDEMIC. Accessed 3 Jan 2021. <https://www.worldometers.info/coronavirus/>
- Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. 2003;426:450–454.
- Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Lett*. 2002;532:107–110.
- Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem*. 2000;275:33238–33243.
- Çayan S, Uğuz M, Saylam B, Akbay E. Effect of serum total testosterone and its relationship with other laboratory parameters on the prognosis of coronavirus disease 2019 (COVID-19) in SARS-CoV-2 infected male patients: a cohort study. *Aging Male*. 2020;23:1493–1503.
- Baldassarri M, Picchiotti N, Fava F, et al. Shorter androgen receptor polyQ alleles protect against life-threatening COVID-19 disease in European males. *EBioMedicine*. 2021;65:103246.
- Bhasin S, Brito JP, Cunningham GR, et al. Testosterone therapy in men with hypogonadism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2018;103:1715–1744.

18. Van Vliet M, Spruit MA, Verleden G, et al. Hypogonadism, quadriceps weakness, and exercise intolerance in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2005;172:1105–1111.
19. Balasubramanian V, Naing S. Hypogonadism in chronic obstructive pulmonary disease: incidence and effects. *Curr Opin Pulm Med*. 2012;18:112–117.
20. Montaña LM, Espinoza J, Flores-Soto E, Chávez J, Perusquía M. Androgens are bronchoactive drugs that act by relaxing airway smooth muscle and preventing bronchospasm. *J Endocrinol*. 2014;222:1–13.
21. Caminiti G, Volterrani M, Iellamo F, et al. Effect of long-acting testosterone treatment on functional exercise capacity, skeletal muscle performance, insulin resistance, and baroreflex sensitivity in elderly patients with chronic heart failure a double-blind, placebo-controlled, randomized study. *J Am Coll Cardiol*. 2009;54:919–927.
22. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395:497–506.
23. Pozzilli P, Lenzi A. Commentary: testosterone, a key hormone in the context of COVID-19 pandemic. *Metabolism*. 2020;108:154252.
24. Mohamad N-V, Wong SK, Wan Hasan WN, et al. The relationship between circulating testosterone and inflammatory cytokines in men. *Aging Male*. 2019;22:129–140.
25. Hak AE, Witteman JC, de Jong FH, Geerlings MI, Hofman A, Pols HA. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. *J Clin Endocrinol Metab*. 2002;87:3632–3639.
26. Khaw KT, Dowsett M, Folkard E, et al. Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men: european prospective investigation into cancer in Norfolk (EPIC-Norfolk) Prospective Population Study. *Circulation*. 2007;116:2694–2701.
27. Whyte CS, Morrow GB, Mitchell JL, Chowdary P, Mutch NJ. Fibrinolytic abnormalities in acute respiratory distress syndrome (ARDS) and versatility of thrombolytic drugs to treat COVID-19. *J Thromb Haemost*. 2020;18:1548–1555.
28. Hessami A, Shamshirian A, Heydari K, et al. Cardiovascular diseases burden in COVID-19: systematic review and meta-analysis. *Am J Emerg Med*. 2020. <https://doi.org/10.1016/j.ajem.2020.10.022>
29. Schroeder M, Tuku B, Jarczack D, et al. The majority of male patients with COVID-19 present low testosterone levels on admission to Intensive Care in Hamburg, Germany: a retrospective cohort study. *medRxiv*. 2020. <https://doi.org/10.1101/2020.05.07.20073817>
30. Bravaccini S, Fonzi E, Tebaldi M, et al. Estrogen and androgen receptor inhibitors: unexpected allies in the fight against COVID-19. *Cell Transplant*. 2021;30:963689721991477.
31. Mohamed MS, Moulin TC, Schiöth HB. Sex differences in COVID-19: the role of androgens in disease severity and progression. *Endocrine*. 2021;71:3–8.

### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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