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Severely low testosterone in males with COVID-19: A case-control study

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Abstract

Background: Circulating androgens could have a relevant pathobiological role in clinical outcomes in men with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (COVID-19).

Objectives: We aimed to assess: (a) circulating sex steroids levels in a cohort of 286 symptomatic men with laboratory-confirmed COVID-19 at hospital admission compared to a cohort of 281 healthy men; and (b) the association between serum testosterone levels (tT), COVID-19, and clinical outcomes.

Materials and Methods: Demographic, clinical, and hormonal values were collected for all patients. Hypogonadism was defined as tT ≤9.2 nmol/l. The Charlson Comorbidity Index (CCI) was used to score health-significant comorbidities. Severe clinical outcomes were defined as patients either transferred to intensive care unit

(ICU) or death. Descriptive statistics and multivariable linear and logistic regression models tested the association between clinical and laboratory variables and tT levels. Univariable and multivariable logistic regression models tested the association between tT and severe clinical outcomes.

Results: Overall, a significantly lower levels of LH and tT were found in patients with COVID-19 compared to healthy controls (all $p < 0.0001$); conversely, healthy controls depicted lower values of circulating E_2 ($p < 0.001$). Testosterone levels suggestive for hypogonadism were observed in 257 (89.8%) patients at hospital admission. In as many as 243 (85%) cases, hypogonadism was secondary. SARS-CoV-2 infection status was independently associated with lower tT levels ($p < 0.0001$) and greater risk of hypogonadism ($p < 0.0001$), after accounting for age, BMI, CCI, and IL-6 values. Lower tT levels were associated with higher risk of ICU admission and death outcomes (all $p \leq 0.05$), after accounting for clinical and laboratory parameters.

Conclusions: We unveil an independent association between SARS-CoV-2 infection status and secondary hypogonadism already at hospital admission, with lower testosterone levels predicting the most severe clinical outcomes.

KEYWORDS
COVID-19, male, SARS-CoV-2, testosterone

1 | INTRODUCTION

The new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced disease (COVID-19) may lead to a complex clinical picture associated with viral multiorgan tropism.[1] Overall, male individuals are more susceptible to the infection than females,[2,3] and have a higher death rate regardless of age.[4] Reasons for sex disparity in COVID-19 severity might be linked to a number of mechanisms associated with the pathogenesis of viral infections, along with biological host factors (eg, sex differences in terms of susceptibility to viral infection and adaptive immune responses).[4-6] Of relevance, type and importance of any immune response strongly rely on the biologic sex (ie, genetic predisposition, circulating sex hormones, and surface/intracellular sex hormone receptors).[5,7-10] Thus, sex-related hormonal milieu might have a significant pathophysiological role after SARS-CoV-2 infection in terms of immune response, viral-induced multiorgan dysregulation, COVID-19-associated clinical severity, and mortality.[10,11]

Moreover, coronavirus entry into host cells is mediated by the transmembrane spike (S) glycoprotein, which needs to be primed to interact with viral receptor angiotensin-converting enzyme 2 (ACE2). SARS-CoV-2 utilizes cell surface protease transmembrane serine protease 2 (TMPRSS2) for viral S protein priming. Both ACE2 and TMPRSS2 are androgen-regulated.[9,12-15] Furthermore, ACE2 expression levels were found to be higher in males than in females, at least in the lungs.[16] Likewise, an expression patterns analysis of ACE2 in adult human testes indicated that ACE2 is predominantly enriched in spermatogonia, Leydig, and Sertoli cells,[16-19] where the receptor is even 3-fold more expressed than in type II alveolar epithelial cells.[16]

Based on the hypothesis that hormone-related biological sex differences may have a relevant impact throughout COVID-19 course, we aimed to (a) investigate the levels of circulating total testosterone (tT) and the rate of patients with tT levels suggestive for hypogonadism [20] in a large cohort of symptomatic SARS-CoV-2 infected male patients compared to a cohort of healthy men; (b) test the association between SARS-CoV-2 infection with tT values and hypogonadism status; and (c) assess the prevalent type of hypogonadism, as well as the likelihood of severe clinical outcomes according to clinical parameters and hormonal levels.

2 | METHODS

Data from a cohort of 286 symptomatic male patients with quantitative RT-PCR laboratory-confirmed SARS-CoV-2 infection, that had not yet taken any steroids or antivirals, and either suggestive chest radiography or computed tomography appearances referred for hospital admission between February 29th and May 2nd, 2020, at a single academic hospital were analyzed.

Data collection followed the principles outlined in the Declaration of Helsinki. All methods were performed in accordance with the relevant guidelines and regulations. On obtaining written individual patient's consent, clinical data from all patients were retrieved using a dedicated case report form, according to an institutional protocol (Covid-BioB, ClinicalTrials.gov NCT04318366; Ethical Committee approval number 34/int/2020).[21]

The Charlson Comorbidity Index (CCI) was used to score health-significant comorbidities, coded using the International Classification

of Diseases, 10th revision.[22] Measured body mass index (BMI) was obtained for each patient.

At hospital admission, patients were subdivided into mild acute respiratory distress syndrome (ARDS) (ratio of the partial pressure of oxygen in arterial blood to the fractional concentration of oxygen in inspired air [PaO2:FiO2] 300–200 mm Hg); moderate ARDS (PaO2/FIO2 100–200 mm Hg); and severe ARDS (PaO2/FiO2 less than 100 mm Hg) according to standard definitions.[23] PaO2:FiO2 was calculated with arterial blood gas.

Baseline chest radiography findings of SARS-CoV-2 pneumonia severity were scored in every patient with the Radiographic Assessment of Lung Edema (RALE) score to evaluate the extent and density of alveolar opacities on chest radiographs the same day of the admission [24]; furthermore, of 286, an artificial intelligence (AI) system (qXR v2.1 c2, Qure.ai Technologies, India) was applied for each lung in 271 (94·8%) patients, using a 3% threshold.[25]

Patients with COVID-19 were divided into four groups according to the outcome after hospital admission: Group 1) patients in good clinical conditions and discharged home from the emergency department; Group 2) patients admitted in the internal medicine unit until possible discharge home; Group 3) patients invasively ventilated in the Intensive Care Unit (ICU), and subsequently successfully extubated and discharged either to the internal medicine unit or home; and Group 4) patients either transferred to ICU or in the internal medicine unit who eventually died.

Moreover, a validated composite risk score based on the characteristics at the time of first hospital admission was calculated for every patient (ie, Critical-ill COVID-19 score) [26]; the score provides an estimate of the risk of developing critical illness for a patient with COVID-19, taking into account the following parameters: (a) chest radiography; (b) age; (c) hemoptysis; (d) dyspnea; (e) unconsciousness; (f) number of comorbidities; (g) cancer history; (h) neutrophil/lymphocytes ratio; (i) lactate dehydrogenase; and (j) direct bilirubin.

Complete data from 309 voluntary healthy blood male donors (aged >18 years) asymptomatic for SARS-CoV-2 infection who consecutively arrived between June 10th and July 23rd, 2020, to the blood donor center of the same hospital were analyzed. According to our research protocol (ethics committee approval number 91/int/2020), healthy controls underwent the same comprehensive clinical and biochemical assessment of the infected counterpart. Of all, 28 healthy controls were excluded since we found SARS-CoV-2 S1/S2 IgM (n = 15, 4.9%) and SARS-CoV-2 S1/S2 IgG (n = 26, 8.4%) positive serological tests, respectively.

2.1 | Biochemical measurements

Baseline venous blood samples were drawn in all patients at hospital admission (between 7 AM and 11 AM, after an overnight fast),[20] and kept at 4°C until serum or plasma were separated by centrifugation. Serum and plasma aliquots were then stored at −80°C until assay. The same experimental protocol was applied in healthy controls. For the specific purposes of this analysis, on each sample, for every patient at baseline, we measured follicle-stimulating hormone (FSH), luteinizing hormone (LH), tT, and 17β-estradiol (E$_2$).

To reflect common practice of a clinical pathology laboratory, we elected to measure circulating hormones using commercially available analytical methods. In all participants, FSH, LH, tT, and E$_2$ were measured by a direct chemiluminescence immunoassay (CLIA) (FSH: LIAISON® FSH ([REF] 312251); LH: LIAISON® LH ([REF] 312201); testosterone: LIAISON® Testosterone ([REF] 310410); and E$_2$: LIAISON® Estradiol II Gen ([REF] 310680); DiaSorin SpA, Saluggia, Italy). Moreover, Interleukin-6 (IL-6) was measured by ECLIA (Elecsys IL-6, COBAS® ROCHE) in every patient.

Likewise, LIAISON® SARS-CoV-2 S1/S2 IgM and LIAISON® SARS-CoV-2 S1/S2 IgG serological tests were used to assess SARS-CoV-2 IgM and IgG in every participant.

2.2 | Outcomes

Primary outcomes were overall rate of patients with tT levels suggestive for hypogonadism (according to a tT threshold of 9.2 nmol/l) [20] compared to healthy controls and the proportion of the different types of hypogonadism in COVID-19 patients, as for the classification criteria reported in the European Male Aging Study (EMAS).[27] To this aim, patients were further stratified into four groups, as follows: eugonadal [normal total testosterone (≥10.5 nmol/l) and normal LH (≤9.4 mUI/ml)]; secondary hypogonadism [low total testosterone (≤10.5 nmol/l) and low/normal LH (≤9.4 mUI/ml)]; primary hypogonadism [low total testosterone (≤10.5 nmol/l) and elevated LH (>9.4 mUI/ml)]; and compensated hypogonadism [normal total testosterone (≥10.5 nmol/l) and elevated LH (>9.4 mUI/ml)].[27] The secondary outcome was the association between tT values and severe clinical outcomes (ie, need for ICU admission or death).

2.3 | Statistical methods

Distribution of data was tested with the Shapiro-Wilk test. Data are presented as medians (interquartile range; IQR) or frequencies (proportions). We used one-way ANOVA on ranks (Kruskal-Wallis test) or Chi-Squared test in order to compare hormonal levels and other demographics, clinical, and laboratory characteristics between COVID-19 patients and healthy controls. The same analyses were used to compare clinical characteristics and hormonal values among patients with different severity in terms of clinical outcomes (ie, groups 1–4).

To test the hypothesis that SARS-Cov-2 infection could be associated with lower tT levels, we used linear and logistic regression models predicting serum tT levels and the probability of hypogonadism, respectively; both models were adjusted for baseline clinical factors and for markers of systemic inflammation (eg, IL-6) which could have influenced the hormonal values.

TABLE 1 Demographic, clinical, and laboratory characteristics of participants at admission (n, 567)

	Healthy controls	COVID−19 patients	P-value*
	281 (49.5)	286 (50.5)	
Age (year)	46.0 (35.0–52.0)	58.0 (49.0–66.0)	<0.0001
Ethnicity			<0.0001
White–European	273 (97.0)	240 (83.9)	
Latin–American	7 (2.5)	31 (10.8)	
African	0 (0.0)	10 (3.5)	
Asian–Far East Asian	1 (0.4)	5 (1.8)	
BMI (kg/m^2)	25.1 (23.5–27.7)	27.8 (25.1–30.8)	<0.0001
BMI (kg/m^2)			<0.0001
<25	141 (50.0)	56 (9.2)	
25–29.9	81 (29.0)	108 (37.7)	
≥30	59 (21.0)	152 (53.1)	
Comorbidities			
CCI	0.0 (0.0–0.0)	0.0 (0.0–1.0)	<0.0001
CCI–age	0.0 (0.0–1.0)	2.0 (1.0–3.0)	<0.0001
CCI (score)			
0	184 (65.5)	154 (53.8)	<0.0001
1	83 (29.5)	65 (22.7)	
≥2	14 (5.0)	67 (23.5)	
Arterial hypertension	34 (12.0)	111 (38.8)	<0.0001
Laboratory parameters			
WBC, 10^9/L	5.8 (4.9–6.6)	7.2 (5.4–10.0)	<0.0001
Neutrophils, 10^9/L	3.1 (2.6–3.8)	5.3 (3.5–7.8)	<0.0001
Lymphocytes, 10^9/L	1.8 (1.5–2.2)	1.0 (0.7–1.4)	<0.0001
NLR	1.7 (1.4–2.1)	5.3 (3.0–8.6)	<0.0001
Platelets, 10^9/L	217.5 (188–251.0)	233.0 (180.0–314.0)	0.01
Creatinine, mg/dL	1.0 (0.9–1.1)	1.0 (0.9–1.2)	0.7
Glycemia, mg/dL	77.0 (70.0–85.0)	104.0 (88.0–122.0)	0.0001
LDH, U/L	186.0 (87.4–202.0)	389.0 (297.0–482.0)	0.001
Total bilirubin, mg/dL	0.7 (0.4–0.7)	0.7 (0.4–0.9)	0.6
Indirect bilirubin, mg/dL	0.3 (0.2–0.4)	0.3 (0.2–0.4)	0.8
Direct bilirubin, mg/dL	0.2 (0.2–0.2)	0.3 (0.2–0.5)	0.2
C–reactive protein, mg/L	2.3 (1.6–3.1)	2.6 (28.2–145.2)	0.023
IL–6, pg/mL	2.5 (2.5–3.6)	37.5 (15.1–88.6)	<0.0001
Ferritin, ng/mL	56.0 (36.5–102.0)	1267.5 (758.0–2514.0)	<0.0001
FSH, mU/mL	6.1 (4.4–8.1)	5.7 (3.9–8.6)	0.5
LH, mU/mL	4.1 (3.0–5.4)	4.7 (3.0–6.7)	0.005
tT, nmol/L	10.4 (8.1–13.4)	2.5 (1.0–4.7)	<0.0001
Hypogonadism (tT <9.2 nmol/L)	42 (14.9)	257 (89.8)	<0.0001
E$_2$, pg/mL	23.3 (19.0–27.9)	35.0 (22.4–44.2)	<0.0001

Note: Data are n (%) or median (IQR); *P-value according to the Kruskal-Wallis test and Chi-Squared test, as indicated. The sum of the percentages may not equal 100% because of rounding.

Abbreviations: BMI, body mass index; CCI, Charlson Comorbidity Index; E$_2$, 17β-estradiol; FSH, follicle-stimulating hormone; IL-6, Interleukin-6; LH, luteinizing hormone; NLR, neutrophil/lymphocytes ratio.

Lastly, the association between tT levels and severe clinical outcomes (ICU admission or death) was tested with logistic regression models. We hypothesized that the association between disease severity and the risk of death could vary according to the various tT levels; therefore, an interaction term was included in the logistic regression model to test this hypothesis.

Statistical analyses were performed using Stata 14.0 (StataCorp, College Station, TX, USA). All tests were two-sided, and statistical significance level was determined at $p < 0.05$.

3 | RESULTS

3.1 | Men with COVID-19 have significantly lower total testosterone than healthy controls

At hospital admission, the entire cohort of 567 participants was subdivided according to SARS-CoV-2 infection status in 286 (50.5%) patients with COVID-19 and 281 (49.5%) healthy controls. Analysis of clinical and laboratory parameters revealed a significantly lower levels of LH and tT in patients with COVID-19 compared to healthy controls (all $p < 0.0001$); conversely, healthy controls depicted lower values of circulating E_2 ($p < 0.001$) (Table 1). Of all, a tT level <9.2 nmol/L was observed in 257 (89.8%) patients and 42 (14.9%) healthy controls ($p < 0.0001$), respectively.

Multivariable linear regression analyses revealed age, BMI, and SARS-CoV-2 infection status as independent predictors of lower tT levels (all $p < 0.0001$), after adjusting for CCI, and IL-6 levels (Table 2). Moreover, SARS-CoV-2 infection status was found to significantly predict tT levels suggestive for hypogonadism almost 6 times more than healthy status ($p < 0.0001$), followed by BMI, age, and IL-6 values (all $p \leq 0.02$), at multivariable logistic regression analyses, after adjusting for CCI (Table 2).

Levels of tT and LH suggestive for secondary, primary, and compensated hypogonadism were found in 243 (85%), 25 (8.8%), and 3 (1%) patients with COVID-19, respectively (Table 3).

3.2 | Total testosterone acts as an early biomarker of clinical severity in patients with COVID-19

Levels of tT were lower in men belonging to more severe outcome groups (groups 3 and 4) compared to patients in groups 1 and 2 (all $p < 0.0001$, Table 3).

At univariable analyses, tT was inversely associated with ICU admission ($p < 0.0001$) and death outcomes ($p < 0.002$) (Table 4). At multivariable analyses, considering tT as a parameter of COVID-19 severity at hospital admission, tT levels were associated both with the need of ICU admission and death outcomes (all $p \leq 0.05$) (Table 4).

The interaction test performed to assess the hypothesis that circulating tT levels could differently impact on the risk of death according to COVID-19 severity, revealed a significant correlation ($p = 0.04$): the lower the tT levels, the higher the risk of death for the same Critical-Ill COVID-19 score (Figure 1).

4 | DISCUSSION

Current findings show that tT levels were significantly lower in symptomatic SARS-CoV-2 infected male patients already at hospital admission compared to healthy controls, with tT levels suggestive for hypogonadism observed in almost 90% of patients with COVID-19. In as many as 85% of cases, hypogonadism was secondary. Notable, SARS-CoV-2 infection status emerged to be independently associated with lower tT levels and tT levels suggestive for hypogonadism, after adjusting for recognized confounders of low tT levels (ie, age, BMI, and CCI) and IL-6 levels (since IL-6 may rise along with the cytokine storm which usually follows the viral infection). Moreover, tT levels were associated with COVID-19 clinical severity already at hospital admission, being tT levels significantly lower in men with greatest need of ICU and highest risk of death.

Our observations corroborate recent findings. Indeed, in a cohort of male patients with COVID-19 admitted in the respiratory ICU, Rastrelli et al. showed that low tT and low calculated free T levels were associated with the need of invasive ventilation or even fatal events.[28] Ma et al. found lower testosterone and higher LH levels in a cohort of reproductive-aged SARS-CoV-2 infected males, compared to age-matched healthy controls.[29] Çayan et al.[30] showed that COVID-19 could decrease circulating T levels, and lower T levels at baseline were associated with a significantly increased risk in terms of ICU and mortality. Similarly, Kadihasanoglu et al. recently reported the findings of a prospective cohort study where male patients with COVID-19 have been compared with

TABLE 2 Linear and logistic regression models predicting total testosterone levels or hypogonadism in the whole cohort of participants (n, 567)

	Testosterone levels	Hypogonadism (tT<9.2 nmol/L)
	MVA	MVA
	B; *P*-value [95% CI]	OR; *P*-value [95% CI]
Age	−0.8; <0.0001 [−0.11, −0.05]	1.05; <0.0001 [1.02, 1.07]
BMI	−0.15; <0.0001 [−0.23, −0.07]	1.15; <0.0001 [1.07, 1.24]
CCI	−0.01; 0.9 [−0.34, 0.33]	2.53; 0.1 [0.67, 9.47]
IL−6	−0.01; 0.1 [−0.01, 0.01]	1.02; 0.02 [1.01, 1.03]
SARS-CoV−2 infection	−6.04; <0.0001 [−6.89, −5.20]	5.94; <0.0001 [2.46, 14.34]

Abbreviations: BMI, body mass index; CCI, Charlson Comorbidity Index; IL-6, Interleukin-6; MVA, multivariable analyses; OR, odds ratio.

TABLE 3 Demographic, clinical, and laboratory characteristics of patients at admission, as divided according to outcome status (n, 286)

	Group 1	Group 2	Group 3	Group 4	*P*-value*
n (%)	27 (9.4)	174 (60.8)	51 (17.8)	34 (8.0)	
Age (year)	49.0 (45.0–55.0)	57.0 (49–65.5)	60.0 (53.0–66.0)	67.0 (59.0–72.0)	<0.0001
Ethnicity					0.9
White-European	20 (74.1)	146 (83.9)	44 (86.2)	30 (88.2)	
Latin-American	4 (14.8)	17 (9.8)	6 (11.8)	4 (11.8)	
African	2 (7.4)	7 (4.0)	1 (2.0)	0 (0)	
Asian-Far East Asian	1 (3.7)	4 (2.3)	0 (0)	0 ()	
BMI (kg/m^2)	26.8 (24.7–31.6)	28.1 (25.6–30.8)	27.7 (25.1–30.9)	26.2 (24.5–28.7)	0.6
Comorbidities					
CCI	0.0 (0.0–1.0)	0.0 (0.0–2.0)	0.0 (0.0–1.0)	1.0 (0.0–2.0)	0.03
CCI-age	1.0 (0.0–1.0)	2.0 (1.0–3.0)	2.0 (1.0–3.0)	3.0 (2.0–5.0)	0.0001
CCI (score)					
0	19 (70.4)	87 (50.0)	34 (66.6)	14 (41.2)	0.1
1	5 (18.5)	42 (24.1)	11 (21.6)	7 (20.6)	
≥2	3 (11.1)	45 (25.9)	6 (11.8)	13 (38.2)	
Arterial hypertension	8 (29.6)	68 (39.0)	19 (37.3)	16 (48.7)	0.6
ARDS (PaO2:FiO2)					<0.0001
None	23 (85.3)	70 (40.2)	(5.8)	4 (11.8)	
Mild ARDS (300–200 mm Hg)	4 (14.8)	73 (42.0)	19 (37.3)	14 (41.2)	
Moderate ARDS (100–200 mm Hg)	0 (0)	17 (9.8)	13 (25.5)	7 (20.6)	
Severe ARDS (<100–200 mm Hg)	0 (0)	14 (8.0)	16 (31.4)	9 (26.5)	
Qure AI right lung [N. 271]	0.0 (0.0–37.0)	31.0 (14.0–44.0)	47.0 (34.0–64.0)	52.0 (30.0–59.0)	<0.0001
Qure AI left lung [N. 271]	3.0 (0.0–27.0)	24.0 (11.0–43.0)	45.0 (28.0–53.0)	46.5 (24.0–55.0)	<0.0001
RALE score	4.0 (0.0–6.0)	6.0 (3.0–13.0)	14.0 (7.0–22.0)	13.0 (8.0–22.0)	<0.0001
Critical-Ill COVID–19 at admission	64.8 (56.3–74.9)	87.8 (69.3–108.6)	100.8 (86.4–115.8)	126.3 (79.9–157.0)	<0.0001
Laboratory parameters					
NLR	4.0 (3.0–5.2)	4.4 (2.6–7.4)	7.0 (4.8–11.6)	10.8 (6.6–15.5)	<0.0001
Creatinine, mg/dL	1.0 (0.8–1.1)	1.0 (0.9–1.1)	1.1 (0.8–1.4)	1.1 (1.0–1.9)	0.01
C-reactive protein, mg/L	27.6 (9.4–92.5)	65.1 (28.8–127.0)	119.2 (36.3–178.9)	165.0 (71.4–278.3)	<0.0001
IL–6, pg/mL	21.2 (9.9–45.0)	30.2 (15.1–63.7)	79.6 (16.7–316.0)	108.0 (60.8–165.0)	<0.0001
FSH, mU/mL	7.0 (3.9–8.3)	6.9 (4.5–9.9)	3.9 (2.6–5.8)	4.6 (3.8–6.4)	<0.0001
LH, mU/mL	3.7 (3.0–5.0)	5.4 (3.8–6.9)	3.5 (2.2–5.5)	3.9 (1.5–6.3)	<0.0001
tT, nmol/L	3.9 (3.0–5.4)	3.0 (1.8–5.6)	1.0 (0.5–1.7)	0.7 (0.3–2.3)	<0.0001
Hypogonadism					
tT <9.2 nmol/L	22 (83)	160 (92)	51 (100)	24 (100)	0.07
Eugonadal	4 (14.8)	10 (5.7)	1 (2.0)	0 (0)	0.4
Secondary hypogonadism	21 (77.8)	146 (84.0)	46 (90.2)	30 (88.2)	
Primary hypogonadism	2 (7.4)	16 (9.2)	4 (7.8)	3 (8.8)	
Compensated hypogonadism	0 (0)	2 (1.1)	0 (0)	1 (3.0)	
E$_2$, pg/mL	24.4 (15.3–36.5)	35.1 (22.3–43.0)	34.5 (20.7–46.5)	53.5 (33.5–74.4)	<0.0001
SARS-CoV–2 S1/S2 IgM	15 (55.6)	139 (79.9)	42 (82.4)	26 (76.5)	0.02
SARS-CoV–2 S1/S2 IgG	11 (40.7)	94 (54.0)	34 (66.7)	19 (55.9)	0.08

Note: Data are n (%) or median (IQR); *P-value according to the Kruskal-Wallis test and Chi-Squared test, as indicated. The sum of the percentages may not equal 100% because of rounding. *Groups were as follows*: Group (1) patients in good clinical conditions and discharged home from the emergency department; Group (2) patients who have been admitted in the internal medicine unit until possible discharge at home; Group (3) patients invasively ventilated in the Intensive Care Unit, and subsequently successfully extubated and discharged either to the internal medicine unit or at home; and, Group (4) patients who eventually died.

Abbreviations: ARDS, Acute Respiratory Distress Syndrome; BMI, body mass index; CCI, Charlson Comorbidity Index; E$_2$, 17β-estradiol; FiO2, fractional concentration of oxygen in inspired air; FSH, follicle-stimulating hormone; IL-6, Interleukin-6; INR, International Normalized Ratio; LH, luteinizing hormone; NLR, neutrophil/lymphocytes ratio; PaO2, partial pressure of oxygen in arterial blood.; Qure AI, deep learning algorithm; RALE, Radiographic Assessment of Lung Edema; tT, total testosterone.

TABLE 4 Univariable logistic regression models testing the association between clinical and laboratory variables and compulsory need for ICU admission or death in the whole cohort

	ICU	Death
(A) Univariable model OR; *P*-value [95% CI]		
Age	1.04; 0.003 [1.01–1.07]	1.09; 0.0002 [1.04–1.14]
Ethnicity	1.15; 0.7 [0.50–2.65]	1.11; 0.9 [0.36–3.41]
BMI	1.01; 0.9 [0.93–1.09]	0.92; 0.2 [0.81–1.05]
CCI	0.84; 0.1 [0.67–1.05]	1.24; <0.05 [1.00–1.54]
ARDS		
None vs. mild	3.62, 0.01 [1.26–10.4]	3.16; 0.09 [0.82–12.13]
None vs. moderate	11.1; <0.0001 [3.37–36.7]	4.72; 0.05 [0.98–22.7]
None vs. severe	16.3; <0.0001 [5.03–53.2]	5.91; 0.02 [1–31–26.6]
Qure AI dx	1.05; <0.0001 [1.03–1.06]	1.03; 0.005 [1.01–1.05]
Qure AI sn	1.04; <0.0001 [1.03–1.06]	1.02; 0.009 [1.01–1.04]
RALE	1.10; <0.0001 [1.07–1.14]	1.07; 0.001 [1.03–1.11]
Critical-Ill COVID-19[*]	1.03; 0.003 [1.01–1.05]	1.04; <0.0001 [1.02–1.05]
NLR	1.10; 0.0002 [1.04–1.14]	1.10; 0.0002 [1.05–1.15]
Creatinine	1.33; 0.06 [0.99–1.77]	1.54; 0.007 [1.13–2.12]
LDH	1.01; <0.0001 [1.00–1.01]	1.00; 0.001 [1.00–1.01]
Direct bilirubin	2.36; 0.005 [1.30–4.31]	1.98; 0.006 [1.22–3.22]
C-reactive protein	1.01; 0.0001 [1.00–1.01]	1.01; <0.0001 [1.00–1.01]
Il-6	1.00; 0.001 [1.00–1.00]	1.00; 0.07 [0.99–1.00]
Ferritin	1.00; 0.08 [0.99–1.00]	0.99; 0.4 [0.99–1.00]
D-dimer	1.30; 0.003 [1.09–1.56]	1.10; 0.1 [0.97–1.24]
Clinostatic renin	1.00; 0.5 [0.99–1.00]	1.00; 0.04 [1.00–1.01]
tT	0.54; <0.0001 [0.43–0.67]	0.68; 0.002 [0.53–0.86]
E_2	1.01; 0.002 [1.01–1.03]	1.03; <0.0001 [1.02–1.05]
(B) Multivariable model OR; *P*-value [95% CI]		
Critical-Ill COVID–19[*]	1.01; 0.085 [1.00–1.03]	1.03; 0.003 [1.01–1.05]
Creatinine	1.17; 0.5 [0.76– 1.79]	1.18; 0.5 [0.74–1.88]
tT	0.53; 0.0001[0.39–0.74]	0.67; <0.05 [0.46–0.99]

Abbreviations: ARDS, Acute Respiratory Distress Syndrome; BMI, body mass index; CCI, Charlson Comorbidity Index; E_2, 17β-estradiol.; ICU, Intensive Care Unit; IL-6, Interleukin-6; OR, odds ratio; Qure AI, deep learning algorithm; RALE, Radiographic Assessment of Lung Edema; tT, total testosterone.

*Based on the score by Liang W, et al.[25]

men with non-COVID-19 respiratory tract infection and age-matched controls; interestingly enough, testosterone deficiency was found in almost 74% of men with COVID-19, and serum tT levels were significantly correlated with a longer hospitalization time.[31]

Here, in a large case-control study, we provide evidence that SARS-CoV-2 infection is significantly associated with low tT levels and confirm that tT correlates with COVID-19 severity. Moreover, in contrast to these former studies suggesting a primary hypogonadism condition, our findings showed hormonal levels compatible with a condition of secondary hypogonadism.[27]

Based on our results, at least five hypotheses might be considered to explain the potential importance of low T levels in terms of sex differences in COVID-19 severity.[6,32,33] First, low T levels may simply be a marker of illness severity, thus recapitulating what has been reported for many other severe illnesses, thus potentially including severe viral infections.[34,35] Second, androgens per se are poorly protective over the immune response in males, whereas estrogens (and progesterone) can provide adequate protection to females, stimulating the humoral response to viral infections [2,3,36]; as a consequence, T levels could not elicit an effective counteracting response to the inflammatory and immunological outcome
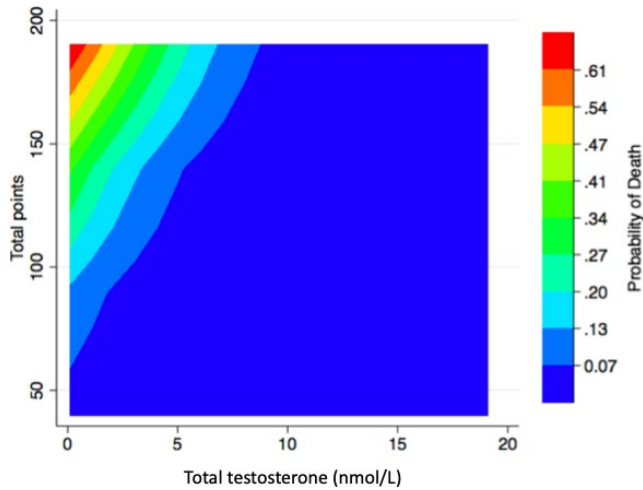
FIGURE 1 Interaction test analysis. The interaction test assessing the hypothesis that circulating total testosterone levels could differently impact on the relationship between the Critical-Ill COVID-19 score, and the risk of death was significant

resulting from viral infection.[5,9,36] Third and opposite, a background condition of chronic low T levels – which is estimated to characterize up to 20% of middle-aged/elderly men [27] – may facilitate overall greater incidence, higher severity, and greater probability of fatal events in men compared to women.[5] Fourth, SARS-CoV-2 needs androgen-regulated proteins to invade host cells, including TMPRSS2 for S priming and ACE2 for viral entry, which are expressed in multiple tissue [9,14,37,38]; thereof, the high ACE2 expression in spermatogonia, Leydig, and Sertoli cells may eventually explain the case of a functional dysregulation in T production [28,29] and spermatogenesis [39,40] at the testicular level. Here, we hypothesize that the virus-host interaction mechanism in males is different, and more acutely linked to SARS-CoV-2 infection per se. Indeed, a sex different sensitivity of the hypothalamic-pituitary-gonadal (HPG) to inflammation [6,41,42] could induce an acute and dramatic drop in circulating tT in SARS-CoV-2 infected males, and the subsequent hypogonadal condition may trigger serious or an even fatal course of the disease.[28-30] According to our speculation, published data showed that circulating tT levels may be negatively affected by unmodifiable conditions (ie, age),[27,35] chronic conditions (ie, obesity, systemic diseases, and overall health status),[35,41] but also by acute illness,[35] including acute viral infections.[37,43] Moreover, we found that SARS-CoV-2 infection status per se emerged to be independently associated with both lower tT levels and hypogonadism up to 6-fold more frequently than the healthy counterpart, further strengthening the speculation on the causal role of the infection in the androgenic collapse. Hence, we may speculate that a condition of acute functional hypopituitarism, following a direct (ie, the virus) or an indirect (ie, the cytokine storm) impact either on the hypothalamus or the pituitary gland, may lead to this acute T depletion, even as a consequence of the multifaceted SARS-CoV-2 neurotropism.[44] We also showed that serum E_2 levels were higher among patients with severe COVID-19 outcomes as compared

with those with less severe clinical conditions. Overall, although it is necessary to underline that laboratory kits may not be as accurate in determining circulating E2 values – or at least less than they are for tT – and therefore, current findings must be taken with adequate caution, high E_2 levels are known to be associated with death in the critically ill patients, regardless of gender.[45] Moreover, not only high E_2 levels at admission but also E_2 changes from baseline in critically ill or injured adults have been demonstrated to be independently associated with mortality.[46] Once again, we could speculate that SARS-CoV-2 infection per se might be responsible for an acute serum tT drop along with E_2 increases in our cohort of patients.

First, strength of this study was the use of a case-control protocol in a relatively large cohort of men, relevant to permit-detailed statistical analyses highlighting the probable causal role of SARS-CoV-2 infection in determining low T levels in most patients with COVID-19. Second, although the definition of hypogonadism usually applied in the literature refers to an established testosterone deficiency condition (namely, late-onset hypogonadism intended as a chronic condition), and, therefore, may not fit perfectly into this context of relevant tT levels reduction acutely observed after SARS-CoV-2 infection, we arbitrarily applied the tT threshold suggested by the Endocrine Society (ie, tT<9.2 nmol/L) [20]; accordingly, patients with COVID-19 depicted tT levels even 10-fold lower than the suggested threshold for normality. Third, and clinically important, tT levels emerged to be inversely associated both with ICU and death outcomes, after adjusting for the Critical-ill COVID-19 composite score,[26] which well recapitulates the complexity of the disease. To this aim, this study was not intentionally designed to prove a causative link between low serum tT levels and adverse clinical outcomes; conversely, a prospective case-control study, including highly comorbid patients but without SARS-CoV-2 infection, would represent the ideal design to test this hypothesis. However, our data showed a relevant association between low tT values and the need for ICU admission or death outcomes already at hospital admission because of symptomatic SARS-CoV-2 infection. Therefore, our findings outline the importance of assessing serum hormones in men presenting for COVID-19 as a potential early sentinel marker for subsequent worse outcomes during hospitalization. We could speculate that the identification of a group of men at higher risk of severe illness might prompt preventive strategies or different intensity of care to even better tailor male patients with COVID-19.

Our study is certainly not devoid of limitations. First, this was a single center-based study, raising the possibility of selection biases and limiting the generalizability of the findings. Second, the control group is represented by voluntary healthy blood male donors asymptomatic and serologically negative for SARS-CoV-2 infection and not by a contemporaneous (or historical)-matched group of same-sex patients admitted to the hospital with a number of comorbidities which overall may resemble COVID-19 in terms of severity. However, we observed that sex hormones levels suggestive for a condition of secondary hypogonadism were clearly found even in groups of patients

with COVID-19 at younger age, with lower amount of comorbid conditions, and with better severity outcomes (Table 3). Moreover, current findings showed that SARS-CoV-2 infection status per se was independently associated with lower tT levels, after adjusting for recognized confounders of established hypogonadism (Table 2). Still, larger studies are needed to assess if the degree of HPG axis suppression correlates with COVID-19 in men, or with the severity of any incidental acute illness, thus including viral infections. As a consequence, current findings might reflect the scenario in which serum tT levels are simply a marker of (any) acute ill health without playing a pathogenic role in SARS-CoV-2 infection. Third, this study is part of an institutional protocol which lacks a pre-infection hormonal milieu assessment in men with COVID-19; in this context, notwithstanding all patients in our cohort had been immediately drawn at hospital admission before any specific medical treatment was implemented, and the baseline assessment has accomplished the most severe inclusion criteria in terms of blood sampling and collection; previous data reported that low circulating T was observed in mechanically ventilated patients with primary ARDS and may contribute to longer ICU stay [47] and in men with critical illness.[35,48] Fourth, we did not consider smoking cigarette among the potential recognized risk factors for outcomes severity in patients with COVID-19, although the literature recently reported not unambiguous findings.[49-51] Finally, we cannot exclude that using different thresholds for hypogonadism [52] our results would have been different; however, we have adopted one of the most established definition of low tT that is endorsed by the Endocrine Society.[20]

5 | CONCLUSIONS

Our findings indicate that most men seeking medical care for symptomatic COVID-19 show low circulating T levels already at the time of hospital admission, which were associated with more severe clinical outcomes. In as many as 85% of cases, sex-hormones levels were suggestive for a condition of secondary hypogonadism. Overall, our case-control study outlines that T levels in males with COVID-19 deserve clinical attention, although it remains to be rigorously established whether T is simply a marker of ill health or measuring it may help to target very high-risk population, while establishing effective counter-active therapeutic measures.

CONFLICTS OF INTEREST

The authors have declared that no conflict of interest exists.

AUTHORS CONTRIBUTION

AS designed and led the study and wrote the report. MP, MT, LB, CC, CA, DC, AMF, GAR, CT, MJ, ML, LS, AC, AZ, FDC, MT, PR-Q, and FC took care of patients and acquired data. AS, PC, SG, WC, IR, GL, FC, and FM analyzed data and drafted the report.

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