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ORIGINAL RESEARCH

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COVID-19 and androgenic status: testosterone or dihydrotestosterone have a pivotal role?

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Abstract

The aim of our study is analysis of the androgenic status including testosterone (T) and dihydrotestosterone (DHT) in men hospitalized with coronavirus disease 2019 (COVID-19) and them relationship with the course of the disease. This is a monocentric prospective study performed on 125 male patients hospitalized for COVID-19. We conducted hematological examination, blood biochemical profile, hemostasis analysis and hormonal examination (T and DHT levels) lung and chest computed tomography and also assessed outcomes of hospitalization. Low DHT serum level was found only in 18 patients (14.4%). Subjects with low DHT were significantly older compare to subjects with normal DHT. At the same time in patients with normal DHT white blood cells (WBC) count, neutrophils at admission were higher than in patients with low DHT. No correlation was observed between T and DHT serum blood levels. C-reactive protein (CRP) has a weak positive correlation of DHT serum blood concentration ($r = 0.22$; $p = 0.016$). The inverse pattern was obtained for T serum blood concentration ($r = -0.285$; $p = 0.001$). After divided all males according to T concentrations we conducted next correlation analysis for DHT and CRP in two different groups: with normal T levels and with low T levels. We found that in males with normal T DHT levels are not correlated with CRP ($r = 0.095$; $p = 0.462$). However, in males with low T DHT and CRP had weak positive correlation with $r = 0.317$ ($p = 0.012$). Higher DHT concentrations are associated with higher CRP levels, however correlation is weak and in patients with normal T is absent, that may indicate anti-inflammatory effect of T and possible proinflammatory effect of DHT.

Keywords

COVID-19; Testosterone; Dihydrotestosterone

1. Introduction

Coronavirus disease 2019 (COVID-19) is a novel infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Already from the first detectable case of COVID-19 in 2019 it became clear that SARS-CoV-2 provide a serious problem for public health. That's why on 11 March 2020 the pandemic was proclaimed by WHO, which had then spread across the globe within a very short time (World Health Organization). As a result, on the April 2022 already almost 500 million people worldwide are infected and already more 6 million people were died (World Health Organization).

The examination of hormonal status under pandemic has faded into the background. However, more and more data starting in 2020 indicated to sex differences of COVID-19, which could be explained by the hormonal characteristics of the male and female [2]. According to meta-analysis published in January 2021 [3] it was shown that the number of infected males was higher than women. When infected, males also had a higher risk for severe COVID-19 disease (relative risk (RR) 1.18, 95% confidence interval (CI) 1.10 to 1.27), a higher need for intensive care (RR 1.38, 95% CI 1.09 to 1.74) and a higher risk of death (RR 1.50, 95% CI 1.18 to 1.91) [3]. In the largest public database of sex-disaggregated data on COVID-19 men and women have the same prevalence for contracting infection. However, the number of male hospitalizations, intensive care unit admissions and confirmed deaths are higher than woman [4]. Consequently, the molecular basis of COVID-19 sex differences has been the subject of active discussion over the past 2 years.

One of potential justifications for sex-biased differences in COVID-19 infection, illness and death among women and men lies in the genetic features associated with the presence of

two X chromosomes in women and one X chromosome in men [5]. First of all, X-chromosome in women can escape X-chromosome inactivation, that provides double dosage of genes with anti-inflammatory properties. Thus, it provides stronger immune response in females [5].

The second part of sex-biased differences can be associated with two main receptors that are essential components for SARS-Cov-2 host cell entry: angiotensin-converting enzyme-2 (ACE2) and transmembrane serine protease-2 (TMPRSS2). ACE2 gene are X-linkage and some data are indicated on its possible escape X-chromosome inactivation, [5]. TMPRSS2 has an androgenic regulation at the gene level and its transcription are mostly activated by active metabolite of testosterone (T)-dihydrotestosterone (DHT). Hence *in vitro* study of DHT function on endothelial cells has shown that DHT causes an increase in the expression of not only TMPRSS2, but also ACE2 [6]. Thus, it became clear that hormonal regulation can affect the expression of various regulatory molecules and a detailed understanding of hormonal features can explain the sex differences of COVID-19. In our previous work we have shown that low T levels among patients with COVID-19 was correlated with lung damage and also associated with high C-Reactive-Protein (CRP) and D-dimer levels [7]. It was also confirmed in other studies: low T level associated with more advance immune activation and increased risk of intensive care unit (ICU) admission or death in patients with COVID 19 [7, 8]. However, about DHT such information are almost absent. Thus, the purpose of our study is analysis of the androgenic status including T and DHT in men hospitalized with COVID-19 and them relationship with the course of the disease.

2. Materials and methods

2.1 Study design and participants

This is a monocentric prospective study performed on 125 male patients hospitalized for COVID-19 at Medical Research and Education Center, Lomonosov Moscow State University, Moscow, Russia between 21 April 2020 and 13 June 2020. The inclusion criteria were: (1) hospitalization for COVID-19 diagnosed by real-time reverse-transcriptase-polymerase-chain-reaction assay of nasal and pharyngeal swab specimens and/or radiological signs of pneumonia; (2) male sex older than 18 years; (3) completed hospital course at study end (discharged or dead).

We used two scores to objectify the severity of the clinical condition and to adequately assess effects of the therapy. The first one was the National Early Warning Score 2 (NEWS-2) distress syndrome severity score updated for patients with COVID-19 [9]. The other one was our original clinical assessment score for patients with coronavirus disease (Symptomatic Hospital and Outpatient Clinical Scale for COVID-19 (SHOKS-COVID)) published earlier and referred to above [10]. We also assessed body mass index (BMI), presence of coronary artery disease (CAD); arterial hypertension, diabetes mellitus, oncological diseases. Clinical data are performed at admission: body temperature, breath rate (BR), heart rate (HR), systolic blood pressure (SBP), oxygen O2 saturation (SaO2) and need of any oxygen support. Patients didn't use of external androgens or antiandrogens before admission or during hospitalization.

2.2 Radiological assessment

Lung and chest computed tomography (CT) scans were produced using a 32 slice SOMATOM Scope CT scanner (Siemens, Munich, Germany). The scans were obtained with 1-mm slices. During the first examination, the standard CT protocol (tube voltage 120 kV, automatic tube current modulation 200–400 mA) was used. All the scans were stored in DICOM (Digital Imaging and Communications in Medicine) format in the radiological information network (PACS/RIS) of the MSU Medical Research and Educational Center. The CT scans were processed and analyzed in the Syngo.via Version VB40A workstations (Siemens, Munich, Germany). A semi-quantitative score for assessing the amount of infiltration and consolidation areas of the lung tissue was used to process and interpret CT findings, as was recommended by the Interim Guidelines of the Russian Ministry of Health Prevention, Diagnosis and Treatment of COVID-19 versions 7–9 (CT1–CT4), and by software for quantitative analysis of the COVID-19-related lung infiltrations, Multivox (Gammamed, Moscow, Russia) and Botkin.AI (Intelogic, Moscow, Russia) [11]. The CT were obtained for 61 males.

2.3 Serum parameters measurement

Plasma samples collected at admission. The following laboratory tests were done: complete blood count, hematological analyzer XN 2000 (Sysmex Corporation, Hyogo, Japan); blood biochemical profile (CRP, creatinine), automatic biochemical analyzer AU480 (Beckman Coulter, Krefeld, Germany); hemostasis analysis (D-dimer), hemostasis analyzer STA-Compact (Diagnostica Stago SAS, Asnières-sur-Seine, France). Neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-C-reactive protein ratio (LCR) and glomerular filtration rate (GFR) were also count.

2.4 Hormone quantification

The levels of total T and DHT were measured in all patients. Serum T levels measured using Elecsys Testosterone II Immunoassay (analyzer Roche Cobas 6000). The test is based using monoclonal antibodies with high binding capacity and specifically directed against T. Endogenous T isolated from the sample using 2-bromoestradiol competes with the added derivative of T labeled with a ruthenium complex for binding sites on a biotinylated antibody. Reference values, as well as all other information on the Elecsys Testosteron II test system for determining the level of T in the blood, are obtained from the manufacturer's instructions attached to the test system. The lower reference value of the normal T in males, in accordance with the instructions for the test system, were 2.49 ng/mL in males younger than 50 years (range—2.49–8.36 ng/mL) and 1.93 ng/mL in males older than 50 years (range—1.93–7.40 ng/mL). Serum DHT levels measured using Elecsys Testosterone II Immunoassay (analyzer Roche

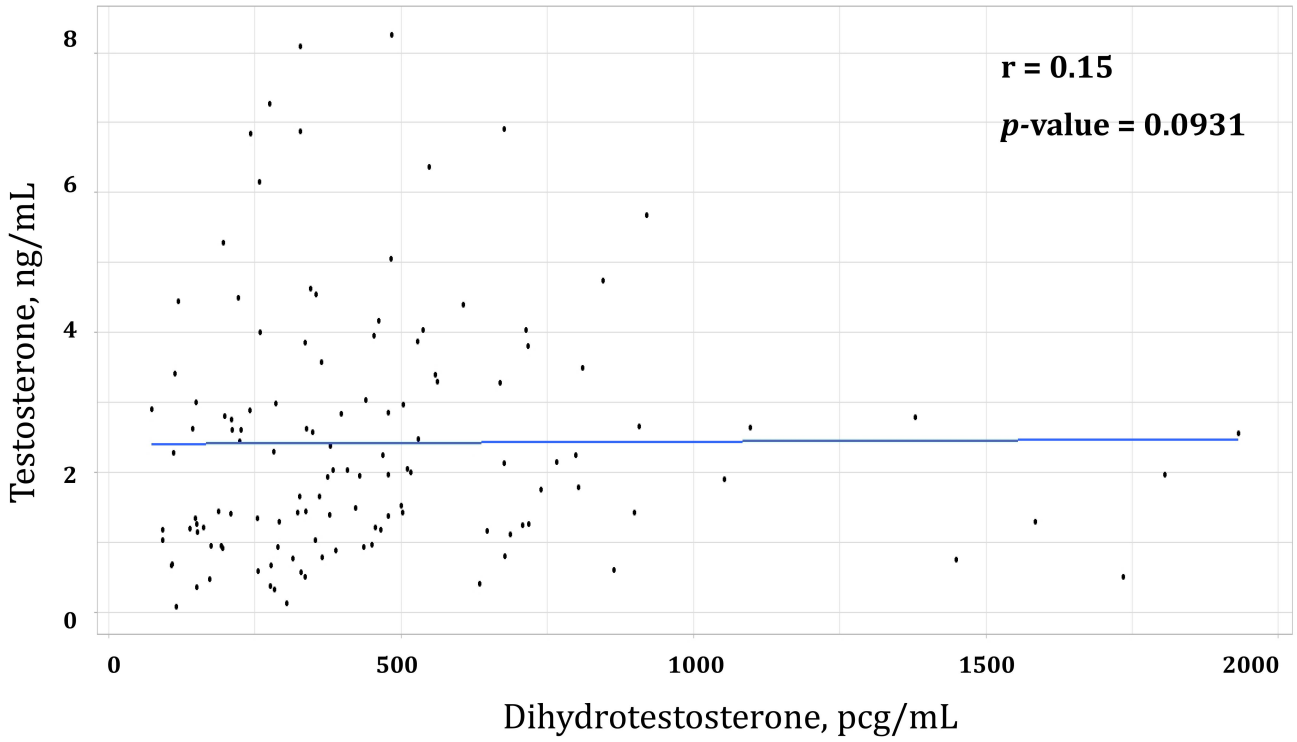

FIGURE 1. Correlation between DHT and T serum blood levels.

Cobas 6000). The lower reference value of the normal DHT in males, in accordance with the instructions for the test system, were 175 pcg/mL (range—175–1204 pcg/mL). Concentrations below or above the limits were defined as low and high, respectively.

2.5 Statistical analysis

Statistical analyses were performed using the R programming language in R Studio version 5.x. The normality of distributions was evaluated with the Shapiro-Wilk test. Quantitative data are described as the median and the interquartile range (IQR 25%; 75%) if the distribution was non-normal and as the mean and the standard deviation (SD) if the distribution was normal. Qualitative data between the two groups were compared with the Mann-Whitney test for non-normal distributions and with the Student's *t*-test for normal distributions. Qualitative data between the more than two groups were compared with the Kruskal-Wallis test for non-normal distributions and with the ANOVA test for normal distributions.

Nominal variable data are presented as absolute and relative values. The significance of intergroup differences in qualitative characteristics was assessed with the χ^2 test and the two-tailed Fisher's exact test. Correlations were estimated using Spearman's correlation coefficient. The threshold for statistical significance was 0.05.

3. Results

The study included 125 male patients diagnosed with COVID-19, with median age of 58.0 years (IQR 43–69 years) and median BMI of 29.1 kg/m^2 (IQR 25.7–32.4). The median T level was 2.0 ng/mL. The median DHT level was 374 pcg/mL. Study participants characteristics are summarized in Table 1.

DHT serum level upper low reference value (normal DHT) was found in 107 patients (85.6%). Low DHT serum level was found only in 18 patients (14.4%). Subjects with low DHT were significantly older compare to subjects with normal DHT (66.0 and 56.0 respectively, $p = 0.004$), however the number of comorbidities and clinical characteristics at admission were comparable. At the same time in patients with normal DHT WBC count, neutrophils and NLR at admission was higher than in patients with low DHT (Table 1). No statistically significant difference outcomes were evident in males with low DHT compared to males with normal DHT.

From the correlation analysis no correlation was observed between T and DHT serum blood levels (r = 0.15; $p = 0.0931$) (Fig. 1).

Next step we divided all patients in 4 groups according to different combinations among T and DHT serum blood levels: group A (Low T + low DHT), group B (Low T + normal DHT), group C (Normal T + normal DHT), group D (Normal T + low DHT). We obtained 12 males with low both T and DHT (group A), 51 males with low T and normal DHT (group B), 56 males with low normal T and normal DHT (group C) and 6 males with normal T and low DHT (group D). All data are summarized in Table 2. We found that patients in group D were older than patients in other groups and had the lowest DHT levels. Males in group A however more often needed any oxygen support and had higher NEWS-2 score. At the same time patients group B have the highest neutrophils and the highest CRP levels at admission, that indicate severer inflammation in this group. However, these patients have more favorable outcomes than males in group A, that reflected in more often corticosteroids prescription, ICU admission, invasive ventilation and longer hospitalization (Table 2).

TABLE 1. Comparative characteristics of men with COVID-19 and low and normal DHT levels.

	All patients (n = 125)	Low DHT (n = 18)	Normal DHT (n = 107)	*p**
General characteristics				
Age, years, median (IQR)	58.0 (43.0–69.0)	66.0 (59.5–74.0)	56.0 (42.0–67.5)	0.004**
BMI, kg/m^2, median (IQR)	29.1 (25.7–32.4)	29.4 (25.9–31.6)	28.7 (25.7–32.4)	0.988**
Dihydrotestosterone, pcg/mL, median (IQR)	374 (243–558)	129 (109–150)	436 (309–658)	<0.001**
Testosterone, ng/mL, median (IQR)	2.00 (1.18–3.04)	1.21 (0.77–2.54)	2.04 (1.25–3.34)	0.031**
Comorbidity				
Arterial hypertension, % (n)	53 (66)	65 (11)	51 (55)	0.447***
Coronary heart disease, % (n)	13 (16)	18 (3)	12 (13)	0.694***
Diabetes mellitus, % (n)	14 (18)	12 (2)	15 (16)	0.999***
Oncological diseases, % (n)	6 (7)	12 (2)	5 (5)	0.250***
Clinical data				
Body temperature, mean (SD)	37.4 (0.8)	37.6 (0.81)	37.4 (0.8)	0.498*
BR, median (IQR)	19.5 (18.0–21.0)	20.0 (19.0–22.0)	19.0 (18.0–21.0)	0.119**
HR, median (IQR)	88.5 (79.8–101.0)	90.0 (81.0–100.0)	87.0 (79.0–102.0)	0.928**
SBP, mm Hg, median (IQR)	125 (118–132)	125 (110–155)	124 (119–130)	0.718**
SaO2, %, median (IQR)	95 (92–97)	94 (92–97)	95 (93–97)	0.367**
Any oxygen support, % (n)	32.8 (40.0)	43.8 (7.0)	31.1 (33.0)	0.474***
SHOCS-COVID, score, median (IQR)	7.0 (4.0–10.0)	8.5 (4.8–10.2)	6.0 (4.0–10.0)	0.506**
NEWS-2, score, median (IQR)	4 (2–7)	5 (3.75–8)	4 (2–7)	0.052**
Laboratory and instrumental data				
HGB, g/dL, median (IQR)	15.5 (14.4–16.6)	15.5 (13.8–15.9)	15.5 (14.4–17.0)	0.330**
WBC, $\times10^9$/L, median (IQR)	5.80 (4.26–7.11)	4.94 (3.96–5.82)	6.07 (4.47–7.20)	0.044**
PLT, $\times10^9$/L, mean (SD)	193 (156–240)	156 (130–191)	201 (160–244)	0.007*
Neutrophils, $\times10^9$/L, median (IQR)	3.88 (2.65–5.39)	2.82 (2.58–3.28)	4.17 (2.71–5.60)	0.011**
Lymphocytes, $\times10^9$/L, median (IQR)	1.19 (0.85–1.65)	1.21 (0.92–1.83)	1.19 (0.84–1.62)	0.552**
CRP, mg/L, median (IQR)	62.8 (21.8–110.0)	48.8 (17.8–64.4)	67.2 (22.5–113.0)	0.145**
NLR, median (IQR)	3.21 (2.12–5.03)	2.21 (1.59–3.29)	3.53 (2.19–5.21)	0.035**
LCR, median (IQR)	20.1 (10.3–62.7)	26.0 (15.8–96.1)	18.0 (9.26–54.2)	0.179**
D-dimer, μg/mL, median (IQR)	0.62 (0.32–1.13)	0.80 (0.50–1.14)	0.56 (0.31–1.12)	0.189**
$GFR_{CKD-EPI}$, mL/min/1.73m^2, mean (SD)	76.3 (18)	69.0 (13.5)	77.5 (18.4)	0.026*
CT lung damage (%), mean (SD)	21.1 (7.4–40.6)	30.9 (8.8–53.9)	21.1 (7.4–37.0)	0.417*
Outcomes				
Corticosteroids, % (n)	19 (23)	31 (5)	17 (18)	0.304***
Hospital stays, days, median (IQR)	11 (8–15)	14 (10–17)	11 (8–15)	0.118**
Pulmonary embolism/thrombosis, % (n)	6% (7)	0% (0)	7% (7)	0.576***
ICU admission, % (n)	17% (21)	28% (5)	15% (16)	0.307***
ICU staying duration, days, median (IQR)	13 (6–21)	16 (13–21)	12 (4–30)	0.535**
Invasive ventilation, % (n)	11% (14)	22% (4)	9% (10)	0.221***
Invasive ventilation, days, median (IQR)	13.0 (9.75–30.0)	12.5 (11.2–13.0)	19.0 (10.0–33.8)	0.285**
In-hospital mortality, % (n)	5% (6)	11% (2)	4% (4)	0.207***

*Abbreviations: BMI, body mass index; IQR, interquartile range; SD, standard deviation; BR, breath rate; HR, heart rate; SBP, systolic blood pressure; SaO2, oxygen O2 saturation; SHOCS-COVID, Symptomatic Hospital and Outpatient Clinical Scale for COVID-19; NEWS-2, National Early Warning Score for COVID-19; HGB, hemoglobin; WBC, white blood cells; PLT, platelets; CRP, C-reactive protein; NLR, neutrophil-to-lymphocyte ratio; LCR, lymphocyte-to-C-reactive protein ratio; $GFR_{CKD-EPI}$, glomerular filtration rate with CKD-EPI formula; CT, computer tomography; ICU, intensive care unit; DHT, dihydrotestosterone. *Student's t-test, **Mann-Whitney U test, *** χ^2 test.*

TABLE 2. Comparative characteristics of men with different combinations of T and DHT serum levels.

	group A (n = 12)	group B (n = 51)	group C (n = 56)	group D (n = 6)	*p*
General characteristics					
Age, years, median (IQR)	64.0 (56.0–72.5)	53.0 (42.5–64.5)	57.0 (42.0–68.2)	73.0 (66.8–75.5)	0.022
BMI, kg/m^2, median (IQR)	29.4 (25.8–31.6)	29.7 (26.3–33.0)	28.2 (24.9–32.3)	29.4 (26.8–30.7)	0.598
Dihydrotestosterone, median (IQR)	143 (108–151)	422 (309–682)	446 (317–559)	116 (111–137)	<0.001
Testosterone, median (IQR)	1.09 (0.62–1.20)	1.21 (0.79–1.44)	3.29 (2.61–4.51)	2.96 (2.70–3.32)	<0.001
Comorbidity					
Arterial hypertension, % (n)	58 (7)	47 (24)	55 (31)	67 (4)	0.519
Coronary heart disease, % (n)	17 (2)	12 (6)	12 (7)	17 (1)	0.999
Diabetes mellitus, % (n)	8 (1)	20 (10)	11 (6)	17 (1)	0.558
Oncological diseases, % (n)	8 (1)	2 (1)	7 (4)	17 (1)	0.251
Clinical data					
Body temperature, mean (SD)	37.60 (0.87)	37.50 (0.80)	37.40 (0.81)	37.60 (0.73)	0.869
BR, median (IQR)	20.0 (19.0–22.0)	20.0 (18.0–22.0)	18.5 (18.0–20.2)	20.0 (19.0–21.0)	0.121
HR, median (IQR)	90.0 (81.0–100.0)	92.0 (80.0–106.0)	86.0 (78.0–95.0)	87.0 (81.0–90.0)	0.155
SBP, mm Hg, median (IQR)	122 (110–135)	120 (116–130)	126 (120–137)	140 (125–160)	0.24
SaO2, %, median (IQR)	98 (92–108)	100 (90 –111)	95 (85–105)	97 (87 –102)	0.301
Any oxygen support, % (n)	64 (7)	37 (19)	25 (14)	0	0.028
SHOCS-COVID, score, median (IQR)	6.50 (4.00–10.00)	7.00 (5.00–10.00)	5.00 (4.00–7.00)	5.00 (4.00–7.00)	0.023
NEWS-2, score, median (IQR)	6.00 (4.00–8.50)	4.00 (2.00–8.00)	3.00 (1.00–5.00)	5.00 (4.00–5.00)	0.047
Laboratory and instrumental data					
HGB, g/dL, median (IQR)	15.8 (15.3–16.2)	15.8 (14.4–126.0)	15.4 (14.4–16.3)	12.6 (10.1–14.2)	0.025
WBC, ×10^9/L, median (IQR)	5.19 (4.41–5.90)	6.20 (4.82–7.39)	5.80 (4.03–7.02)	4.06 (3.49–5.47)	0.087
PLT, ×10^9/L, mean (SD)	168 (154–188)	193 (156–242)	212 (166–246)	90 (40–189)	0.045
Neutrophils, ×10^9/L, median (IQR)	2.86 (2.70–3.38)	4.50 (3.11–5.72)	3.98 (2.60–5.26)	2.49 (1.93–3.19)	0.023
Lymphocytes, ×10^9/L, median (IQR)	1.11 (0.89–1.86)	1.22 (0.81–1.61)	1.12 (0.93–1.63)	1.25 (1.02–1.67)	0.901
CRP, mg/L, median (IQR)	43.6 (15.8–69.4)	87.1 (43.8–138.0)	52.0 (17.0–91.0)	54.0 (25.5–64.4)	0.022
NLR, median (IQR)	2.47 (1.62–3.48)	3.51 (2.23–5.32)	3.62 (2.09–4.57)	2.07 (1.55–2.62)	0.137
LCR, median (IQR)	32.4 (17.6–128.0)	16.5 (7.4–33.9)	23.6 (11.2–88.1)	23.2 (16.1–47.4)	0.093
D-dimer, μg/mL, median (IQR)	0.77 (0.55–0.90)	0.59 (0.34–1.14)	0.50 (0.29–1.04)	1.07 (0.58–1.59)	0.401
$GFR_{CKD-EPI}$, mL/min/1.73m^2, mean (SD)	68.4 (15.6)	75.0 (18.0)	80.0 (18.7)	70.2 (8.9)	0.136
CT lung damage (%), mean (SD)	16.40 (4.65–48.90)	25.75 (7.90–49.70)	13.10 (4.30–30.70)	19.90 (10.20–42.40)	0.109

TABLE 2. Continued.

	group A (n = 12)	group B (n = 51)	group C (n = 56)	group D (n = 6)	*p*
Outcomes					
Corticosteroids, % (n)	45.5 (5)	21.6 (11)	12.7 (7)	0	0.045
Hospital stays, days, median (IQR)	15.0 (12.5–25.0)	12.0 (10.0–16.0)	10.0 (7.00–13.2)	11.0 (9.25–13.5)	0.022
Pulmonary embolism/thrombosis, % (n)	0	6 (3)	7 (4)	0	0.768
ICU admission, % (n)	42 (5)	20 (10)	11 (6)	0	0.032
ICU staying duration, days, median (IQR)	16.0 (13.0–21.0)	13.5 (5.8–34.0)	9.00 (3.8–15.0)	-	0.596
Invasive ventilation, % (n)	33 (4)	14 (7)	5 (3)	0	0.038
Invasive ventilation, days, median (IQR)	12.5 (11.2–13.0)	17.0 (11.0–27.5)	33.0 (20.5–35.0)	-	0.553
In-hospital mortality, % (n)	17 (2)	6 (3)	2 (1)	0	0.133

Abbreviations: BMI, body mass index; IQR, interquartile range; SD, standard deviation; BR, breath rate; HR, heart rate; SBP, systolic blood pressure; SaO2, oxygen O2 saturation; SHOCS-COVID, Symptomatic Hospital and Outpatient Clinical Scale for COVID-19; NEWS-2, National Early Warning Score for COVID-19; HGB, hemoglobin; WBC, white blood cells; PLT, platelets; CRP, C-reactive protein; NLR, neutrophil-to-lymphocyte ratio; LCR, lymphocyte-to-C-reactive protein ratio; $GFR_{CKD-EPI}$*, glomerular filtration rate with CKD-EPI formula; CT, computer tomography; ICU, intensive care unit; p for all groups.*

The paired comparison of more important data is shown on Fig. 2.

From the correlation analysis of C-reactive protein, as most common biomarker of COVID-19 severity, a weak positive correlation of DHT serum blood concentration was found (r = 0.22; $p = 0.016$) (Fig. 3A). The inverse pattern was obtained for T serum blood concentration (r = −0.285; $p = 0.001$) (Fig. 3B). After divided all males according to T concentrations we conducted next correlation analysis for DHT and CRP in two different groups: with normal T levels and with low T levels. We found that in males with normal T DHT levels are not correlated with CRP (r = 0.095; $p = 0.462$) (Fig. 3C). However, in males with low T DHT and CRP had weak positive correlation with r = 0.317 ($p = 0.012$) (Fig. 3C).

4. Discussion

Since there are sex differences in the death rate from COVID-19, it can be assumed that some biomarkers can predict the severity of the disease depending on sex. In our previous study was shown that patients with low T levels at admission have more severe COVID-19, that was associated with higher CRP, D-dimer, neutrophils count, higher SHOCS-COVID score and NEWS-2 score and longer hospitalization [7]. However, about DHT such information is almost absent. We firstly conducted the study about both DHT and T association of COVID-19 severity and outcomes among males. We have found that in patients with normal DHT WBC count, neutrophils and NLR at admission was higher than in patients with low DHT, that can indicate more severe inflammation in this group. Previously it was reported about inverse relationship between serum levels of interleukin-1beta and T in men, that shows anti-inflammatory effect of T [12], that has been proven in our study [7]. Now we need understand DHT effects on inflammation responses.

The effects of T and DHT on interleukin-6 (IL-6) production (as a major inflammation marker) by human gingival fibroblasts were accessed *in vitro* by Russell A. Gornstein *et al.* [13] in 1999. Both T and DHT were able to inhibit IL-6 production in males. The inhibition observed for T has been described as dose dependent and seemed to reach a maximum level at concentration 10^{-7}M. For DHT was observed interesting pattern. At 10^{-10}M, 10^{-9}M and 10^{-8}M DHT levels IL-6 production was significantly less than in control male cells. However, at 10^{-7}M DHT IL-6 production was the same as in control male cells. Thus, it can also describe as dose dependent, but in the reverse manner [13]. Such results confirmed protective and anti-inflammatory effect of T, however about DHT still not clear.

The results of Seline Zurfluh *et al.* [14] (2018) showed that in males higher serum levels of DHT on admission were associated with higher long-term mortality after community-acquired pneumonia. After Cox regression multivariate analyses adjusted for age and comorbidities it was found that males with higher DHT concentrations have 2.84 times higher risk of 6-year mortality (95% CI 1.15–6,99; $p = 0.023$). In females this association was not significant [14]. According to results of Seline Zurfluh *et al.* [14] (2018), in males there was an inverse correlation of T levels (but not DHT) with acute inflammatory markers, namely CRP (r = −0.39, $p < 0.001$). In this study DHT had weak positive correlation with CRP during COVID-19 (r = 0.22; $p = 0.016$), but not with bad or good outcome. For DHT and CRP found positive correlation (r = 0.22; $p = 0.016$). This effect was higher in group of low T (r = 0.317; $p = 0.012$), which may be explained by anti-inflammatory effect of T, that are also higher that possible

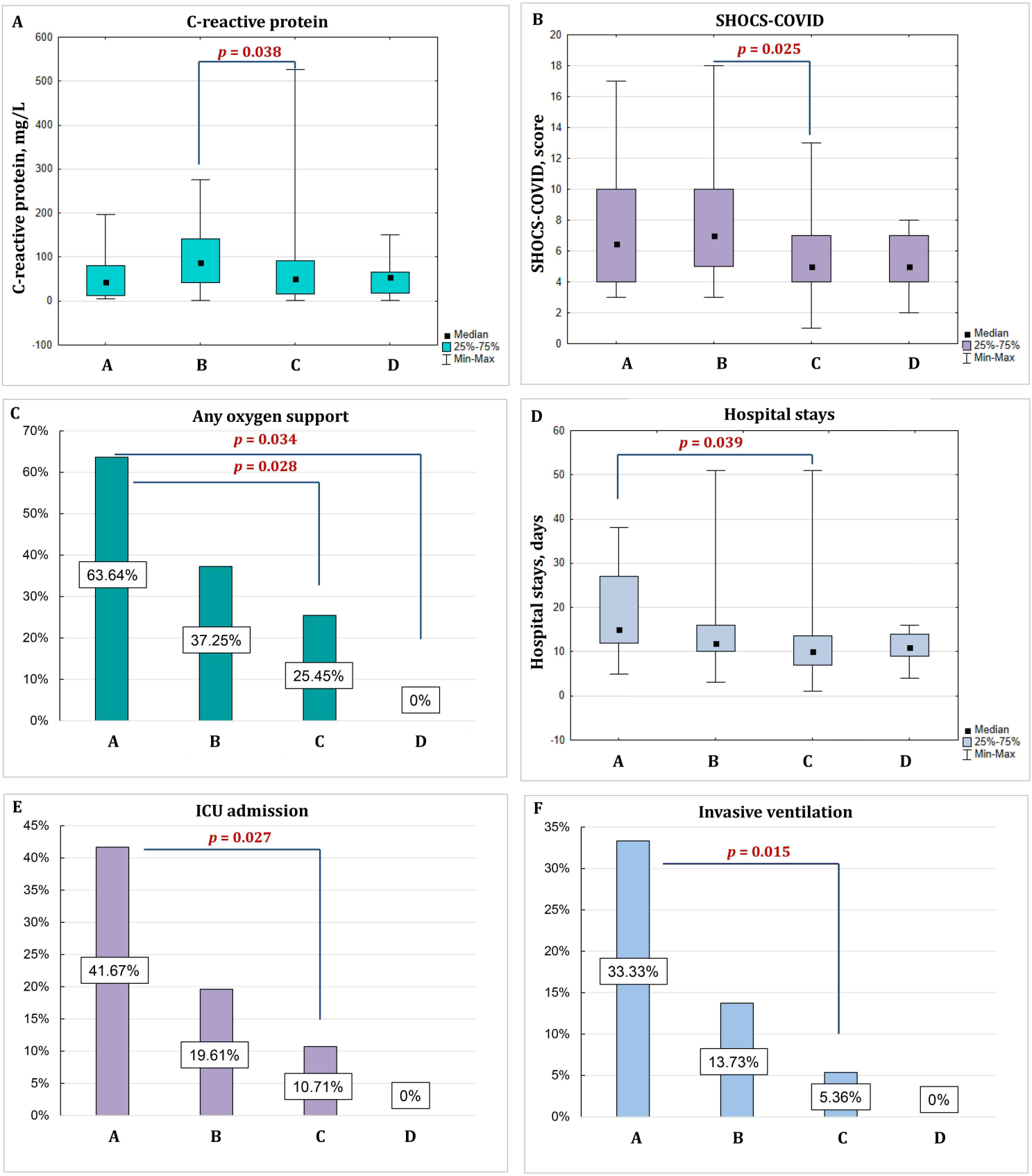

FIGURE 2. Paired comparison of most important data between four groups depending on androgenic status. (A)—CRP depending on androgenic status. (B)—SHOCS-COVID depending on androgenic status. (C)—any oxygen support depending on androgenic status. (D)—hospital stay depending on androgenic status. (E)—ICU admission depending on androgenic status. (F)—invasive ventilation depending on androgenic status. SHOCS-COVID, Symptomatic Hospital and Outpatient Clinical Scale for COVID-19; ICU, intensive care unit.

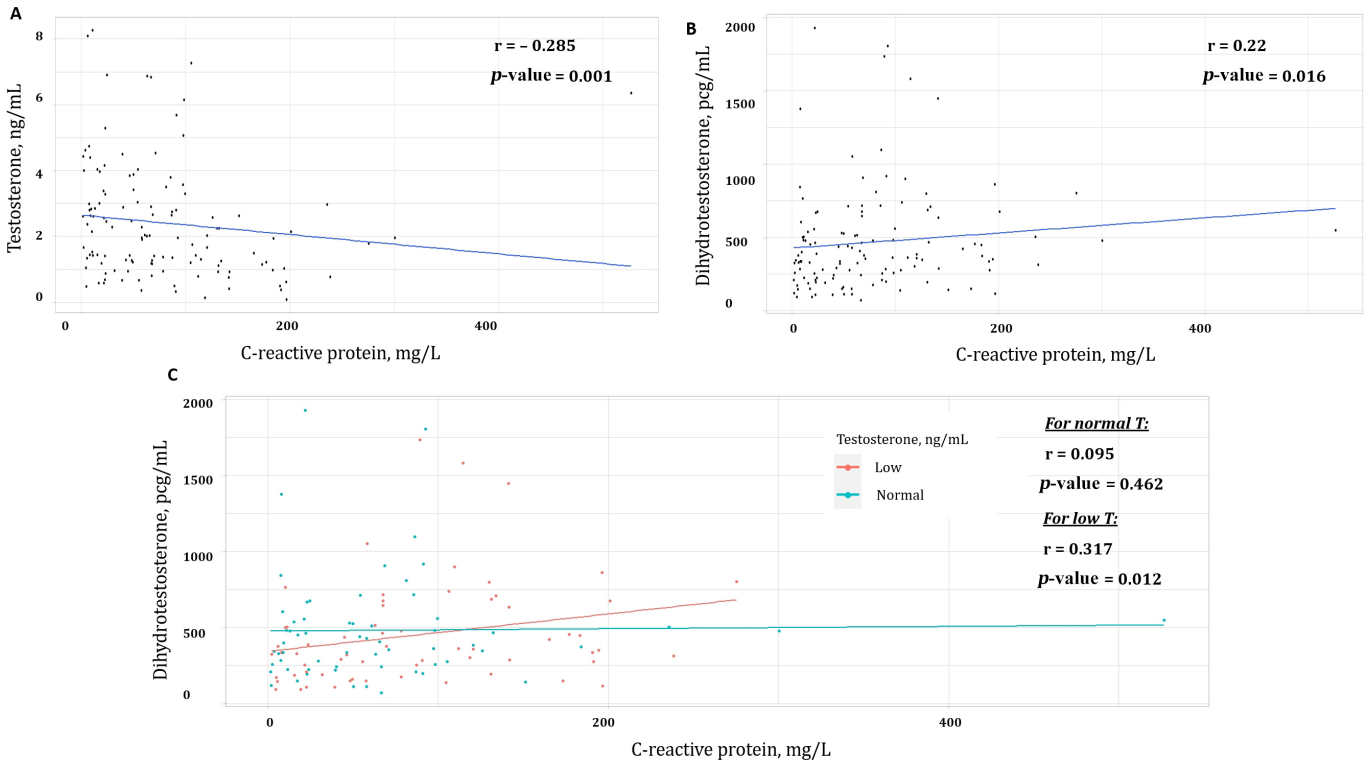

FIGURE 3. Correlation analysis for T, DHT and CRP. (A)—correlation between T and CRP. (B)—correlation between DHT and CRP. (C)—correlation between DHT and CRP in 2 groups according to T levels.

pro-inflammatory effect of DHT, because of there are not correlation between DHT and CPR in group of normal T (r = 0.095; $p = 0.462$).

The potential mechanism of productive infection sustenance under DHT influence was proposed by Laximan Sawant *et al.* (2021) [15]. It was demonstrated that androgen receptors (AR) via DHT activation can increase Krüppel like transcription factor 4 (KLF4) protein levels and these two factors regulate each other through a reciprocal feedback mechanism. In turn, AR + KLF4 + DHT cooperatively stimulate productive infection of bovine herpesvirus 1 (BoHV-1) and transactivate key viral regulatory promoters augments viral spread and influences the incidence of reactivation from latency higher than AR + DHT and AR or KLF4 [15]. At the same time DHT-treated mice have increased levels of virus entry proteins: ACE2 and the cellular proteases TMPRSS2, furin, cathepsin L (last two can cleave SARS-CoV-2 S1 spike protein as well as TMPRSS2) in kidney, especially in high fat diet group mouse at both mRNA and protein levels [16]. Similar results under DHT exposure were obtained in the lung, cecum, heart [17].

Data regarding the direct interaction of SARS-CoV-2 and DHT are very limited. The first and only *in vitro* study about the effects of SARS-CoV-2 spike protein-mediated endothelial injury under conditions of exposure to DHT was conducted by Nitin Kumar *et al.* [6] (2021). DHT exposure was associated with increased transcript expression of the endothelial cell adhesion molecules E-selectin and Intercellular Adhesion Molecule 1 (ICAM-1) in endothelial cells and significantly decreased tissue plasminogen activator (tPA), while plasminogen activator inhibitor-1 (PAI-1) transcript expression was not affected. It was also found that DHT led to a 1.5-fold increase in ACE2 and 2.8-fold increase in TMPRSS2 transcript expression [6]. Thus, endothelial injury was induced by the SARS-CoV-2 S1 spike protein *in vitro*, and this effect was exacerbated in the presence of the DHT, that is notably expressed at higher levels in men. It can be may be explained by higher expression of ACE2 and TMPRSS2 that leads to higher infection and as a result to severe cases of COVID-19 among men [6].

It was known that the effect of 5α-reductase inhibitors is resulted in reduced DHT levels without change in T concentration [18]. Even before the pandemic was started it was reported about protective function of 5α-reductase inhibitors on the inflammatory response and pulmonary damage. So triple administration of finasteride (time points 0 hour, 12 hours and 48 hours) after trauma-hemorrhage and polymicrobial sepsis induction in male mice was associated with reduced post-traumatic cytokine secretion by alveolar macrophages as well as with decreased expression of plasma cytokine (Monocyte Chemotactic Protein 1 (MCP-1) and Macrophage inflammatory protein 1β (MIP-1β)) in lung tissue [19]. The similar results were obtained in males with COVID-19 treated with 5α-reductase inhibitors. On day 7 dutasteride treatment, 64.7% of men from the dutasteride group and 11.8% of men from the placebo group had undetectable nasopharyngeal SARS-CoV-2 virus or viral fragments. At the same time men from dutasteride group had higher mean oxygen saturation, lower CRP, lactate, lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR) and troponin. D-dimer level differences were only observed on day 14 and it was significantly lower in dutasteride group [20]. Absolute risk for SARS-Cov-2 infection among males treated with 5α-reductase inhibitors (mean duration of use was 60.4 months) was also

significantly lower compared to control group (absolute risk reduction 4.9%; OR 0.81; 95% CI 0.67–0,97, p = 0.026) [21]. So, DHT reduction under 5α-reductase inhibitors effect can associated with lower viral entry proteins expression and, respectively, viral shedding and inflammatory markers, as well as stable T concentrations act as anticytokine protective agent.

Thus, the first *in vivo* study about DHT among patients with COVID-19 was published by Maria Schroeder *et al*. [22] in December 2021. It was shown that in male ICU-COVID-19 patients (39 patents) DHT were reduced compare to healthy individuals (30 patients) ($p < 0.0001$). A substantial proportion of plasma DHT in COVID-19 males was even below the lowest reference range. In contrast, DHT levels were comparable and within clinical references in female COVID-19 and healthy individuals (p = 0.957) [22]. In our study 85.6% males had DHT about upper reference level. Higher serum DHT levels were associated with stronger inflammation response, that reflected in higher WBC count, neutrophils and NLR at admission. Moreover, DHT alone was not associated with worse outcome of COVID-19. After divided of males on 4 groups according to T and DHT combinations we found that worse outcome of COVID-19 was in patients with low both T and DHT, that can be explained by absence of protective T effect and COVID-19 is severe and long. Meanwhile, the highest CRP level was observed in group with low T and normal DHT, that can be connected with from the one side possible pro-inflammation effect of DHT and from another side with higher expression of ACE2 and TMPRSS2 under DHT exposure with then generalization of infection.

The results of our study take some limitations. First and foremost is the relatively small number of group with low DHT level, as well as groups with low T/DHT and normal T/low DHT. Second, the sex hormone analysis was based on a single measurement upon hospital admission. Third, patients with low and normal DHT levels have different baseline age. However, it is the first study about T and DHT association in COVID-19 males, that can explain some clinical features of this disease.

5. Conclusions

Higher DHT concentrations are associated with higher CRP levels, however correlation is weak and in patients with normal T is absent, that may indicate anti-inflammatory effect of T and possible proinflammatory effect of DHT.

AVAILABILITY OF DATA AND MATERIALS

Due to privacy and ethical concerns (the lack of patient consent to data exchange), neither the data nor the digital location of the data can be made available.

AUTHOR CONTRIBUTIONS

KA—designed the research study, performed the research; MV—designed the research study, wrote the manuscript; OI—designed the research study, wrote the manuscript; MY—designed the research study, wrote the manuscript; BY—designed the research study; PZ—performed the research; PA—performed the research; SL—performed the research; ME—performed the research; OD—performed the research; NO—performed the research, wrote the manuscript; SA—performed the research; TD—performed the research; All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the local ethics committee of MSU University clinic (approval number 8 and approval date 15 April 2020). The patients provided their written informed consent to participate in this study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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