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Aromatase, testosterone, TMPRSS2: determinants of COVID-19 severity

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Abstract

Background Male sex has been identified as a risk factor for worse COVID-19 outcomes. This sex difference has been mostly attributed to the complex role of sex hormones. Cell surface entry of SARS-CoV-2 is mediated by the transmembrane protease serine 2 (TMPRSS2) which is under transcriptional regulation by androgens. P450 aromatase enzyme converts androgens to estrogens. This study measured concentrations of aromatase enzyme, testosterone, estradiol, and TMPRSS-2 in plasma of hospitalized COVID-19 patients to elucidate the dynamics of sex-linked disparity in COVID-19 and correlate them with disease severity and mortality.

Methods In this prospective cohort study, a total of 265 patients (41% women), age 18 years and older, who had a positive COVID-19 PCR test and were hospitalized for COVID-19 at Memorial Hermann Hospital in Houston, (between May 2020 and May 2021) were enrolled in the study if met inclusion criteria. Plasma concentrations of Testosterone, aromatase, TMPRSS-2, and estradiol were measured by ELISA. COVID-19 patients were dichotomized based on disease severity into moderate-severe ($n = 146$) or critical ($n = 119$). Mann Whitney U and logistic regression were used to correlate the analytes with disease severity and mortality.

Results TMPRSS2 (2.5 ± 0.31 vs. 1.73 ± 0.21 ng/mL, $p < 0.01$) and testosterone (1.2 ± 0.1 vs. 0.44 ± 0.12 ng/mL, $p < 0.01$) were significantly higher in men as compared to women with COVID-19 after adjusting for age in a multivariate model. There was no sex difference seen in the level of estradiol and aromatase in COVID-19 patients. TMPRSS2 and aromatase were higher, while testosterone was lower in patients with increased COVID-19 severity. They were independently associated with COVID-19 severity, after adjusting for several baseline risk factors in a multivariate logistic regression model. In terms of mortality, TMPRSS2 and aromatase levels were significantly higher in non-survivors.

Conclusions Our study demonstrates that testosterone, aromatase, and TMPRSS2 are markers of COVID-19 severity. Estradiol levels do not change with disease severity in COVID-19. In terms of mortality prediction, higher aromatase and TMPRSS-2 levels can be used to predict mortality from COVID-19 in hospitalized patients.

Plain English Summary COVID-19 has caused over a million deaths in the U.S., with men often getting sicker than women. Testosterone, a male hormone, helps control a protein called TMPRSS-2, which allows the COVID-19 virus to spread more easily in the body. A protein called aromatase converts the male hormone testosterone into the

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female hormone estrogen. It is thought that female hormone estrogen helps protect women from getting seriously ill from COVID-19. To understand the role of these hormones in COVID-19 and sex differences, we measured levels of testosterone, estrogen, aromatase (which turns testosterone into estrogen), and TMPRSS-2 in hospitalized COVID-19 patients. We also checked how this level might reflect the severity of the disease. We found that critically ill COVID-19 patients (the ones in ICU) had higher levels of TMPRSS-2 and aromatase, and lower testosterone levels. When we used these hormone levels to predict death in hospitalized COVID-19 patients, higher levels of TMPRSS-2 and aromatase were linked to a lower chance of survival.

Highlights

- COVID-19 Disease Severity: In hospitalized patients with COVID-19, higher TMPRSS-2, aromatase and lower total testosterone are markers of disease severity.
- COVID-19 Mortality: In hospitalized patients with COVID-19, TMPRSS2 and aromatase levels are significantly increased in COVID-19 non survivors.

Keywords COVID-19, Sex differences, CRP, Aromatase

Introduction

Coronavirus disease 19 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to over 1 million deaths in the United States [1]. Initial studies identified age as a risk factor for symptomatic infection, as seen with other respiratory illnesses [2]. Meta-analyses of 70 clinical reports from around the world, detailing COVID-19 infections by sex, have shown that while the number of infected men (47.9%) and women (51.5%) were similar, men had a higher mortality rate, 56.1% as compared to women, 42.5% [3]. In intensive care unit admissions, women had significantly fewer admissions, 30.4% compared to men, 69.6% [3]. Therefore, male sex has been recognized to be a risk factor for COVID-19 severity [4]. This may be due to the complex role of sex chromosomal complement or epigenetic factors, but a link with sex hormones has appeared to be more plausible [5]. This is related to the mechanism of entry for the SARS-CoV-2 virus. Cell surface entry of SARS-CoV-2 is mediated by the transmembrane protease serine 2 (TMPRSS2). The spike protein of the SARS-CoV-2 virus binds to TMPRSS2 on the cell surface where it is then cleaved by TMPRSS2, leading to membrane fusion of the viral capsid with the eukaryotic membrane, internalizing the free-floating viral RNA into the cytoplasm [6]. Interestingly, hormone testosterone has been shown to regulate TMPRSS2 expression in different human and animal models [7]. Also, in vitro studies of A549 human lung cancer cells have shown that 24 h of testosterone treatment results in the upregulation of the TMPRSS2 gene, upregulating TMPRSS2 protein on the cell surface [8]. Interestingly, testosterone concentration is regulated by the enzyme P450 aromatase (aromatase) that converts testosterone to 17 β -estradiol via a sequence of redox, dehydration, and elimination reactions [9]. Aromatase's role is critical to sexual differentiation [10]. In peripheral blood, aromatase is synthesized by blood leukocytes and helps modulate sex hormone

concentrations in circulation [8]. On the other hand, the female sex hormone, estrogen has been known to have anti-inflammatory and protective effects in COVID-19 [11]. Many studies have focused on quantifying the level of these hormones individually in small cohorts of only men or women. To understand the dynamics of aromatization and the role of sex hormones, the goal of this study was to quantify the endogenous sex hormones (estradiol, testosterone, and aromatase) in hospitalized COVID-19 patients. Another secondary objective was to determine the association of the levels of these analytes with COVID-19 outcomes (disease severity, in-hospital mortality).

Methods

Study population and patient inclusion/exclusion criteria

This study screened 3045 patients who were admitted to Memorial Hermann Hospital, Houston, Texas, USA with a positive nasopharyngeal swab confirmed by real-time polymerase chain reaction for COVID-19 between May 2020 to May 2021 [12, 13]. Of the 3045 patients, we excluded 2377 patients in which COVID-19 was not the primary diagnosis for admission but was incidental. Patients with steroids, hormone replacement therapy, immunosuppressants, or chemotherapy on home medication history ($n=431$) were further excluded from the study. 265 patients met our inclusion and exclusion criteria, and peripheral blood samples were collected after patient consent. 33% of the patients were admitted to the ICU. Patients were then categorized based on WHO COVID-19 severity classification, as either moderate-severe (hospitalized and requiring supplemental oxygen) or critical (in ICU on ventilator/ artificial life support) [2]. All procedures were approved by the Institutional Review Board at The University of Texas Health Science Center at Houston, Houston, Texas, USA.

Sample collection

Patients were consented and enrolled within 48 h of admission. Blood samples were collected fasting, with morning labs, around 4–5 am for all patients. Peripheral blood was collected in appropriate sterile vacutainers and transported on ice for processing. Blood plasma was isolated by centrifuging samples at 1,200 x g for 10 min at 4 °C, followed by plasma supernatant isolation and further centrifugation at 10,000 x g for 10 min at 4 °C to generate plasma. Samples were stored at -80 °C in aliquots until analysis.

ELISA analysis

We used enzyme-linked immunosorbent assay (ELISA) to measure the levels of total testosterone (Millipore Sigma, Inc, Burlington, MA, USA), aromatase (Cloud-Clone Corp, Houston, TX, USA), TMPRSS2 (Novus, Centennial, CO, USA), and estradiol (Calbiotech, El Cajon, CA, USA). CRP values were obtained from hospital records at the time of admission.

Statistical analysis

Descriptive statistics were calculated for demographic variables and hormone levels in control and COVID-19 subjects. Results are reported as mean \pm standard error of the mean (SEM). To describe differences in demographics, χ^2 -test, Fisher's exact test, student's t-test, and the Mann-Whitney U test were used where appropriate. The Mann-Whitney U test was used to test for differences in analyte levels across different groups. A p-value of ≤ 0.05

was considered statistically significant. In constructing the multivariate models, we selected variables based on their relevance to COVID-19 severity and mortality, as well as their statistical significance within our dataset [14]. Logistic regression (LR) method was used, and the predictive performance was measured using area under the curve (AUC) analysis. The receiver operating characteristics (ROC) curves were computed and the area under the AUC of each model was obtained. The ROC curves were compared using the De-Long method [15]. Figures were made using GraphPad Prism version 8. All statistical analyses were performed using open-source software packages in R (v3.1.3) and MedCalc for Windows, version 15.0 (MedCalc Software, Ostend, Belgium).

Results

Sex Differences in COVID-19

We analyzed samples from 265 hospitalized COVID-19 patients, where 41% of the COVID-19 patients were women. The mean age of women was 54.3 years, (range, 19 to 95 years), with 58.7% of women enrolled being above 51 years (average age of menopause [16]). The mean age of men was 52.8 years of age, (range, 22 to 101 years), with 56.4% of men enrolled being over the age of 51 years. There was no significant difference in the demographics, past medical history, and mortality between men and women with COVID-19 in the hospitalized patients (Table 1: Demographics of Hospitalized COVID-19 Patients; Women vs. Men). CRP levels after COVID-19 admission were significantly higher in men compared

Table 1 Demographics of Hospitalized COVID-19 Patients; Women vs Men. Sex differences in testosterone, TMPRSS2, Estradiol and aromatase levels were assessed. Testosterone and TMPRSS2 were significantly higher in men vs. women

	Women (n = 109)	Men (n = 156)	p-value	Sample Size
Demographics				
Age (mean,sd)	54.3(16)	52.8(15.7)	0.4	
Race (n,%)				
African American	22(20)	26(16.6)	0.35	
White	53(48)	84(53)	0.47	
Other	34(31)	46(29)	0.87	
Ethnicity, Hispanic (n,%)	62(56)	93(59)	0.75	
Past Medical History				
Hypertension (n,%)	51(46)	74(47)	1	
Diabetes (n,%)	41(37)	56(36)	0.87	
Hyperlipidemia (n,%)	20(18)	32(20)	0.78	
Obesity (n,%)	59(54)	82(52)	0.89	
Smoking(n,%)	5(4.5)	18(11)	0.07	
Mortality at discharge (n,%)	25(23)	37(23)	0.99	
Hormones				
Testosterone, ng/ml (mean \pm sem)	0.44 \pm 0.12	1.2 \pm 0.1	< 0.01*	n = 182 (153 vs 129)
TMPRSS2, ng/ml (mean \pm sem)	1.73 \pm 0.21	2.5 \pm 0.31	0.04*	n = 220 (92 vs 128)
Estradiol, pg/ml (mean \pm sem)	48 \pm 8.3	53 \pm 7.75	0.66	n = 108 (44 vs 64)
Aromatase, ng/ml (mean \pm sem)	7 \pm 0.42	7.3 \pm 0.27	0.4	n = 259 (108 vs 151)

* Significant after adjusting for age

to women (107 ± 7 vs. 86 ± 7.3 mg/L, $p < 0.05$). Plasma samples were available from 109 women and 156 men, and the availability of sex hormone levels are as follows: testosterone for 182 samples (53 women and 129 men); TMRSS2 for 220 samples (92 women and 128 men), estradiol for 108 samples (44 women and 64 men) and aromatase for 259 samples (108 women and 151 men).

TMRSS2 (2.5 ± 0.31 vs. 1.73 ± 0.21 ng/mL, $p < 0.01$) and testosterone (1.2 ± 0.1 vs. 0.44 ± 0.12 ng/mL, $p < 0.01$) were both significantly higher in men compared to women. Both were independently associated with sex after adjusting for age in a multivariate model (Table 1). There was no sex difference seen in the level of estradiol and aromatase in the hospitalized COVID-19 patients.

Clinical Severity and Sex Hormones

COVID-19 patients were dichotomized based on disease severity into either moderate-severe ($n = 146$) or critical ($n = 119$) disease. Patients with critical disease were older and had higher CRP levels at admission (Table 2: Demographics of Hospitalized COVID-19 Patients; Moderate-Severe vs. Critical). There were no differences in comorbidities, history of hypertension, diabetes, hyperlipidemia, obesity, or smoking between the two groups. We analyzed levels of sex hormones stratified by critical vs. moderate-severe subjects. TMRSS2 (3.46 ± 0.38 vs. 1.15 ± 0.12 ng/mL, $p < 0.01$), estradiol (67 ± 9.6 vs. 34 ± 4.8 pg/mL, $p < 0.01$) and aromatase (7.9 ± 0.4 vs. 6.5 ± 0.24 ng/mL, $p < 0.01$) were significantly higher and testosterone

(0.7 ± 0.1 vs. 1.27 ± 0.13 ng/mL, $p < 0.01$) was significantly lower in patients with critical disease, as compared to the ones with moderate-severe disease. Testosterone, TMRSS2, and aromatase were independently associated ($p < 0.01$) with severity after adjusting for several baseline risk variables (including age, sex, CRP, obesity, hypertension, hyperlipidemia, and diabetes) in a multivariate logistic regression model (Table 2).

Given a significant difference in hormone levels was seen with severity, we next investigated if any of these hormones can predict COVID-19 severity (Fig. 1: Clinical Severity and Hormones). PCA analysis with baseline variables (including age, sex, and CRP) and hormones (testosterone, TMRSS2, and aromatase) indicated excellent segregation between patients with moderate-severe vs. critical disease (Fig. 1A). We developed two multivariate models: a baseline model (including age, sex, CRP, obesity, hypertension, hyperlipidemia, and diabetes) and a hormone model (that included the testosterone, TMRSS2, and aromatase level in addition to the variables in the baseline model). The baseline model included variables that are known predictors of adverse COVID-19 outcomes, such as age, CRP levels, hypertension, hyperlipidemia, and diabetes mellitus. We performed univariate analysis on these known variables, and only those with a p -value < 0.1 were included in the multivariate model (age, hypertension, diabetes, hyperlipidemia, and CRP were all $p < 0.05$ in the univariate model).

Table 2 Demographics of Hospitalized COVID-19 Patients; Moderate-Severe vs. Critical. Testosterone was decreased and TMRSS2, Aromatase were significantly increased with disease severity after adjusting for age, sex, CRP, obesity, hypertension, hyperlipidemia, diabetes mellitus (‡)

	Moderate-Severe (n = 146)	Critical (n = 119)	p-value	Sample Size
Demographics				
Age (mean, sd)	51(15)	56(16.8)	0.007	
Sex (female, %)	67(46)	42(35)	0.1	
Past Medical History				
Hypertension (n, %)	67(45)	58(48.7)	1	
Diabetes (n, %)	52(35.6)	45(38)	0.8	
Hyperlipidemia (n, %)	29(19.8)	23(19)	1	
Obesity (n, %)	76(52)	65(54)	0.89	
Smoking (n, %)	14(9.5)	9(7.5)	0.6	
Severity				
CRP, mg/L (mean \pm se)	55 \pm 3.2	152 \pm 8.4	< 0.01	
Mortality at discharge (n, %)	0(0)	62(52)	< 0.01	
Hormones				
Testosterone, ng/ml (mean \pm sem)	1.27 \pm 0.13	0.7 \pm 0.1	< 0.01*~‡	n = 182 (96 vs 86)
TMRSS2, ng/ml (mean \pm sem)	1.15 \pm 0.12	3.46 \pm 0.38	< 0.01*~‡	n = 220 (123 vs 97)
Estradiol, pg/ml (mean \pm sem)	34 \pm 4.8	67 \pm 9.6	< 0.01*	n = 108 (152 vs 56)
Aromatase, ng/ml (mean \pm sem)	6.5 \pm 0.24	7.9 \pm 0.4	< 0.01*~‡	n = 259 (146 vs 113)

*Significant after adjusting for Age and Sex

~Significant after adjusting for Age, Sex and CRP

‡Significant after adjusting for Age, Sex, CRP, Obesity, Hypertension, Hyperlipidemia, Diabetes Mellitus

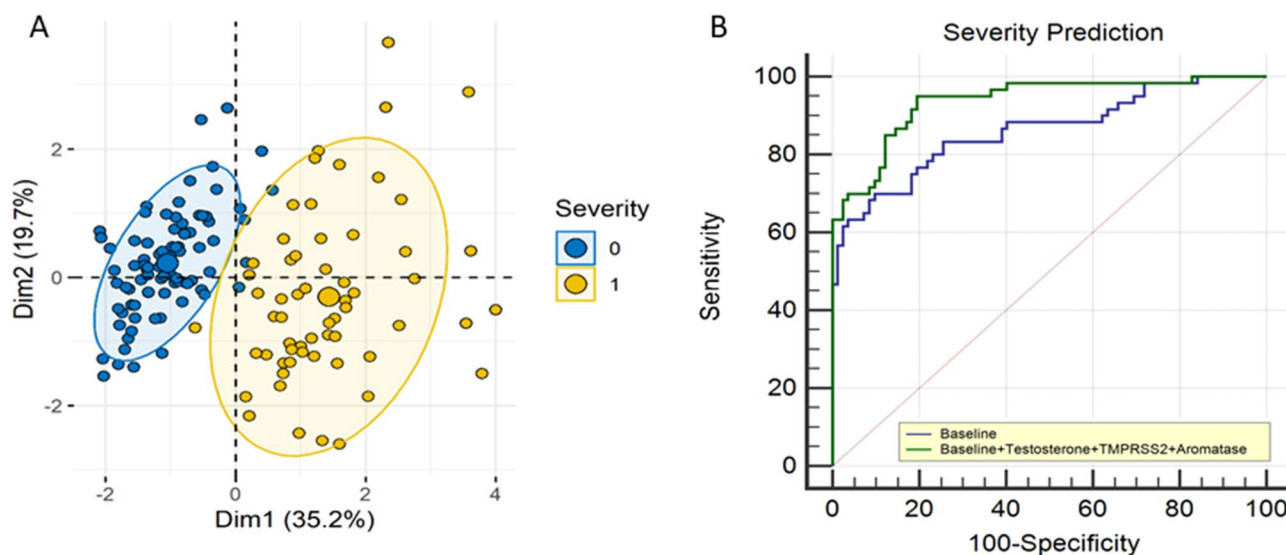


Fig. 1 Clinical severity and Hormones: **(A)** Using PCA analysis, we report a panel of hormones (testosterone, TMPRSS2 and Aromatase) was able to segregate COVID-19 patients who ate at risk of severe clinical course (1-Clinical, 0-Moderate-Severe) **(B)** Inclusion of hormone panel (including testosterone, TMPRSS2 and Aromatase) in addition to baseline risk factors in a logistic regression model significantly improved the prediction of severity course over the baseline model by 8.7% (0.94 ± 0.02 [95% CI: 0.88 to 0.97] vs. 0.86 ± 0.03 [95% CI: 0.79 to 0.91], $p < 0.01$, De-Long Test)

Although several studies have identified smoking as a risk factor for adverse COVID-19 outcomes, in our cohort, smoking was not significantly associated with mortality in a univariate analysis ($p = 1.0$). The prevalence of smoking was low among both survivors and non-survivors (7% vs. 7.8%), leading to its exclusion from the multivariate model to preserve model parsimony. The hormone model significantly improved the prediction of clinical severity compared to the baseline model by 8.7% (0.94 ± 0.02 [95% CI: 0.88 to 0.97] vs. 0.86 ± 0.03 [95% CI: 0.79 to 0.91], $p < 0.01$, De-Long Test) (Fig. 1B).

Mortality and sex hormones

In-hospital mortality data were extracted for these patients hospitalized with COVID-19. No mortality was reported in moderate-severe COVID-19 patients. Critical COVID-19 patients were dichotomized based on mortality: Non-survivors ($n = 64$) vs. survivors ($n = 55$) as per discharge disposition. Mortality was higher with advanced age, co-morbidity of hypertension, and hyperlipidemia (Table 3: Mortality Analysis of Critical COVID-19 Patients). Interestingly, among the patients with critical disease, the survivors had significantly higher CRP levels than non-survivors (182 ± 9.3 vs. 127 ± 16 mg/L, $p < 0.1$). There was no difference in diabetes, obesity, and smoking in the two groups. We analyzed the levels of hormones stratified by mortality in the patients that had critical COVID-19 and found that TMPRSS2 (4.3 ± 0.59 vs. 2.3 ± 0.4 ng/mL, $p < 0.01$), estradiol (81 ± 13.7 vs. 43 ± 10.6 pg/mL, $p = 0.05$) and aromatase (9.3 ± 0.74 vs. 6.4 ± 0.3 ng/mL, $p < 0.01$) were significantly higher in non-survivors. Testosterone was higher in survivors, but this trend was

not statistically significant ($p = 0.33$). TMPRSS2 and aromatase were both increased in non-survivors and independently associated ($p < 0.01$) with mortality after adjusting for several baseline risk variables (including age, sex, CRP, hypertension, hyperlipidemia, and diabetes) in a multivariate logistic regression model.

We investigated if the hormone levels on admission can predict mortality (Fig. 2: Mortality and Hormones). PCA analysis with baseline variables (including age and CRP) and hormones (testosterone, TMPRSS2, and aromatase) indicated reasonable segregation between survivors and non-survivors (Fig. 2A). We developed two multivariate models: a baseline model (including age, sex, CRP, hypertension, hyperlipidemia, and diabetes) and a hormone model (that included the testosterone, TMPRSS2 and aromatase levels at admission in addition to the variables in the baseline model). The hormone model significantly improved AUC over the baseline model by 13% (0.86 ± 0.04 [95% CI: 0.74 to 0.93] vs. 0.76 ± 0.06 [95% CI: 0.63 to 0.86], $p < 0.01$, De-Long Test (Fig. 2B).

Discussion

Our study was designed to measure the levels of sex steroids in hospitalized COVID-19 patients at admission and associate them with disease severity and mortality. We found that P450 aromatase, testosterone, and TMPRSS-2 can be markers of COVID-19 severity. Additionally, TMPRSS-2 and aromatase can be predictors of mortality in patients with critical COVID-19 disease. TMPRSS-2 is a transmembrane serine protease that facilitates SARS-COV-2 viral entry in target cells [17–19], and androgen receptors are transcription

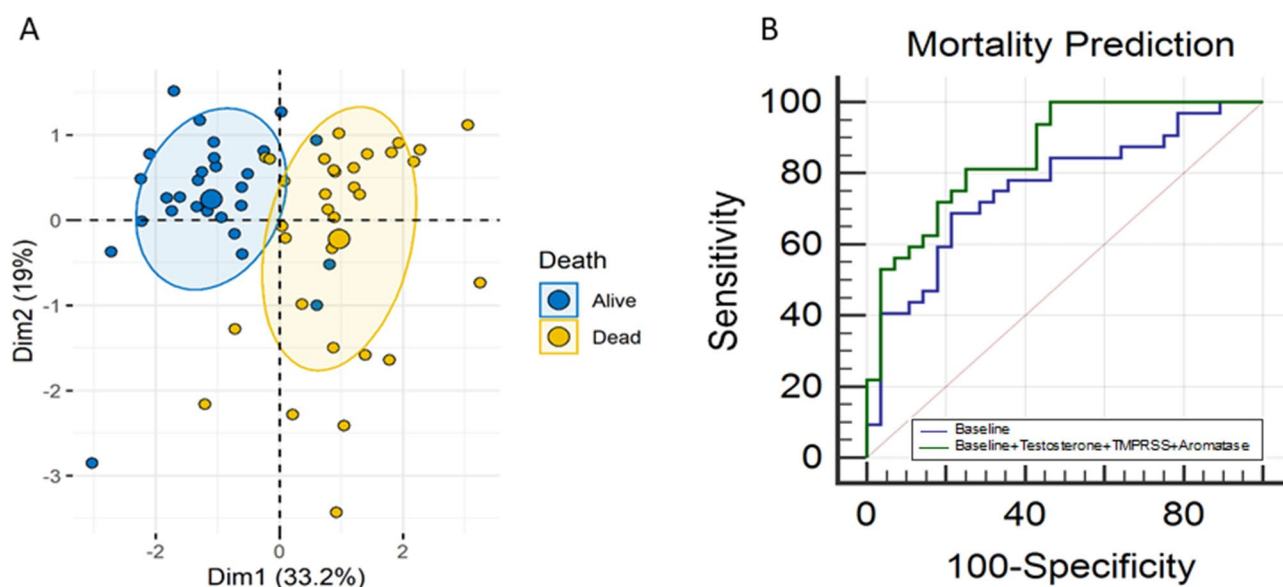
Table 3 Mortality Analysis of Critical COVID-19 Patients. TMPRSS2 and aromatase were significantly increased in survivors vs. non-survivors, after adjusting for age, sex, CRP, obesity, hypertension, hyperlipidemia, diabetes mellitus (‡)

	Survivors (n = 55)	Non-Survivors (n = 64)	p-value	Sample Size
Demographics				
Age (mean,sd)	50.4(15.8)	61.5(16)	< 0.01	
Sex (female,%)	16(29)	26(40)	0.2	
Race (n,%)				
African American	7(12)	11(17)	0.67	
White	31(56)	32(50)	0.61	
Other	17(31)	21(32)	0.98	
Ethnicity, Hispanic (n,%)	38(69)	41(64)	0.7	
Past Medical History				
Hypertension (n,%)	21(38)	37(57)	0.05	
Diabetes (n,%)	16(29)	29(45)	0.1	
Hyperlipidemia (n,%)	5(9)	18(28)	0.01	
Obesity (n,%)	34(62)	31(48)	0.2	
Smoking (n,%)	4(7)	5(7.8)	1	
Severity				
CRP,mg/L (mean ± se)	182 ± 9.3	127 ± 12	< 0.01	
Hormones				
Testosterone, ng/ml (mean ± sem)	0.84 ± 0.15	0.64 ± 0.12	0.33	n = 86 (42 vs 44)
TMPRSS2, ng/ml (mean ± sem)	2.3 ± 0.4	4.3 ± 0.59	< 0.01*‡	n = 97 (41 vs 56)
Estradiol, pg/ml (mean ± sem)	43 ± 10.6	81 ± 13.7	0.05	n = 56 (22 vs 34)
Aromatase, ng/ml (mean ± sem)	6.4 ± 0.3	9.3 ± 0.74	< 0.01*~‡	n = 113 (54 vs 59)

*Significant after adjusting for Age

~Significant after adjusting for Age, Hypertension, Hyperlipidemia, Diabetes Mellitus

‡Significant after adjusting for Age, CRP, Hypertension, Hyperlipidemia, Diabetes Mellitus

**Fig. 2** Mortality and Hormones: **(A)** Using PCA analysis, tested the same panel of hormones (including testosterone, TMPRSS2, and Aromatase) used to segregate COVID-19 severity to segregate mortality among critical subjects. **(B)** The same panel of hormones in addition to baseline risk factors improved the prediction of mortality at admission over the baseline model by 13% (0.86 ± 0.04 [95% CI: 0.74 to 0.93] vs 0.76 ± 0.06 [95% CI: 0.63 to 0.86], $p < 0.01$, De-Long Test

promoters for this protease [17]. Thus, androgens like testosterone activate the androgen receptor, promoting TMPRSS2 transcription and increased viral entry into cells. Higher levels of TMPRSS-2 have been postulated to

be the etiology of increased disease severity in men with COVID-19 [20, 22] contributing to the observed sex differences in COVID-19. In our enrolled patients who were hospitalized with COVID-19, there were higher levels of

testosterone and TMPRSS-2 in men as compared with women, consistent with prior studies [23]. Estradiol and aromatase levels did not differ between sexes in patients with COVID-19, which may be secondary to the enrollment of more post-menopausal women in our study (58.7% were above 51 years old). Patients with critical disease had higher levels of aromatase, TMPRSS2, and lower levels of testosterone as compared to patients with moderate-severe disease. This suggests that TMPRSS2, testosterone, and aromatase can be used as markers of poor prognosis or increased disease severity in COVID-19 hospitalized patients.

Cytochrome P450 aromatase is an enzyme involved in the biosynthesis of estrogens and sexual differentiation [24]. It converts androgens into estrogens maintaining the balance of sex hormones in the body and its activity has been detected in ovaries, adipose tissue, brain, breast, and placenta [25]. Our analysis of patients with COVID-19 has revealed a significant increase in aromatase levels and decreased testosterone levels in patients with critical disease, suggesting increased aromatase activity with disease severity. This is consistent with a recent study that used exome sequencing and demonstrated that CYP19A1 (aromatase) activity increasing mutation Thr201Met is associated with increased disease severity in men [26]. This is the first study to measure protein aromatase in hospitalized COVID-19 patients (both men and women) and demonstrate its association with disease severity. It is speculated that increased aromatization of androgens to estrogens may be the protective response to SARS-COV-2. We found a trend, but no significant increase, in estradiol levels in patients with severe COVID-19 after controlling for variables such as age, sex, and comorbidities. Estradiol, a sex steroid is known to have anti-inflammatory effects in COVID-19 [27]. Sex differences in COVID-19 outcomes have been attributed to these protective effects of estrogens. In a Sweden-based registry, estrogen supplementation in post-menopausal women was associated with decreased mortality from COVID-19 [28, 29]. This can be due to the anti-inflammatory effects of estrogens, for example, reduction in proinflammatory cytokines, increased T cell response, and increased antibody production from B Cells [28]. Aromatization of androgens to estrogens is a dynamic process, and it is possible that the two-day post-admission time point was too early to detect significant changes in estradiol levels in our study. It is also possible that the elevation of estradiol may be at the tissue level and the measured plasma estradiol may not be reflective of the tissue level increase. Future preclinical studies can provide more insights into the dynamics of this sex-hormonal milieu.

Consistent with several other studies [30–33], testosterone concentrations in patients with critical COVID-19 were found to be significantly lower than those in

moderate-severe disease patients. This further supports the theory that, in severe inflammatory states caused by COVID-19, testosterone is actively converted to estradiol by aromatase as a compensatory defense mechanism. Alternatively, the reduction in testosterone levels may represent transient hypogonadism or due to transient fasting, a stress response that has also been documented in other severe illnesses [34, 35]. TMPRSS2 levels increased with disease severity in patients with COVID-19, consistent with prior reports [7, 19, 36, 37]. Testosterone can upregulate TMPRSS2, a protease that is important for viral entry and activation [38]. The paradoxical decrease in testosterone levels with increased TMPRSS2 levels in patients with severe COVID-19 can potentially be secondary to negative feedback inhibition. This further highlights the complex dynamics of sex steroid aromatization and androgen receptor activity on TMPRSS2. Longitudinal studies at different time points may help further understand the role of this aromatization pathway.

Limitations

Our study had some limitations. Being a single-center study, it was designed to predict associations of sex steroids, aromatase, and TMPRSS2 levels with disease severity and mortality. Association does not imply causation; therefore, these results should be interpreted with caution. Due to the limited availability of human plasma for each analyte, the number of patients tested for each analyte varied. Moreover, when the data was dichotomized based on disease severity, our study lacked a sufficient sample size to perform a sex-based subgroup analysis. All our enrolled patients were hospitalized, so results should be interpreted carefully as they pertain to moderate-severe and critical cases of hospitalized patients and not to COVID-19 patients that recover in the outpatient and do not need supplemental oxygen (classified as mild per WHO classification). Furthermore, 58.7% of the women in our study were above 51 years, potentially peri or post-menopausal, which may affect the levels of estradiol. Although the analysis of analytes in our hospitalized patients may not change with vaccination or COVID-19 strain, it is important to realize that our study participants were enrolled between May 2020 and May 2021 when most patients were unvaccinated and the dominant strains were the original strain, D614G and B.1.17 mutant. We measured these markers at a single time point, within 48 h of admission, using ELISA. Future studies incorporating measurements at multiple time points and utilizing more advanced techniques like mass spectrometry may provide deeper insights into the complex dynamics of these hormones and markers.

Conclusion

This study shows that testosterone, aromatase, and TMPRSS2 are markers of COVID-19 severity in hospitalized patients. Estradiol levels do not change with disease severity in COVID-19. In terms of mortality prediction, high aromatase and TMPRSS-2 levels measured within 48 h of hospital admission can be used to predict mortality from COVID-19.

Author contributions

EM, LM and BM were involved in the conception and design of the study. EM, GC, DM, CF, SJ, AM and HA were involved in the acquisition of patient samples and sample processing. JS was involved in analysis of data. EM, JS, LM and BM contributed substantially in preparing the manuscript and figures.

Funding

This work was supported by an award from the Huffington Foundation and NIMH R01MH127856 to LDM. American Heart Association, 20CDA35310306 and NIA R21AG070860, R01 AG079314 to BM.

Data availability

Data available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

Our study involves human plasma samples. Consent was obtained for all participants as part of the enrollment process.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 10 July 2024 / Accepted: 4 October 2024

Published online: 24 October 2024

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