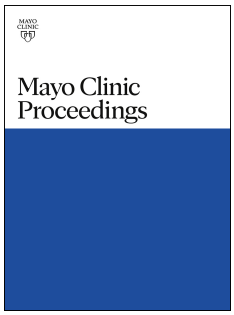

Estradiol and Dihydrotestosterone Levels in COVID-19 Patients

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Estradiol and Dihydrotestosterone Levels in COVID-19 Patients

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ABSTRACT:

Objective: To determine differences in plasma sex hormone levels in male and female COVID-19 patients and healthy volunteers (HVs), since cell entry of SARs-CoV-2 virus occurs via the ACE-2 receptor which is downregulated by 17β-Estradiol.

Patients and Methods: Citrated plasma samples were collected from 101 COVID-19 patients upon presentation to the Emergency Department and from 40 HVs between November 1, 2020, through May 30, 2021. Plasma 17β-Estradiol and 5α-dihydrotestosterone (DHT) levels were measured using ELISA (pg/mL). Data presented as median and quartiles [Q1, Q3]. Wilcoxon rank sum test, $p<.05$ significant.

Results: COVID-19 patients (median age 49 years) included 51 males and 50 females (25 post-menopausal). Hospital admission was required for 59% of male patients and 48% of female patients (16 post-menopausal). HVs (median age 41 years) included 20 males and 20 females (9 post-menopausal). Female COVID-19 patients were found to have decreased 17β-Estradiol levels (18.5 [10.5, 32.3]; 41.4 [15.5, 111.0], $p=.025$), and lower 17β-Estradiol to DHT ratios (0.073 [0.052, 0.159]; 0.207 [0.104, 0.538], $p=.015$) than female HVs. Male COVID-19 patents were found to have decreased DHT levels (302.8 [249.9, 470.8]; 457.2 [368.7, 844.3], $p=.005$), compared to male HVs. DHT levels did not differ between female COVID-19 patients and female HVs, while 17β-Estradiol levels did not differ between male COVID-19 patients and male HVs.

Conclusions: Sex hormone levels differ between COVID-19 patients and HVs, with sex-specific patterns of hypogonadism in males and females. These alterations may be associated with disease development and severity.

Key Words: COVID-19, Sex, Testosterone, Estrogen, Cytokines

Abbreviations:

ACE, angiotensin converting enzyme; COVID-19, coronavirus disease 2019; DHT, 5α-dihydrotestosterone; ED, emergency department; IFN-γ, interferon-γ; IL, interleukin

Introduction:

Males with coronavirus disease 2019 (COVID-19) infection have been consistently shown to have increased disease severity (including need for mechanical ventilation) and greater mortality than females[1]. Sex hormones, specifically estrogen and testosterone, impact viral cell entry and systemic immune response to COVID-19 infection. SARs-CoV-2 viral cell entry is dependent on the Angiotensin Converting Enzyme (ACE)-2 receptor, and males are known to have greater ACE-2 levels in plasma and on pneumocytes[2-5]. Females, meanwhile, are known to mount more robust immune responses to viral infection and vaccines, which is at least partly related to X-linked regulation of immune factors. Furthermore, 17β-estradiol may downregulate ACE-2 mRNA expression [2, 4, 6].

In response to COVID-19, female patients demonstrate greater activation and terminal differentiation of T-cells, while male patients have greater levels of pro-inflammatory cytokines including interleukin (IL)-8, IL-18 and chemokine ligand (CCL)-5[7]. Of note, 17β-Estradiol suppresses expression of the pro-inflammatory cytokine IL-6 and upregulates the anti-inflammatory interferon-γ (IFN-γ)[2, 8]. High levels of 17β-Estradiol and anti-müllerian hormone have recently been associated with decreased disease severity and lower levels of pro-inflammatory cytokines, including IL-6[9]. Elucidation of the interplay between sex hormones and cytokines is necessary, especially with emerging evidence that IL-6 mediated cytokine release contributes to systemic inflammation and multi-organ failure in COVID-19 [10]. Clinically, the protective effects of estrogen in COVID-19 infection have been repeatedly demonstrated[3, 11].

There is limited data on the association of 5α-dihydrotestosterone (DHT) in COVID-19 infection and clinical outcomes. Several studies have found that males with

COVID-19 infection have low testosterone levels, especially those with severe disease[12-14]. Testosterone is synthetized from cholesterol and is a common intermediate between 17β-estradiol and DHT. Males and females both produce DHT and 17β-estradiol, although the quantities and ratios between these hormones differ between sexes. Given the immunosuppressive effects of DHT, low testosterone levels may predispose patients to greater systemic inflammation due to decreased conversion to 17β-estradiol and thus increase susceptibility to the deleterious effects of cytokine storm[12]. Alternatively, the observed low testosterone levels may reflect a transient state of primary hypogonadism driven by direct virally mediated damage to the testicular epithelium[14].

To assess the impact of sex hormones on outcomes in COVID-19 patients, we measured 17β-Estradiol and DHT levels from plasma samples obtained from COVID-19 patients presenting to the Emergency Department (ED) at our institution. We also measured levels of IL-6 and IFN-γ in these same patients to explore how sex hormones influence inflammatory response in a heterogenous group of patients. We hypothesized that patients with greater estradiol levels, particularly female patients, may have some protection from more severe infection, and that levels of pro-inflammatory cytokines will be lower in such patients.

Materials and Methods:

Study Design and Sample Collection

This study was approved by the Mayo Clinic Institutional Review Board and all subjects provided written informed consent. Patients presenting to the ED at Mayo Clinic in Rochester, MN between November 1, 2020, and May 30, 2021, who tested positive for

COVID-19, were prospectively enrolled. A single blood draw was obtained from each patient at the time of presentation. Only patients that had a positive COVID-19 Polymerase Chain Reaction (PCR) test documented in the electronic medical record within two weeks of presentation were considered for enrollment. Exclusion criteria included: Refusal or inability to obtain informed consent from a patient or their Legal Authorized Representative (LAR), therapeutic anticoagulation (e.g., warfarin, dabigatran etexilate, rivaroxaban, apixaban), an inherited or acquired coagulation disorder, active malignancy, renal failure, or high-dose immunosuppression (including active chemotherapy, biologic medications, chronic immune suppression, and high dose steroids). Patients that did not have a documented positive COVID-19 PCR test, as well as those who were greater than 14 days out from positive test were also excluded. Patients on anti-platelet medications, including aspirin, clopidogrel, ticagrelor, and non-steroidal anti-inflammatory medications were included.

A total of 176 patients were screened for this study. Fifty-three (30.1%) were excluded based on one or more criteria, 7 (3.9%) declined to consent, and 15 of 116 (12.9%) enrolled patients were not included due to insufficient sample volume to appropriately complete all assays. We analyzed a total of 101 citrated plasma samples from enrolled COVID-19 patients and 40 citrated plasma samples from healthy volunteers. Volunteer samples utilized in this study were collected under a parent study, as previously described, and samples were randomly selected to include in this cohort [15]. Volunteers were recruited, prior to the pandemic, from the local community, screened for major medical conditions, and written informed consent was obtained prior to sample

collection. The electronic medical record was reviewed to obtain information on patient demographics and clinical course.

Sample Collection, Processing, and Storage:

Whole blood was collected by venipuncture or via existing indwelling catheters into 4.5 mL citrated Vacutainer tubes (0.105M buffered sodium citrate, 3.2% Becton Dickinson, Plymouth, UK) and processed (within 1 hour of collection) to platelet-poor plasma by double centrifugation (3000g, 15 minutes), and stored in multiple aliquots at -80°C until analysis.

Sex Hormone Level Measurements:

Estradiol levels were measured in citrated plasma samples from COVID-19 patients and volunteers by ELISA assay for 17β-Estradiol (ABCAM Kit AB108667, ABCAM, Waltham, MA) per the manufacturer's instructions. DHT levels were measured by ELISA assay for DHT (DRG international, Springfield, NJ), per the manufacturer's instructions.

Cytokine Level Measurements:

IL-6 and IFN-γ levels were measured using the Invitrogen ProcartaPlex Luminex ELISA assay (Invitrogen™ EPX01010420901, Fischer scientific, Waltham, MA), per the manufacturer's instruction's using separate antibodies for each cytokine. Cytokines were multiplexed and analyzed using the MagPlex Microsphere technology on the Luminex 200 Instrument (Luminex Corporation, Austin, TX).

Statistical Analysis:

Data analysis was performed using SAS Version 9.4 (Cary, NC). The Wilcoxon rank sum test was used to detect differences in values between COVID-19 patients and healthy volunteers, as well as between different subgroups of COVID-19 patients. Chi-square or Fisher's exact test was performed to detect differences in categorical variables between groups. Results for continuous variables are presented as median and quartiles [Q1, Q3], unless otherwise specified. A p-value of <.05 was considered statistically significant.

Results

Baseline Demographics

A total of 101 patients with COVID-19 and 40 healthy volunteers were analyzed in this study. Baseline demographic characteristics of all subjects are described in **Table 1**. Notably, the COVID-19 patients were older and had greater BMI than volunteers. COVID-19 and volunteer groups had an approximately even sex distribution, and there was no significant difference in the proportion of post-menopausal females, or the number of female patients on hormonal supplementation (i.e., hormonal birth control or hormone replacement therapy) between the two groups.

COVID-19 Patients: Clinical Characteristics of Males vs. Females

Clinical characteristics of the COVID-19 patients are described in **Table 2.** There were no significant differences in the proportion of male vs. female patients requiring hospital or intensive care unit (ICU) admission. A greater number of male patients required supplemental oxygen than female patients. Male and female patients had comparable baseline levels hypertension, diabetes, and chronic kidney disease, but a greater proportion of female patients had chronic obstructive pulmonary disease (COPD) or asthma at baseline. There was no significant difference in the number of symptomatic days prior to patient presentation and sample collection between males and females. Information on which variant of COVID-19 each patient was infected with was not available in the electronic medical record. The majority of patients in this cohort were unvaccinated, with only six patients (5.9%) being partially vaccinated at the time of sample collection, with one dose of an mRNA-based vaccine (4 Pfizer Comirnaty, 1 Moderna Spikevax) and one patient being fully vaccinated with one dose of the Johnson & Johnson Jcovden viral vector vaccine.

Sex Hormone Levels: COVID-19 Patients and Healthy Volunteers

Levels of 17β-Estradiol and DHT in COVID-19 patients and volunteers are described in **Table 3.** Compared to female volunteers, female patients with COVID-19 had significantly lower levels of estradiol, as well as a lower Estradiol to DHT ratio, particularly among the pre-menopausal COVID-19 patients (**Table 3**). In an effect size model, menopausal status and COVID-19 were shown to have similar effects on estradiol levels with no significant interaction between the two factors (**Supplemental Table 1**).

Similarly, male patients with COVID-19 had significantly lower levels of DHT than male volunteers. COVID-19 patients who were admitted to the hospital were older than those who were discharged from the ED, but we did not find any significant differences in 17β-Estradiol or DHT levels between COVID-19 patients who required hospital admission and those who did not.

Cytokine Levels: COVID-19 Patients and Healthy Volunteers

IL-6 and IFN-γ levels were measured in all COVID-19 patients and 33 of the 40 healthy volunteers. Cytokines were not performed on seven volunteers due to limited sample availability. Among the COVID-19 group, 11 (10.9%) patients had IL-6 levels above the lower limit of detection (LLD, 11.8 pg/mL) for the assay, which is considered a positive result per the manufacturer's instructions. Overall median IL-6 level for those with positive levels was 29.6 pg/mL [18.4, 75.9], with no significant difference in levels between males and females (52.7 pg/mL [25.3, 95.3] vs. 18.4 pg/mL [16.2, 43.3], *p=.100*). No volunteers had a positive IL-6 level. Only one (1.0%) of the COVID-19 patients had a positive IFN-γ level of 14.8 pg/mL (assay LLD of 14.2 pg/mL), and that patient also had a positive IL-6 level. None of the volunteers had positive IFN-γ levels.

Patients with positive cytokine levels were older and more likely to be admitted than those with normal cytokine levels (**Table 4**). In fact, 10/11 (90.9%) of patients with positive cytokines required hospital admission, compared with 44/90 (48.9%) of those with normal cytokines (*p=.008*, **Table 4**). Furthermore, COVID-19 patients with high cytokines had a significantly longer length of hospital stay than COVID-19 patients with

normal cytokines (*p=.003*, **Table 4**). There were no significant differences in 17β-Estradiol levels, DHT levels, or Estradiol to DHT ratio between the patients with high cytokines and those with normal cytokines, even when stratified by sex and menopausal status. Of note, there were no pre-menopausal females with high cytokine levels.

Logistic Regression Models of COVID Status as a Function of Sex Hormone Levels

Multivariable logistic regression models stratified by sex were performed to determine the association between sex hormone levels and COVID-19 status (**Table 5**). For female patients, estradiol levels were negatively correlated to COVID-19 status while DHT levels had the opposite effect (positive correlation to COVID-19 though not reaching statistical significance) (**Table 5a**). In a second model, estradiol to DHT ratio was negatively associated with COVID-19 status in female subjects (**Table 5b**). Both multivariable models revealed no significant association of age with COVID-19 status. For male patients, age was positively correlated to COVID-19 status while estradiol and DHT levels did not have a significant association with COVID-19 status in a multivariable model (**Table 5c**).

Discussion:

Baseline characteristics, including sex, impact an individual's response to COVID-19 infection. Though our cohort had a low number of severely ill patients, males in this pilot study had some evidence of more severe disease, with greater need for

supplemental oxygen than female patients. We also found that males and females with COVID-19 had different patterns of circulating sex hormones, with female COVID-19 patients having lower levels of 17β-Estradiol than female volunteers, and male COVID-19 patients having lower levels of DHT than male volunteers. In female patients, estradiol levels and estradiol to DHT ratio are predictive of COVID status while age, not sex hormones, is a predictor of COVID status in males. However, we were unable to find any correlations between sex hormone levels and pro-inflammatory or anti-inflammatory cytokine levels to explain the clinical dimorphisms between sexes.

Recently, Dhindsa et al. demonstrated an inverse association between testosterone levels and concentrations of IL-6 and IFN-γ in male COVID-19 patients[13]. It is likely that we were unable to replicate these results due to the lack of severely ill patients in our cohort. It should also be noted that our patient cohort was heterogenous in terms of the number of days from COVID-19 symptom onset to sample collection. Thus, it remains unknown if there are any progressive alterations in the levels of hormones and cytokines after the initiation of infection until the time of blood sampling in these patients.

Our findings highlight the potential protective role of estrogen in COVID-19 infection, with females having lower levels of supplemental oxygen requirement, and no pre-menopausal females having high cytokine levels. Similar trends have been demonstrated by others, with females having better clinical outcomes than males, and hormonal supplementation appearing to have a protective effect in both younger and older female patients[4,9]. This may be due in part to estrogen downregulating pro-inflammatory cytokines, including IL-6, an important mediator of the cytokine storm seen after infection[8,16]. Although we did not find any significant differences in 17β-Estradiol or

DHT levels between patients with high cytokines vs. low cytokines, all (5/5) of the female patients with high cytokines, specifically IL-6, were post-menopausal. As no pre-menopausal females fell into the high cytokine category, this further suggests a role of estrogen in downregulating the cytokine storm and potentially mediating some anti-inflammatory effects in COVID-19. The significance and mechanisms for male COVID-19 patients having decreased levels of DHT are less clear. It may be that lower levels of testosterone are predisposing some male patients to a greater inflammatory response, making them more susceptible to cytokine mediated effects such as pulmonary tissue injury[12,17]. Alternatively, this may be a transient hypogonadal response to COVID-19 infection either due to a direct effect of the virus on ACE-2 receptors in the testicular epithelium, or due to a centrally mediated response to physiologic stress[14]. While DHT levels were not predictive of COVID status in our multivariable models, the current literature suggests that males with known low testosterone levels should be considered at increased risk for COVID-19 infection and potentially worse outcomes if infected[17]. There were also no differences in sex hormone levels for admitted patients vs. those discharged from the ED, even when stratified by sex. This suggests that sex and presence/absence of disease, rather than disease severity, are the primary determinants of 17β-Estradiol and DHT levels.

Cytokines IL-6 and IFN-γ are both known to be mediated by estradiol levels and are known to play a role in COVID-19[8,16]. We found a small proportion (11/101) of the COVID-19 patients to have elevated cytokine levels, all of whom had a positive IL-6 reading and only one individual having both a positive IL-6 and IFN-γ result. Despite these small numbers, the patients with high cytokines were expectedly significantly older with

>90% requiring hospital admission for COVID-19 and had longer hospital length of stay than those with normal cytokines. We did not find any significant differences in sex hormone levels between the high and normal cytokine groups, as we had originally hypothesized. This may be again due to the small number of critically ill patients in this cohort.

There are several limitations to this pilot study. This study was designed to collect samples from COVID-19 patients using a single blood draw at the time of ED presentation. Therefore, we captured a heterogenous group of patients at different phases of infection and convalescence. We are thus unable to make any conclusions about time course of sex hormone and cytokine levels in COVID-19. Additionally, as we do not have baseline sex hormone levels for each patient, we are unable to discern whether the patterns we are seeing reflect the patients' baseline hormonal status, are a result of the infection, or a combination thereof. In female patients, 17β-Estradiol levels can certainly be influenced by phase in the menstrual cycle, on which we have limited information [11]. A comparable portion of female patients in the COVID-19 and healthy volunteer groups were on oral contraceptives or hormone replacement therapy at the time of sample collection, but we cannot theorize as to how these therapies may have influenced our results as we are unable to control for hormonal treatment between the groups. Given the large percentage of females in the general population who are on such medications, it was important to include them, in order to increase this study's generalizability[18]. We also acknowledge that there is a relatively small number of critically ill patients in this cohort, and that this may be limiting our ability to detect differences in sex hormone and cytokine levels. With a more severely ill group of patients, our results

may have varied, as has been shown in other work [13]. Finally, we elected to use citrated plasma samples in this study. Though our results were within acceptable ranges for citrated plasma samples, we acknowledge that sex hormone levels may vary based on collection and processing of samples.

Conclusion:

Sex hormone levels differ between COVID-19 patients and healthy volunteers, with discrete patterns between males and females. As the COVID-19 pandemic wears on, it is important to understand how the prevailing hormonal milieu in various adverse circulatory conditions and disease states may impact infection and disease severity. In this pilot study, we have demonstrated that 17β-Estradiol may have some protective effect against COVID-19 infection. The precise role of DHT in the response to COVID-19 remains unclear, but males with low testosterone levels may be at greater risk with this infection, making it an important clinical risk factor to consider. Cytokine release does not fully explain the mechanisms by which sex hormones impact COVID-19 disease course. Ongoing work will be needed to further delineate the mechanistic reasons for the sex differences that have been consistently observed in this disease process.

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References:

1. C. Mussini, A. Cozzi-Lepri, M. Menozzi, et al. Better prognosis in females with severe COVID-19 pneumonia: possible role of inflammation as potential mediator. *Clin Microbiol Infect. 2021;*27(8):1137-1144. DOI: 10.1016/j.cmi.2020.12.010

2. N. Gadi, S. C. Wu, A. P. Spihlman and V. R. Moulton. What's Sex Got to Do With COVID-19? Gender-Based Differences in the Host Immune Response to Coronaviruses. *Front Immunol. 2020;*11:2147. DOI: 10.3389/fimmu.2020.02147

3. N. Khan. Possible protective role of 17beta-estradiol against COVID-19. *J Allergy Infect Dis.* 2020;1(2):38-48. DOI: 10.46439/allergy.1.010

4. S. Wray and S. Arrowsmith. The Physiological Mechanisms of the Sex-Based Difference in Outcomes of COVID19 Infection. *Front Physiol. 2021;*12:627260. DOI: 10.3389/fphys.2021.627260

5. H. Song, B. Seddighzadeh, M. R. Cooperberg and F. W. Huang. Expression of ACE2, the SARS-CoV-2 receptor, and TMPRSS2 in prostate epithelial cells. *bioRxiv*. 2020. DOI: 10.1101/2020.04.24.056259

6. K. E. Stelzig, F. Canepa-Escaro, M. Schiliro, S. Berdnikovs, Y. S. Prakash and S. E. Chiarella. Estrogen regulates the expression of SARS-CoV-2 receptor ACE2 in differentiated airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol.* 2020;318(6):L1280-L1281. DOI: 10.1152/ajplung.00153.2020

7. T. Takahashi, M. K. Ellingson, P. Wong, et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature.* 2020;588(7837):315-320. DOI: 10.1038/s41586-020-2700-3

8. F. Mauvais-Jarvis, S. L. Klein and E. R. Levin. Estradiol, Progesterone, Immunomodulation, and COVID-19 Outcomes. *Endocrinology.* 2020;161(9). DOI: 10.1210/endocr/bqaa127

9. C. Cattrini, M. Bersanelli, M. M. Latocca, B. Conte, G. Vallome and F. Boccardo. Sex Hormones and Hormone Therapy during COVID-19 Pandemic: Implications for Patients with Cancer. *Cancers (Basel)* 2020;12(8):2325. DOI: 10.3390/cancers12082325

10. Y. Leyfman, T. K. Erick, S. S. Reddy, et al. Potential Immunotherapeutic Targets for Hypoxia Due to COVI-Flu. *Shock.* 2020;54(4):438-450. DOI: 10.1097/SHK.0000000000001627

11. T. Ding, J. Zhang, T. Wang, et al. Potential Influence of Menstrual Status and Sex Hormones on Female Severe Acute Respiratory Syndrome Coronavirus 2 Infection: A Cross-sectional Multicenter Study in Wuhan, China. *Clin Infect Dis.* 2021;72(9):e240-e248. DOI: 10.1093/cid/ciaa1022

12. V. A. Giagulli, E. Guastamacchia, T. Magrone, et al. Worse progression of COVID-19 in men: Is testosterone a key factor? *Andrology.* 2021;9(1):53-64. DOI: 10.1111/andr.12836

13. S. Dhindsa, N. Zhang, M. J. McPhaul, et al. Association of Circulating Sex Hormones With Inflammation and Disease Severity in Patients With COVID-19. *JAMA Netw Open.* 2021;4(5):e2111398. DOI: 10.1001/jamanetworkopen.2021.11398

14. M. Camici, P. Zuppi, P. Lorenzini, et al. Role of testosterone in SARS-CoV-2 infection: A key pathogenic factor and a biomarker for severe pneumonia. *Int J Infect Dis.* 2021;108:244-251. DOI: 10.1016/j.ijid.2021.05.042

15. M. S. Park, G. M. Spears, K. R. Bailey, A. Xue, M. J. Ferrara, A. Headlee, S. K. Dhillon, D. H. Jenkins, S. P. Zietlow, W. S. Harmsen, A. A. Ashrani and J. A. Heit: Thrombin generation profiles as predictors of symptomatic venous thromboembolism after trauma: A prospective cohort study. *J Trauma Acute Care Surg* 83(3):381-387, 2017.

16. T. Haitao, J. V. Vermunt, J. Abeykoon, et al. COVID-19 and Sex Differences: Mechanisms and Biomarkers. *Mayo Clin Proc.* 2020;95(10):2189-2203. DOI: 10.1016/j.mayocp.2020.07.024

17. G. Rastrelli, V. Di Stasi, F. Inglese, et al. Low testosterone levels predict clinical adverse outcomes in SARS-CoV-2 pneumonia patients. *Andrology.* 2021;9(1):88-98. DOI: 10.1111/andr.12821

18. M. L. Kavanaugh and J. Jerman: Contraceptive method use in the United States: trends and characteristics between 2008, 2012 and 2014. *Contraception* 97(1):14-21, 2018.

Table 1: Baseline Demographic Information for COVID-19 Patients and Volunteers

	COVID-19 (n = 101)	**Volunteers** (n = 40)	**p-value**
Age (Years)	49 [37, 66]	41 [30, 52]	**.003**
Sex (Male/Female)	51 Male 50 Female	20 Male 20 Female	.958
BMI (kg/m^2)	30.8 [26.7, 37.5]	28.7 [23.3, 31.3]	**.027**
Post-Menopausal Females (%)*	25 (50%)	9 (45%)	.705
Females on Hormonal Supplementation (%)*	11 (22%)	5 (25%)	.763

Table 1 Footnotes:

[a] Results are presented as median with quartiles [Q1, Q3], or raw counts with percentages of total patients except where specified.

[b] Wilcoxon rank sum (Mann Whitney), Chi-Square, or Fisher's exact testing for differences between groups. $p < .05$ considered statistically significant.

*Percent of female patients only.

Table 2: Clinical Characteristics of Female vs. Male Covid-19 Patients

	COVID-19 Patients Females (n = 50)	**COVID-19 Patients Males (n = 51)**	**p-value**
Age (years)	49 [34, 67]	51 [37, 66]	.804
Admitted to Hospital	24 (48.0%)	30 (58.8%)	.276
% Post-Menopausal	16 (66.7%)*	-	
ICU Admission (% of admitted)	1 (4.2%)	3 (10.0%)	.620
Supplemental oxygen requirement (%)	10 (20.0%)	23 (45.1%)	**.007**
Mechanical Ventilation (%)	0 (0%)	1 (2.0%)	>.999
90-day VTE (%)	3 (6.0%)	2 (3.9%)	.678
90-day Mortality (%)	1 (2.0%)	0 (0%)	.495
Symptomatic days prior to presentation	Mean: 7.9 (+/- 5.1) Median: 7 [4, 11]	Mean: 7.6 (+/- 4.6) Median: 8 [4, 10]	.938

Hospital Length of stay (days)- admitted patients only	Median: 3 [2, 5]	Median: 4 [3, 5]	.163
Current Smoker (%)	2 (4.0%)	0 (0%)	.243
Hypertension (%)	17 (34.0%)	18 (35.3%)	.891
Diabetes (%)	10 (20.0%)	9 (17.6%)	.762
Chronic Kidney Disease (%)	5 (10.0%)	5 (9.8%)	>.999
COPD or Asthma (%)	18 (36.0%)	7 (13.7%)	**.010**

Table 2 Footnotes:

[a] Results are presented as median with quartiles [Q1, Q3], or raw counts with percentages of total patients, unless otherwise specified.

[b] COPD = chronic obstructive pulmonary disease, ICU = intensive care unit, VTE = venous thromboembolism.

[c] Wilcoxon rank sum (Mann Whitney), Chi-Square, or Fisher's exact testing for differences between groups.

[d] $p < .05$ considered statistically significant.

*Percent of admitted female patients only.

Table 3: 17β-Estradiol and DHT Levels in COVID-19 Patients vs. Volunteers

	Volunteers **20 Male** **20 Female**	**COVID-19** **51 Male** **50 Female**	**p-value**
17β-Estradiol Levels			
(pg/mL)	18.1 [13.5, 27.8]	19.1 [14.5, 21.6]	.798
Males only	41.4 [15.5, 111.0]	18.5 [10.5, 32.3]	**.025**
Females only	47.8 [15.0, 120.8]	22.5 [18.4, 57.6]	.175
Pre-menopausal	26.0 [15.9, 101.3]	11.8 [8.2, 18.9]	.070
Post-menopausal			
DHT Levels (pg/mL)			
Males only	457.2 [368.7,	302.8 [249.9,	**.005**
Females only	844.3]	470.8]	.648
Pre-menopausal	224.5 [129.9,	232.0 [123.4,	.279
Post-menopausal	385.5]	407.9]	.644
	244.0 [112.3,	303.3 [214.3,	
	389.7]	557.2]	
	204.4 [131.6,	147.7 [104.3,	
	277.3]	345.3]	
Estradiol: DHT Ratio			
Males only	0.037 [0.023,	0.056 [0.040,	.118
Females only	0.072]	0.078]	**.015**

Pre-menopausal Post-menopausal	0.207 [0.104, 0.538] 0.225 [0.104, 0.538] 0.201 [0.084, 0.533]	0.073 [0.052, 0.159] 0.073 [0.055, 0.159] 0.073 [0.046, 0.133]	**.035** .166

Table 3 Footnotes:

[a] Results are presented as median with quartiles [Q1, Q3].

[b] Wilcoxon rank sum (Mann Whitney), Chi-Square, or Fisher's exact testing for differences between groups.

[c] $p < .05$ considered statistically significant.

Table 4: Clinical Characteristics of Cytokine High COVID-19 Patients vs. Normal Cytokine COVID-19 Patients:

	COVID-19 Patients with High Cytokines (n = 11)	**COVID-19 Patients with Normal Cytokines (n = 90)**	**p-value**
Age (years)	66 [64, 80]	47 [36, 64]	**.002**
Sex (M/F)	6 M (54.5%) 5 F (45.5%)	45 M (50%) 45 F (50%)	.776
% Post-menopausal Females	5 (100% of F)	20 (44.4% of F)	.050
BMI (kg/m^2)	28.5 [26.1, 34.0]	31.3 [27.0, 37.8]	.366
Symptomatic Days Prior to Presentation	Mean: 8.2 (+/- 5.4) Median: 7 [5, 11]	Mean: 7.7 (+/- 4.8) Median: 7 [4,10]	.814
90-day VTE (%)	2 (18.2%)	3 (3.3%)	.090
Admitted (%)	10 (90.9%)	44 (48.9%)	**.008**
ICU Requirement (% of admitted)	2 (20.0%)	2 (4.5%)	.152

Supplemental Oxygen Requirement (%)	6 (54.5%)	27 (30.0%)	.170
Mechanical Ventilation (%)	0 (0%)	1 (1.1%)	>.999
Length of Stay (days)- admitted patients only	Median: 8 [5, 12]	Median: 3 [2, 4]	**.003**

Table 4 Footnotes:

[a] All 11 of the COVID-19 patients with high cytokines had plasma IL-6 levels above the lower limit of detection (LLD) for the assay, and one of those patients also had a plasma IFN- γ level above the assay LLD.

[b] Results are presented as median with quartiles [Q1, Q3] or raw counts with percentages of total patients.

[c] Wilcoxon rank sum (Mann Whitney), Chi-Square, or Fisher's exact test for differences between groups.

[d] p-value < .05 considered statistically significant.

[e] BMI = body mass index, F = female, ICU = intensive care unit, M = male, VTE = venous thromboembolism.

Table 5a: Logistic Regression Model for COVID status (COVID vs. Volunteer) for Female Subjects

	Estimate	Standard Error	Wald Chi-Square	p-value
Intercept	-2.253	2.879	0.612	.4343
Age (yrs)	0.029	0.021	1.900	.168
Log (Estradiol)	-0.670	0.309	4.707	**.030**
Log (DHT + 20)	0.737	0.445	2.736	.098

Table 5b: Logistic Regression Model for COVID status (COVID vs. Volunteer) for Female Subjects

	Estimate	Standard Error	Wald Chi-Square	p-value
Intercept	-1.843	1.072	2.956	.086
Age (yrs)	0.028	0.018	2.334	.127
Log (Estradiol/(DHT + 20))	-0.685	0.293	5.467	**.019**

Table 5c: Logistic Regression Model for COVID status (COVID vs. Volunteer) for Female Subjects

	Estimate	Standard Error	Wald Chi-Square	p-value
Intercept	5.811	4.143	1.967	.161
Age (yrs)	0.043	0.022	3.982	**.046**
Log (Estradiol)	-0.362	0.573	0.400	.527
Log (DHT + 20)	-0.934	0.647	2.083	.149

Table 5 Footnotes:

[a] Logistic regression models for COVID status (COVID vs. Volunteer) as outcome variable, stratified by sex ((a) and (b) are models for female subjects while (c) is model for male subjects).

[b] DHT = 5α-dihydrotestosterone, Estradiol = 17β-Estradiol.

[c] Values log transformed to normal distribution.

[d] $p < .05$ considered significant.

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