Sex hormone dysregulations are associated with disease severity in critically ill male COVID-19 patients - a retrospective analysis

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Abstract

BACKGROUND

Male sex was repeatedly identified as a risk factor for death and intensive care admission. However, it is yet unclear whether sex hormones are associated with disease severity in COVID-19 patients. We sought to characterize sex differences in hormone levels and cytokine responses in critically ill COVID-19 patients.

METHODS

We performed a retrospective cohort study of critically ill COVID-19 patients. Males and females were compared. Multivariate regression was performed to assess the association between sex hormones, cytokine responses and the requirement for extracorporeal membrane oxygenation (ECMO) treatment.

RESULTS

We analyzed sex hormone levels (estradiol and testosterone) of \( n = 181 \) male and female individuals. These consisted of \( n = 50 \) critically ill COVID-19 patients (\( n = 39 \) males, \( n = 11 \) females), \( n = 42 \) critically ill non-COVID-19 patients (\( n = 27 \) males, \( n = 15 \) females), \( n = 39 \) non-COVID-19 patients with coronary heart diseases (CHD) (\( n = 25 \) males, \( n = 14 \) females) and \( n = 50 \) healthy individuals (\( n = 30 \) males, \( n = 20 \) females). We detected highest estradiol levels in critically ill male COVID-19 patients compared to non-COVID-19 patients (\( p = 0.0123 \)), patients with CHD (\( p = 0.0002 \)) or healthy individuals (\( p = 0.0007 \)). Lowest testosterone levels were detected in critically ill male COVID-19 patients compared to non-COVID-19 patients (\( p = 0.0094 \)), patients with CHD (\( p = 0.0068 \)) or healthy individuals (\( p < 0.0001 \)). No statistically significant differences in sex hormone levels were detected in critically ill female COVID-19 patients, albeit similar trends in estradiol levels were observed. In critically ill male COVID-19 patients, cytokine and chemokine responses (IFN-\( \gamma \), \( p = 0.0301 \); IL-1RA, \( p = 0.0160 \); IL-6, \( p = 0.0145 \); MCP-1, \( p = 0.0052 \); MIP-1\( \alpha \), \( p = 0.0134 \)) were significantly elevated in those with higher Sequential Organ Failure Assessment (SOFA) scores (8-11). Linear regression analysis revealed that herein IFN-\( \gamma \) levels correlate with estradiol levels in male and female COVID-19 patients (\( R^2 = 0.216, = 0.0009 \)). Male COVID-19 patients with elevated estradiol levels were more likely to receive ECMO treatment in the course of their ICU stay (\( p = 0.0009 \)).

CONCLUSIONS

We identified high estradiol and low testosterone levels as a hallmark of critically ill male COVID-19 patients. Elevated estradiol levels in critically ill male COVID-19 patients were positively associated with IFN-\( \gamma \) levels and increased risk for ECMO requirement.

Background

The current SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus Type 2) pandemic continues taking its toll on human health with currently 3.12 million lives lost worldwide (as of 27th April 2021).
SARS-CoV-2 was first reported in humans in December 2019 in Wuhan, China [1]. On February 11th 2020, the World Health Organization (WHO) named the disease caused by SARS-CoV-2, COVID-19 (coronavirus disease 2019). One month later, on 11th March 2020, the WHO declared COVID-19 as a pandemic. The clinical spectrum of SARS-CoV-2 infection is broad, ranging from mild upper respiratory illnesses to severe primary pneumonia with respiratory failure, multi-organ failure and death [2]. Retrospective cohort studies revealed risk factors associated with disease severity and death. A study from Wuhan, China, which enrolled 191 inpatients on hospital admission since its first occurrence in China in December 2019, reported that older age and comorbidities, such as hypertension, diabetes and coronary heart diseases being among the top three, present poor prognostic markers at an early stage [2]. Another study from the UK linked 10,926 COVID-19-related deaths pseudonymously to primary care records of 17 million individuals, identifying being male, older age, diabetes, asthma, obesity as well as chronic heart diseases among the comorbidities associated with COVID-19 related death [3, 4].

Thus, there is increasing evidence that being male constitutes a major risk factor associated with SARS-CoV-2 fatality. However, the underlying factors of sex disparity observed in COVID-19 remain unclear yet. We have recently shown using the golden hamster model, that SARS-CoV-2 infection attacks the reproductive organs and causes massive dysregulation of sex hormones in infected male and female animals [5]. In the young and lean golden hamsters without comorbidities, the males had reduced plasma testosterone levels combined with elevated plasma estradiol levels, unlike females who showed reduced plasma estradiol levels upon SARS-CoV-2 infection [5]. Thus, we wanted to study whether the observed dysregulation in sex hormones upon SARS-CoV-2 infection is also present in COVID-19 patients and poses a risk factor for disease severity.

Therefore, we herein compared sex hormone levels and cytokine responses in critically ill male and female COVID-19 patients to critically ill non-COVID-19 patients, patients with coronary heart diseases as one of the top comorbidities present in COVID-19 patients and healthy individuals. Analysis within the critically ill cohort was stratified by sex, age and Sequential Organ Failure Assessment (SOFA) scores.

**Methods**

**Study design and participants**

This retrospective matched cohort study includes the first 50 laboratory-confirmed (qRT-PCR) SARS-CoV-2 patients who were admitted to the Department of Intensive Care Medicine at the University Medical Center Hamburg-Eppendorf from March 9th to April 29th, 2020. A total of 39 male and 11 female patients were included. For controlled analysis, we included patients with coronary heart diseases as one of the most prevalent comorbidities in our COVID-19 cohort (Institute for Pathology and Neuropathology, University Hospital Tübingen; 25 males, 14 females) and healthy donors (Institute for Transfusion Medicine, University Medical Center Hamburg-Eppendorf; 30 males, 20 females). Additionally, we included 42 patients with laboratory-confirmed negative SARS-CoV-2 PCR, who were admitted to the ICU (Intensive
Care Unit). All cohorts were sex and age matched, the ICU control group were also SOFA (Sequential Organ Failure Assessment) Score matched (supplementary figure s1).

Setting

The University Medical Center Hamburg-Eppendorf is a tertiary care hospital with 1,738 hospital beds. The Department of Intensive Care Medicine includes 12 multidisciplinary ICUs with a total of 140 ICU beds, regularly. Since the first wave of SARS-CoV-2 pandemic in March 2020, a minimum of two and up to four intensive care units treat up to 40 patients, simultaneously, suffering from COVID-19 or suspected COVID-19. The in-house crisis management team has developed a step-by-step plan for this purpose. Normally, the Department of Intensive Care Medicine has units for the care of all medical and surgical specialties, including specialized units for the treatment of acute respiratory distress syndrome (ARDS), which routinely use extracorporeal membrane oxygenation (ECMO).

Data collection

The following demographic and clinical variables were collected retrospectively for the COVID-19 and non-COVID-19 cohort from the electronic patient data management system (PDMS) (ICM, Dräger, Lübeck, Germany): age, sex, body mass index, comorbidities, admission type and diagnosis, Acute Physiology and Chronic Health Score (APACHE II) and Simplified Acute Physiology Score II (SAPS II) on admission, Sequential Organ Failure Assessment Score (SOFA) within the first 24 hours, classification of Acute Respiratory Distress Syndrome (ARDS) using the Berlin definition and the need for mechanical ventilation and extracorporeal membrane oxygenation. Additionally, we recorded antiviral treatment, supportive and experimental COVID-19 therapies for the COVID-19 cohort. Furthermore, we followed the course of the patients and recorded discharge or death.

Hormone quantification

A panel of 13 hormones was measured in plasma samples (total testosterone, free testosterone, dihydrotestosterone, androstenedione, 17-β-estradiol (E2), estrone, sex hormone-binding globulin, thyroid-stimulating hormone, free triiodothyronine (T3), free thyroxine (T4), luteinizing hormone, follicle-stimulating hormone and cortisol by an external laboratory accredited for measurements of human samples (Labor Lademannbogen, Hamburg, Germany). Cortisol, TSH, T4, LH, FSH, TT, E2 and SHBG were analyzed by electro-chemiluminescence immunoassay (ECLIA). Free TT was analyzed by enzyme-linked immunosorbent assay and DHY-TT was measured by liquid chromatography–mass spectrometry (LC-MS/MS). Estrone levels were measured by a radioimmunoassay (RIA).

Cytokine and chemokine measurement

A panel of 27 cytokines and chemokines (eotaxin, fibroblast growth factor (FGF), granulocyte-colony stimulating factor (G-CSF), interferon-γ (IFN-γ), interferon γ-induced protein (IP-10), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-13 (IL-13), interleukin-15 (IL-15), interleukin-17 (IL-17), interleukin-1β (IL-1β), interleukin 1 receptor antagonist (IL-1RA), monocyte
chemoattractant protein-1 (MCP-1), platelet-derived growth factor-BB (PDGF-BB), regulated upon activation, normal T-cell expressed and presumably secreted chemokine (RANTES), tumor necrosis factor-α (TNF-α), and vascular endothelial growth factor (VEGF)) was measured in plasma samples of COVID-19 patients. Cytokine and chemokine levels were measured using a Bio-Plex Pro™ multiplex assay (#M500KCAF0Y, Bio-Rad, Feldkirchen, Germany) according to the manufacturer's instructions in a Bio-Plex 200 System with high-throughput fluidics (HTF; Bio-Rad, Feldkirchen, Germany).

**Statistical analysis**

Continuous variables are expressed as median with 1st to 3rd quantile. Categorical variables are given as absolute and relative numbers. The distribution of data was visually interpreted using histograms. Variables were compared between groups (COVID-19 and non-COVID-19) with the Wilcoxon–Mann–Whitney U test, Student-T test and the Fisher’s exact test as appropriate. All given p-values in the tables are of descriptive nature and not adjusted for multiple testing.

Statistical evaluation for quantitative data was performed with two-way-ANOVA including the cohort and sex as independent variable as well as its interaction. For non-normal data unpaired Mann-Whitney or Kruskal-Wallis test ignoring any multiple comparisons were used. Statistical significance was defined as \( p < 0.05 \) \( (*) \ p < 0.05, \ (**) \ p < 0.01 \) and \( *** \ p < 0.001 \).

All statistical evaluations mentioned above were performed with SAS®, version 9.4 TS level 1M5 (SAS Institute Inc., Cary, NC, United States) and IBM® SPSS® Statistics 27. Graphical representation of the data was performed via GraphPad Prism 8 v. 8.4.2 (GraphPad Software, Inc.).

**Results**

A total of \( n = 274 \) SARS-CoV-2 PCR-positive patients were admitted to the Department of Intensive Care Medicine, at the University Medical Center Hamburg-Eppendorf within the period 9th March to 18th April 2021. Of these, \( n = 144 \) (53%) were patients secondarily admitted from referring intensive care units from all over Northern Germany. In summary, \( n = 181 \) (66%) of all COVID-19 patients were male and \( n = 93 \) (34%) of all COVID-19 patients were female. A total of \( n = 95 \) (35%) patients died, of which \( n = 65 \) (68%) were male and \( n = 30 \) (32%) were female. These data are in line with large epidemiological data analysis identifying being male as a high-risk factor for COVID-19 related death [3].

Critically ill COVID-19 patients were compared to the respective control group of critically ill non-COVID-19 patients with negative SARS-CoV-2 PCR (Table 1). In the COVID-19 group, 78% of the patients were male, in the control group 64.3%. The median age was 63 (IQR: 58–73) years for COVID-19 males and 67 (IQR: 59–71) years for COVID-19 females. The ICU non-COVID-19 group was older with a median age of 67 (IQR: 58–75) years for the men and 71 (IQR: 69–82) years for the women. All COVID-19 patients admitted to the ICU presented at least one comorbidity with obesity, arterial hypertension, coronary heart diseases and diabetes mellitus type II being among the most frequent (Table 1). The body mass index was higher in the COVID-19 cohort (median, male 27.4, female 25) than in the non-COVID-19 group (median, male
25.2, female 23.5). Accordingly, the diagnosis of obesity was more frequent among COVID-19 sufferers. There were no significant differences between the two groups (COVID-19 cohort vs. male AND female non-COVID-19 group) with regard to the admission diagnoses of arterial hypertension, bronchial asthma, chronic obstructive pulmonary disease (COPD), coronary heart disease (CHD) and diabetes mellitus type II. Of the patients in the COVID-19 group, 39 (78%) were hospitalized due to COVID-19 disease, while the remaining 11 (22%) patients were hospitalized for their underlying oncological disease prior to ICU admission. The ICU non-COVID-19 group contains critically ill patients from all medical specializations. 26 (61.9%) of these patients were admitted after major surgical interventions. 32 (64%) patients meets the ARDS criteria [6] in the COVID-19 group, in the ICU non-COVID-19 group 11 (26.2%) of the patients. There were no statistical relevant differences in the need of mechanical ventilation (invasive and non-invasive) in both groups \( (p = 1.0) \). In the ICU non-COVID-19 group, 14 (33.3%) patients had a suspected bacterial infection on admission to the ICU. CRP was significantly higher in COVID-19 patients \( (p < 0.001) \). 15 (30%) of the COVID-19 patients deceased, in the non-COVID-19 control group 5 (11.9%) patients.

First, we wanted to assess whether sex hormone levels are altered within the critically ill male and female COVID-19 patients potentially providing a link to sex-dependent disease severity. However, diabetes type II \( (p = 1.0) \) and obesity \( (p = 1.0) \) showed same frequencies in male and female COVID-19 patients (supplementary Table 1). Albeit statistically not significant \( (p = 0.6) \), more coronary heart diseases patients were present in the male compared to the female COVID-19 cohort, which might reflect extended frequency of CHD in men [7]. Since obesity and type II diabetes were evenly distributed between male and female COVID-19 patients, sex hormone analysis might be shifted due to this unbalance of CHD presence. Therefore, we recruited age- and sex-matched male and female SARS-CoV-2 negative CHD patients \( (n = 39) \) as an internal control. As an additional healthy control, we recruited age-and sex-matched SARS-CoV-2 negative male and female blood donors (HC) \( (n = 50) \). Furthermore, we recruited SARS-CoV-2 PCR-negative ICU patients \( (n = 42) \) as an additional control for critical illness independently of COVID-19 (herein referred as ICU non-COVID-19).

Male, critically ill COVID-19 patients presented the highest estradiol levels detected when compared to critically ill male non-COVID-19 patients \( (p = 0.0123) \) or male patients with CHD \( (p = 0.0002) \) or healthy males \( (p = 0.0007) \) (Fig. 1a). This finding is further confirmed that estradiol levels of male critically ill COVID-19 patients were far above clinical reference values [8, 9]. No significant differences in estradiol levels were detected within the control cohorts, all being within clinical reference values. Conversely, testosterone levels were lowest in critically ill male COVID-19 patients compared to critically ill male non-COVID-19 patients \( (p = 0.0094) \) or male patients with CHD \( (p = 0.0068) \) or healthy males \( (p < 0.0001) \) (Fig. 1b). In line, testosterone levels of male critically ill COVID-19 patients were below clinical reference values, albeit not significant, some critically ill COVID-19 patients also presented testosterone levels below clinical references [8, 9]. No significant differences in testosterone levels were detected within the HC and CHD control cohorts. Female, critically ill COVID-19 patients presented a trend towards elevated estradiol levels, albeit statistically not significant (Fig. 1c). Testosterone levels were not significantly altered within the female critically ill COVID-10 patients, albeit a trend towards elevated levels was observed comparable to CHD female controls above clinical references [8, 9] (Fig. 1d).
Collectively, these findings show that male COVID-19 patients present significantly increased estradiol and reduced testosterone levels compared to non-COVID-19 males.

**Sex hormone levels in COVID-19 patients, non-COVID-19 patients, patients with coronary heart diseases and healthy individuals.** Estradiol (a,c) and testosterone (b,d) levels were measured in plasma obtained from critically ill COVID-19 patients (ICU\textsubscript{COVID−19}), critically ill non-COVID-19 patients (ICU\textsubscript{non−COVID−19}), patients with coronary heart diseases (CHD) and healthy individuals (HC). Male data sets are shown in blue color-toned columns and female data sets are shown in red color-toned columns. The laboratory assessed hormone reference ranges are indicated in grey. Percentile boxplots represent 25–75% of values, with the median value indicated by a crossline, and mean values by a plus icon. Statistical significance was assessed via One-Way-ANOVA.

To shed light on the origin of severe testosterone deficiency in male COVID-19 patients, we further analyzed related hormones (Table 2). Free testosterone levels were reduced in 66.7% of male COVID-19 patients compared to reference values. Conversely, 54.5% of female COVID-19 patients presented elevated levels of free testosterone. Thus, changes in total testosterone levels reported above correlate with levels of free bioavailable testosterone levels in the respective sex. We then measured levels of the sex hormone-binding globulin (SHBG) since the majority (98%) of total testosterone is bound to SHBG and only 2% is in its free, bioavailable form. Thus, in some cases, testosterone deficiencies might be masked by elevated SHBG levels. In 28.2% of male COVID-19 patients, SHBG levels were elevated, which might suggest masked testosterone deficiencies in some patients. Luteinizing hormone (LH) levels were elevated in 30.8% of male COVID-19 patients, while being within the normal range in all female patients. Interestingly, 7 out of the 28 male patients with low total testosterone levels presented elevated LH levels at the same time (data not shown), suggesting impairment of Leydig cell steroidogenesis in 25% of the male patients. Follicle stimulating hormone (FSH) levels were elevated in 12.8% of male patients. Elevated FSH levels in these male patients were combined with elevated LH levels. In 45.5% of female patients, FSH levels were reduced, which may indicate loss of ovarian function. This would be in line with the postmenopausal status of the 10 out of 11 COVID-19 females in our cohort. Other hormones, such as thyroid stimulating hormone (TSH) and T4 were within normal ranges in the majority of male and female patients. Cortisol levels were elevated in 56.4% of male and 81.8% of female COVID-19 patients.

These findings suggest that in 25% of the male COVID-19 patients with low total testosterone levels, testosterone deficiency is likely of testicular origin. Thus, in 75% of male patients, the origin of testosterone deficiency is likely of hypothalamic-hypopituitary origin.

Testosterone is further metabolized to dihydrotestosterone by 5-α reductase. Dihydrotestosterone also acts as an androgen and plays a key role in activating the transcription of various genes and activation of various immune cells similar to testosterone [10]. Thus, we wanted to assess whether alterations in testosterone levels detected in male COVID-19 patients are also reflected in its most potent metabolite. In male COVID-19 patients, dihydrotestosterone levels were reduced compared to HC males ($p$ < 0.0001) (supplementary Figure s2a). In line, a substantial proportion of plasma dihydrotestosterone levels in
COVID-19 males was even below the lowest reference range, confirming dihydrotestosterone deficiency in men. In contrast, dihydrotestosterone levels were comparable between female COVID-19 and HC cohorts within clinical references \( (p = 0.9568) \) \( \text{(supplementary Figure s2c)} \). To assess whether the increase in estradiol levels is attributed to a general increase in estrogens, we next measured estrone concentrations. Estrone levels in the plasma of COVID-19 males were higher compared to HC males \( (p < 0.0001) \) \( \text{(supplementary Figure s2b)} \). Similarly, estrone levels were elevated in the plasma of female COVID-19 patients unlike HC females \( (p = 0.0009) \) \( \text{(supplementary Figure s2d)} \).

Thus, male critically ill COVID-19 patients present additionally elevated estrone levels accompanied by severely reduced dihydrotestosterone levels. Female critically ill COVID-19 patients present elevated estrone levels, while dihydrotestosterone levels remain unchanged.

Next, we analyzed whether sex hormone levels correlate with an increased risk for severe disease outcome as assessed by the Sequential Organ Failure Assessment Score (SOFA) scores or the requirement for extracorporeal membrane oxygenation (ECMO) later during their ICU stay. In male critically ill COVID-19 patients, estradiol levels increased with disease severity (Fig. 2a) unlike in non-COVID-19 males (Fig. 2b) \( (p = 0.0245 \text{ and } p = 0.0273) \). In female COVID-19 patients, a trend towards higher estradiol levels with increasing SOFA scores was also observed (Fig. 2c). Male COVID-19 patients with elevated estradiol levels were more likely to be require ECMO treatment than those having estradiol levels within normal clinical references \( (p = 0.0307) \) (Fig. 2d). Within the female COVID-19 cohort only 1 patient required ECMO treatment; thus, not allowing statistical analysis (data not shown). Testosterone levels did not show statistical significant changes comparing groups with different disease severity or those requiring ECMO treatment \( \text{(supplementary Figure s3a-d)} \). This might be due to the fact that most male patients presented low testosterone levels below clinical references [8, 9].

These data show that critically ill male COVID-19 patients with elevated estradiol levels are more likely to require ECMO treatment.

**Estradiol levels in dependency of disease severity.** Estradiol levels were measured in plasma obtained from critically ill male (blue columns) and female (red columns) COVID-19 or non-COVID-19 patients are displayed in dependency of disease severity as assessed by SOFA scores (a-c). Male COVID-19 patients were subdivided into patients requiring ECMO therapy (+ ECMO) and patients not requiring ECMO therapy (-ECMO) (d). Percentile boxplots represent 25–75% of values, with the median value indicated by a crossline, and mean values by a plus icon. The laboratory assessed hormone reference ranges are indicated in grey. Values are shown as median and interquartile range. Statistical significance in males was assessed by NON-parametric tests (Kruskal-Wallis test and Dunn's test for multiple comparisons). Statistical significance in females was evaluated by unpaired, two-tailed NON-parametric Student’s t-test (Mann-Whitney test).

We then compared cytokine and chemokine patterns in male and female COVID-19 patients. Therefore, we analyzed a panel of 27 different cytokines and chemokines in the plasma of COVID-19 patients and correlated to disease severity as assessed by the Sequential Organ Failure Assessment Score (SOFA)
In male COVID-19 patients, particularly IFN-γ \( (p = 0.0301) \), IL-1RA \( (p = 0.0160) \), IL-6 \( (p = 0.0145) \), MCP-1 \( (p = 0.0052) \) and MIP-1α \( (p = 0.0134) \) levels were elevated in those with higher SOFA scores (8–11) compared to those with lower SOFA scores (2–3) (Fig. 3a-e). In female COVID-19 patients, TNF-α levels were higher in those with high SOFA scores compared to those with low SOFA scores \( (p = 0.0476) \) (Fig. 3f). Albeit statistically not significant, IFN-γ showed a trend towards elevation with increasing SOFA scores (Fig. 3g).

These findings show that cytokine and chemokine responses, particularly IFN-γ, IL-1RA, IL-6, MCP-1 and MIP-1α are generally elevated in dependency of disease severity in critically ill male and female COVID-19 patients.

**Chemokine and cytokine responses in COVID-19 patients.** Cytokine and chemokines were measured in plasma obtained from critically ill male (a-e) and female (f and g) COVID-19 patients by using a 27-plex immunoassay. Here, those with significant differences are shown. Cytokine and chemokine levels of male and female COVID-19 patients are displayed in dependency of disease severity as assessed by SOFA scores (2–3; 4–7; 8–11).

We next addressed the question whether changes in cytokine and chemokine responses in critically ill COVID-19 patients might correlate with their respective sex hormone levels given that most immune cells possess androgen and estrogen receptors [10–12]. Performing linear regression analysis between all 27 cytokine and chemokines assessed, only IFN-γ presented a significant correlation to estradiol in male and female COVID-19 patients \( (R^2 = 0.216, p = 0.0009); \) Fig. 4). Testosterone levels did not significantly correlate with changes in IFN-γ levels \( (R^2 = 0.133, p = 0.3111); \) supplementary Fig. 4).

These findings show a direct association of estradiol levels and IFN-γ induction in line with previous studies reporting on the estradiol-controlled transcription of IFN-γ due to the presence of an estrogen responsive element (ERE) in its promoter region [13–15].

**Correlation of IFN-γ levels in male and female COVID-19 patients to estradiol levels.**

Estradiol levels were measured in plasma of critically ill male and female COVID-19 patients and were plotted over the expression levels of all assessed cytokines and chemokines. Here, only IFN-γ is displayed, which showed significant correlations in regression analysis with estradiol among 27 different cytokines and chemokines assessed. Statistical significance was assessed by generalized linear regression.

**Discussion**

In this study, we show for the first time that critically ill male COVID-19 patients have higher estradiol and lower testosterone levels on the day of admission to the ICU. These changes in sex hormones are not only significantly different from healthy or pre-diseased patients, but are also higher (estriadiol) or lower (testosterone), respectively, than in critically ill patients without SARS-CoV-2, of the same disease severity. In addition, this study shows a direct association between elevated estradiol levels and disease severity
as measured by the SOFA score. The association between very high estradiol levels in men on the day of admission and a very severe COVID-19 course with subsequent need for ECMO therapy is considerable. We were able to confirm the study hypothesis that SARS-CoV-2 infection leads to dysregulation of sex hormones in humans. These changes are differently pronounced in male and female patients and provide a groundbreaking impulse to explain the sex-dependent differences in the COVID-19 course and outcome.

The small number of cases is clearly a limitation of this study. Also the monocentric design, which is responsible for the small number of cases. This is because over 50% of the patients admitted to the intensive care units of the University Hospital Hamburg-Eppendorf after the first wave, had already been treated in other intensive care unit before. A mixture of these patients would lead to a distortion of the results. The change in all hormones after longer intensive care treatment has been described several times in the literature [16–18]. However, our patient collective shows normal hormone values (TSH, Free T4 and Cortisol) at the time of admission on ICU, so that a selective attack on the sex-specific hormones and/or organs must be assumed.

First, we detected severely reduced testosterone levels in COVID-19 males compared to HC or CHD patients. Estradiol levels were not changed in male healthy controls or male CHD patients analyzed herein but were strongly elevated in COVID-19 males. Testosterone levels in male non-COVID-19 ICU patients are lower, but nowhere near as low as in SARS-CoV-2 infected individuals. A recently published study from Italy also showed transferable results. It was shown in 25 patients that higher testosterone levels are associated with a shorter SARS-CoV-2 clearance [19]. The vast majority (95%) of testosterone is produced in Leydig cells of the testes depending on stimulation by luteinizing hormone (LH). Only small amounts (5%) are produced in the adrenal glands. Low levels of testosterone may either be of testicular origin (primary hypogonadism), of hypothalamic-pituitary origin (secondary hypogonadism) or a combination of both, which is predominantly found in the aging male population as late onset hypogonadism [20, 21]. Hypogonadism with and without elevated estradiol levels was reported before in patients with cardiovascular diseases as a risk factor for increased mortality in men [22–24]. Thus, extrapolating from these reports on hypogonadism in males with cardiovascular diseases, the more severely reduced testosterone levels in the male COVID-19 cohort identified herein, further highlights the high risk for males. Furthermore, it is tempting to speculate whether an initial comorbidity-driven hit with respect to low testosterone levels (also reported for patients with obesity and type II diabetes [25, 26], all top COVID-19 comorbidities) might put males at higher risk to develop severe COVID-19.

Noteworthy, the herein identified reduced testosterone levels were likely of testicular origin in only 25% (primary hypogonadism) of all male COVID-19 cases, suggesting that 75% can likely be attributed to hypothalamic-hypopituitary origin (secondary hypogonadism). We furthermore observed that some female COVID-19 patients also presented elevated testosterone levels albeit statistically not significant. In our female COVID-19 cohort, all except one patient, were postmenopausal. However, the low female COVID-19 cohort size in our study is a potential limitation with respect to conclusions on female COVID-19 outcome. This was due to the fact that more men than women were admitted to the ICU in the time
period of recruitment further highlighting the importance of sex on critical COVID-19 outcome. Despite these limitations in the female COVID-19 cohort, others postulated an elevated COVID-19 risk for women with polycystic ovary syndrome (PCOS), a condition characterized by increased androgen levels \[27\]. This highlights the need for further investigations to understand the impact of elevated testosterone levels in women in the context of COVID-19.

Second, we found that similar to males, female COVID-19 patients also present elevated estradiol levels. Future in-depth investigations are required to understand the role of estradiol in postmenopausal COVID-19 patients. However, elevated estradiol levels seem to be a risk factor for severe COVID-19 outcome in male and female patients. Male COVID-19 patients on the other hand, additionally suffer from severely depleted testosterone and dihydrotestosterone levels, which might present an additional hit putting them at increased risk compared to females.

Third, by analyzing 27 different cytokines/chemokines and correlating their levels to testosterone or estradiol levels using regression analysis, we identified interferon-\(\gamma\) (IFN-\(\gamma\)) as a key cytokine that positively correlated with estradiol levels. Our findings in this study are in line with the estradiol-controlled transcription of IFN-\(\gamma\) since it possesses an estrogen responsive element (ERE) in its promoter region \[13–15\]. IFN-\(\gamma\) is a key activator of macrophages \[15, 28\] and macrophage activation was repeatedly reported as a hallmark of COVID-19 severity \[29\]. Macrophages contain membrane-bound as well as nuclear androgen- and estrogen-receptors. Thus, it will be of high interest to dissect the role of sex hormones and IFN-\(\gamma\) in orchestrating e.g. macrophage activation and cytokine storm in future studies (supplementary Fig. 5).

**Conclusions**

Collectively, our findings herein highlight that monitoring sex hormones pose a hallmark of severe COVID-19 outcome \[30\]. Longitudinal hormone surveillance studies might be warranted during acute and recovery phases in COVID-19 patients to predict short- and potentially long-term risks \[31–33\].

**Abbreviations**

| Abbreviation | Description                        |
|--------------|------------------------------------|
| APACHE II    | Acute Physiology and Chronic Health Score |
| ARDS         | Acute Respiratory Distress Syndrome |
| CHD          | Coronary heart diseases             |
| COVID-19     | Coronavirus disease 2019            |
| DZIF         | German Center for Infection Research |
| E2           | 17-b-estradiol                      |
Declarations

Ethical Approval and Consent to participate

The study with laboratory-confirmed COVID-19 and non-COVID-19 ICU patients was reviewed and approved by the ethics committee at the Hamburg State Chamber of Physicians (registration no.: WF-053/20 and WF-073/21). The need for an informed consent for the ICU cohort, healthy blood donors (HC) and patients with coronary heart diseases (CHD) was waived by the ethics committee because data were retrieved anonymously and retrospectively from electronic health records.
Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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The study funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Author contributions

GG conceived the study. SK, MS, DJ, KR, GdH and AN were responsible for COVID-19 patient management and recruitment. GG, SK, MS and BS (Berfin Schaumburg) designed and overviewed the study design. BS, HJ, MZ and SSB conducted the cytokine and chemokine assays. ZK, KK, TR and BS performed hormone assays. GG, BS, MS, AK and TB analyzed data and developed the figures. LK, BetS (Bettina Schneider) and FS conducted ANOVA models and statistical tests. JG helped with data management and the analysis. JA, AM, TB, JH, HS, KK, TR critically reviewed the manuscript and were involved in study design. GG and MS wrote the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1.
Baseline demographic and clinical characteristics, admission diagnosis and treatment-related variables in critically ill patients with COVID-19 and without COVID-19. Continuous variables are given as median (1st to 3rd quantile), categorical variables are given as n (%).
## COVID-19 Demographics

|                      | COVID-19 | NON-COVID-19 | \( p \) |
|----------------------|----------|--------------|--------|
|                      | male     | female       | male   | female |
| Age                  | 63 (58-73) | 67 (59-71) | 67 (58-75) | 71 (69-82) | 0.168 |
| Body mass index      | 27.4 (24.8-31.9) | 25 (22.8-30.7) | 25.2 (23-26.8) | 23.5 (18.5-27.4) | 0.003 |
| Demographics         |          |              |        |        |
| Comorbid conditions on admission |          |              |        |        |
| Adipositas           | 28 (71.8) | 6 (54.5) | 19 (57.6) | 5 (33.3) | 0.035 |
| Arterial hypertension| 20 (51.3) | 5 (45.5) | 19 (70.4) | 9 (60) | 0.139 |
| Bronchial Asthma     | 1 (2.6) | 0 | 3 (11.1) | 0 | 0.328 |
| COPD                 | 0 | 1 (9.1) | 3 (11.1) | 1 (6.7) | 0.174 |
| Coronary heart disease | 8 (20.5) | 1 (9.1) | 6 (22.2) | 4 (26.7) | 0.607 |
| Diabetes mellitus    | 13 (33.3) | 3 (27.3) | 5 (18.5) | 4 (26.7) | 0.347 |
| HbA1C V%             | 6.4 (5.9-7.1) | 6.2 (6-7) \( n=9 \) | 6.8 \( n=1 \) | 6.5 \( n=1 \) |        |
| Admission type       |          |              |        |        |
| medical              | 39 (100) | 11 (100) | 12 (44.4) | 4 (26.7) |        |
| surgical             | 0 | 0 | 15 (55.6) | 11 (73.3) |        |
| Admission diagnosis  |          |              |        |        |
| Pneumological        | 28 (71.8) | 9 (81.8) | 8 (29.6) | 3 (20) |        |
| Neurosurgical        | 0 | 0 | 5 (18.5) | 4 (25) |        |
| Neurological         | 0 | 0 | 6 (22.2) | 1 (6.7) |        |
| Abdominal surgery    | 0 | 0 | 3 (11.1) | 0 |        |
| Traumatological      | 0 | 0 | 2 (7.4) | 2 (13.3) |        |
| Oncological          | 11 (28.2) | 2 (18.2) | 1 (3.7) | 1 (6.7) |        |
| Oral and maxillofacial surgery | 0 | 0 | 0 | 1 (6.7) |        |
| Thoracoscopic        | 0 | 0 | 1 (3.7) | 0 |        |
| Vascular medicine    | 0 | 0 | 1 (3.7) | 0 |        |
| Endocrinological     | 0 | 0 | 0 | 1 (6.7) |        |
| Gastroenterological  | 0 | 0 | 0 | 1 (6.7) |        |
| Nephrological        | 0 | 0 | 0 | 1 (6.7) |        |
| ICU stay             |          |              |        |        |
| ARDS                 |          |              |        |        |
| None                 | 9 (23.1) | 2 (18.2) | 16 (59.3) | 14 (93.3) |        |
| Mild                 | 4 (10.3) | 1 (9.1) | 4 (14.8) | 1 (6.7) |        |
| Moderate             | 16 (41) | 3 (27.3) | 7 (25.9) | 0 |        |
| Severe               | 10 (25.6) | 5 (45.5) | 0 | 0 |        |
| Respiratory support  |          |              |        |        |
| Mechanical ventilation | 27 (69.2) | 9 (81.8) | 14 (51.6) | 8 (53.3) | 1.0 |
| Non-invasive ventilation | 0 | 0 | 6 (22.2) | 2 (13.3) |        |
| High flow oxygen therapy | 9 (23.1) | 1 (9.1) | 4 (14.8) | 0 | 0.245 |
| ECMO                 | 5 (12.8) | 2 (18.2) | 1 (3.7) | 0 |        |
| COVID-19 Treatment   |          |              |        |        |
| Lopinavir / Ritonavir | 6 (15.4) | 2 (18.2) |        |        |        |
| Hydrochloroquin      | 0 | 1 (9.1) |        |        |        |
| Adrecizumab          | 6 (15.4) | 0 |        |        |        |
| Cytosorb Filtration  | 3 (7.7) | 0 |        |        |        |
| Suspected bacterial infection on | 9 (33.3) | 5 (33.7) |        |        |        |
### Admission Scores

|                | APACHE II | SASP II | SOFA |
|----------------|-----------|---------|------|
|                | 27 (24-30)| 40 (32-47)| 7 (4-9) |
|                | 29 (25-32)| 42 (36-56)| 7 (5-10) |
|                | 23 (17-25)| 36 (30.5-48, n=21)| 4 (3-7) |
|                | 24 (21-31)| 39 (34-58, n=11)| 5 (3-7) |
| p-value        | < 0.001   | 0.505   | 0.001 |

### Laboratory parameters on admission

| Parameter                        | Admission 1 | Admission 2 | p-value |
|----------------------------------|-------------|-------------|---------|
| White blood cell count (10⁹/l)   | 9.2 (4.8-12.2) | 6.5 (0.4-12.9) | 0.009   |
| Thrombocytes (10⁹/l)             | 204 (70-293) | 143 (49-222) | 0.237   |
| Procalcitonin (µg/l)             | 0.4 (0.2-2.3) | 0.5 (0.1-1.6) | 0.119   |
| C-reactive protein (mg/dl)       | 200 (105-278) | 174 (60-293) | < 0.001 |
| Lactate (mmol/l)                 | 1.1 (0.8-1.6) | 0.7 (0.6-1)  | 0.36    |

### Hormone levels

| Parameter      | Admission 1 | Admission 2 | p-value |
|----------------|-------------|-------------|---------|
| Testosterone nmol/l | 4.2 (2.5-8.6) | 1.9 (0.8-3.1) | 0.07 (0.4-1.9) |
| Estradiol pg/ml  | 38.5 (26.9-72) | 36.7 (25.6-69.3) | 31.4 (25.9-42.3) | 25.9 (8.7-47.2) |

### Outcome

| Outcome | Recovered | Deceased |
|---------|-----------|----------|
|         | 27 (69.2) | 12 (30.8) |
|         | 8 (72.7)  | 3 (27.3)  |
|         | 24 (88.9) | 3 (11.1)  |
|         | 13 (86.7) | 2 (13.3)  |

**Note:** Wilcoxon–Mann–Whitney U or student T-test was performed for continuous variables, as appropriate and Fisher’s exact test for categorical variables. APACHE II: Acute Physiology and Chronic Health Score, COPD: Chronic obstructive pulmonary disease, COVID-19: Corona Virus Disease 2019, ECMO: Extracorporeal membrane oxygenation, SAPS II: Simplified Acute Physiology Score II, SOFA score: Sepsis-related organ failure assessment score.

**Table 2.**

Hormone levels in critically ill patients with COVID-19. Continuous variables are given as median (1st to 3rd quantile), categorical variables are given as n (%).
| Test                                      | Male (n=39) | Female (n=11) |
|-------------------------------------------|-------------|---------------|
| **Free testosterone pg/ml**              | 2.6 (1.7-3.7) | 2.3 (0.9-3.6) |
| Normal males:                             |             |               |
| 20-39 yr: 7-22.7 pg/ml                   |             |               |
| 40-60 yr: 6.3-17.8 pg/ml                 |             |               |
| ≥61 yr: 2.5-17.8 pg/ml                   |             |               |
| Low (all age groups, below reference)    | 13 (33.3)   |               |
| Normal females:                           |             |               |
| 40-60 yr: ≤2.3 pg/ml                     |             |               |
| ≥61 yr: ≤2.1 pg/ml                       |             |               |
| High (all age groups, above reference)   | 6 (54.5)    |               |
| **Sex hormone-binding globulin nMol/l**  | 31.3 (19.2-49.9) | 33.2 (22.6-58.6) |
| Normal males: 10-40 nMol/l               | 27 (69.2)   |               |
| Low: <10 nMol/l                           | 1 (2.6)     |               |
| High: 41-100 nMol/l                      | 7 (17.9)    |               |
| Very High: ≥101 nMol/l                   | 4 (10.3)    |               |
| Normal females: 26-110 nMol/l             |             |               |
| Low: <26 nMol/l                           | 3 (27.3)    |               |
| High: ≥110 nMol/l                        | 1 (9.1)     |               |
| **Luteinizing hormone mIU/ml**            | 5.5 (2.8-11.8) | 6.5 (2.6-16.4) |
| Normal males: 0-8.6 mIU/ml                | 27 (69.2)   |               |
| High: ≥8.7 mIU/ml                        | 12 (30.8)   |               |
| Normal females: <58.5 mIU/ml              |             | 11 (100)      |
| **Follicle stimulating hormone**          | 4.2 (2-9.9) | 20.1 (6.5-33.7) |
| Normal males: 1.5-12.4 mIU/ml             | 32 (82.1)   |               |
| Low: <1.5 mIU/ml                          | 2 (5.1)     |               |
| High: 12.5-25 mIU/ml                     | 5 (12.8)    |               |
| Normal females: 25.8-134.8 mIU/ml        | 6 (54.5)    |               |
| Low: 10-25.7 mIU/ml                      | 5 (45.5)    |               |
| **Thyroid stimulating hormone µU/ml**    | 0.8 (0.4-1.5) | 0.45 (0.4-1.5) |
| Normal: 0.27-4.2 µU/ml                   | 30 (76.9)   | 7 (63.6)      |
| Low: <0.27 µU/ml                          | 7 (18.0)    | 3 (27.3)      |
| High: >4.2 µU/ml                          | 2 (5.1)     | 1 (9.1)       |
| **Free T4**                               | 13.2 (11.2-14.7) | 11.1 (10.3-15.1) |
| Normal: 8-17 ng/dl                        | 38 (97.4)   | 11 (100)      |
| High: >17 ng/dl                           | 1 (2.6)     |               |
| **Cortisol µg/dl**                        | 20.6 (14.8-26.5) | 26.7 (14.8-26.5) |
Wilcoxon–Mann–Whitney U- test was performed, the difference between gender isn`t significant.

**Figures**

**Figure 1**

Sex hormone levels in COVID-19 patients, non-COVID-19 patients, patients with coronary heart diseases and healthy individuals. Estradiol (a,c) and testosterone (b,d) levels were measured in plasma obtained from critically ill COVID-19 patients (ICUCOVID-19), critically ill non-COVID-19 patients (ICUnon-COVID-19), and healthy controls (HC).
patients with coronary heart diseases (CHD) and healthy individuals (HC). Male data sets are shown in blue color-toned columns and female data sets are shown in red color-toned columns. The laboratory assessed hormone reference ranges are indicated in grey. Percentile boxplots represent 25–75% of values, with the median value indicated by a crossline, and mean values by a plus icon. Statistical significance was assessed via One-Way-ANOVA.

**Figure 2**

Estradiol levels in dependency of disease severity. Estradiol levels were measured in plasma obtained from critically ill male (blue columns) and female (red columns) COVID-19 or non-COVID-19 patients are displayed in dependency of disease severity as assessed by SOFA scores (a-c). Male COVID-19 patients were subdivided into patients requiring ECMO therapy (+ECMO) and patients not requiring ECMO therapy (-ECMO) (d). Percentile boxplots represent 25–75% of values, with the median value indicated by a crossline, and mean values by a plus icon. The laboratory assessed hormone reference ranges are
indicated in grey. Values are shown as median and interquartile range. Statistical significance in males was assessed by non-parametric tests (Kruskal-Wallis test and Dunn's test for multiple comparisons). Statistical significance in females was evaluated by unpaired, two-tailed non-parametric Student’s t-test (Mann-Whitney test).

**Figure 3**

Chemokine and cytokine responses in COVID-19 patients. Cytokine and chemokines were measured in plasma obtained from critically ill male (a-e) and female (f and g) COVID-19 patients by using a 27-plex immunoassay. Here, those with significant differences are shown. Cytokine and chemokine levels of male
and female COVID-19 patients are displayed in dependency of disease severity as assessed by SOFA scores (2-3; 4-7; 8-11).

**Figure 4**

Correlation of IFN-γ levels in male and female COVID-19 patients to estradiol levels. Estradiol levels were measured in plasma of critically ill male and female COVID-19 patients and were plotted over the expression levels of all assessed cytokines and chemokines. Here, only IFN-γ is displayed, which showed significant correlations in regression analysis with estradiol among 27 different cytokines and chemokines assessed. Statistical significance was assessed by generalized linear regression.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SchroederetalSupplementaryFigures27042021.pdf