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The majority of male patients with COVID-19 present low testosterone levels on admission to Intensive Care in Hamburg, Germany: a retrospective cohort study.

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Summary

Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to spread worldwide and pose a major public health burden. There is increasing evidence that men are more likely to die from SARS-CoV-2 infection than women. However, underlying factors that mediate the observed sex bias in coronavirus disease 2019 (COVID-19) remain unknown.

Methods. In this retrospective cohort, we included COVID-19 patients who were admitted to an Intensive Care Unit at the University Hospital Hamburg-Eppendorf, Germany. We obtained demographic data of all patients who were discharged or had died by 29th April 2020. We systematically analyzed sex hormones as well as cytokine and chemokine responses in male and female patients with laboratory-confirmed SARS-CoV-2 infections upon hospital admission. We used uni- and multivariable linear regression methods to identify potential risk factors for disease severity in males and females.

Findings. All enrolled patients (*n*=45; *n*=35 males and *n*=10 females) presented comorbidities with hypertension being the most common (45.7% in males; 40% in females), followed by cancer (35% in males; 40% in females), obesity (34.3% in males and 30% in females), type II diabetes (25.7% in males and 20% in females) and chronic heart diseases (8.6% in males and 0% in females). We detected that the vast majority of male COVID-19 patients present low testosterone (68.6%) and low dihydrotestosterone (48.6%) levels. In contrast, most female COVID-19 patients have elevated testosterone levels (60%) without alterations in dihydrotestosterone levels. Both, female and male COVID-19 patients may present elevated estradiol levels (45.7% in males and 40% in females). Disease severity defined by SOFA score correlates with elevated cytokine responses (e.g. IL-6) in males and IL-2 in females. In male COVID-19 patients, testosterone levels negatively correlate with inflammatory IL-2 and IFN-γ, whereas estradiol levels positively correlate with the inflammatory cytokine IL-6. Vice versa, in female COVID-19 patients, testosterone levels positively correlate with inflammatory cytokines (e.g. IL-6).

Interpretation. We here show that critically ill male COVID-19 patients suffer from severe testosterone and dihydrotestosterone deficiencies. Both androgens are required to mount antiviral immune responses to combat infection in males.

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Introduction

The current SARS-CoV-2 pandemic continues taking its toll on human health and health care systems worldwide. First SARS-CoV-2 infections in humans were reported in December in Wuhan, China [1]. It is believed to have a zoonotic origin, albeit the animal reservoir is still under investigation. However, the newly emerging SARS-CoV-2 possessed the ability to transmit from human-to-human, thereby rapidly spreading worldwide. 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 appears to be broad, ranging from mild upper respiratory illnesses to severe primary pneumonia with respiratory failure and death [2]. A retrospective cohort study, which enrolled 191 inpatients with COVID-19 in Wuhan, China, analyzed patient demographics and laboratory findings on hospital admission since its first occurrence in December 2019 in China. They reported that older age, high SOFA score, d-dimers greater than 1µg/ml as well as comorbidities (e.g. hypertension, type II diabetes) present poor prognostic markers at an early stage [2]. In January 2020, first patients in Europe were diagnosed with the COVID-19, describing 3 male and 2 female patients, all of whom had travelled from China [3]. In New York City, a case study on 5,700 hospitalized patients also revealed hypertension, obesity and diabetes as the most common comorbidities [4]. A total of 373 patients (33.5% female) were treated in intensive care units and 320 of them received invasive mechanical ventilation [4]. With SARS-CoV-2 continuing to infect humans worldwide, it was repeatedly reported that men with COVID-19 are at higher risk to develop severe and even lethal outcome compared to women, independent of age [5]. Thus, it has become of utmost importance to understand why men are more likely to die from COVID-19 than women.

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Methods

Ethics statement

Sampling from laboratory-confirmed COVID-19 patients was reviewed and approved by the ethics committee at the Hamburg State Chamber of Physicians (registration no.: WF-053/20). The need for an informed consent was waived by the ethics committee, because data were retrieved retrospectively from electronic health records.

Study design, setting and participants

This retrospective cohort study included serum and plasma samples collected from the first *n*=45 laboratory-confirmed COVID-19 patients who were admitted to the ICU at the University Medical Center Hamburg-Eppendorf from March 8th to April 29th, 2020. A total of 35 male and 10 female patients were included in this study. 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. During the SARS CoV-2 pandemic, 3 intensive care units were initially designated as dedicated units for the treatment of COVID-19 patients. Up to 30 patients suffering from COVID-19 are currently being treated here. A further ward treats critically ill patients with suspected COVID-19 until laboratory confirmation of SARS-CoV-2. 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 postoperative care following cardiac, neurosurgery, traumatologic, vascular or visceral surgery. Our department routinely treats patients following solid organ or allogeneic stem cell transplantations. In addition, the department has specialized units for the treatment of acute brain injury, major trauma, acute respiratory distress syndrome or cardiac failure that routinely use extracorporeal membrane oxygenation and cardiac assist devices, as well as a specialized liver-failure unit using extracorporal liver support. All serum and plasma samples were collected from COVID-19 patients on the day of admission (except one sample, which was collected 1 day later) and stored at -80°C until further analyses.

Data collection

The following demographic and clinical variables were collected from the electronic patient data management system (PDMS) (ICM, Dräger, Lübeck, Germany): age, sex, body mass index, comorbidities, Simplified Acute Physiology Score II (SAPS II) at admission, Sequential Organ Failure Assessment Score (SOFA) at admission, and classification of Acute Respiratory Distress Syndrome (ARDS) using the berlin definition[6]. Additionally, we recorded antiviral treatment, supportive and

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experimental COVID-19 therapies, the need for mechanical ventilation and extracorporeal membrane oxygenation (ECMO). Furthermore, we followed the course of the patients and noted when they could be discharged from the intensive care unit or deceased.

Hormone quantification

Serum or plasma samples of COVID-19 patients were analyzed for a panel of 12 hormones: Cortisol, thyroid-stimulating hormone (TSH), free triiodothyronine (T3), free thyroxine (T4), luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone (TT), dihydrotestosterone (DHY-TT), 17-β-estradiol (E2) and sex hormone binding globulin (SHBG). The serum and plasma hormone levels were measured externally in the clinical laboratory of Lademannbogen (Hamburg, Germany). Cortisol, TSH, T3, 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).

Cytokine and chemokine measurement

Serum or plasma samples of COVID-19 cases were analyzed for a panel of 24 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 agonist (IL-1rα), 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). Cytokine and chemokine levels were measured using a Bio-Plex Pro^{TM} 27-plex assay (#M500KCAF0Y, Bio-Rad) according to manufacturer's instructions in a Bio-Plex 200 System with high-throughput fluidics (HTF; Bio-Rad).

Statistical analysis

Statistical significance was determined with GraphPad Prism 8 v. 8.4.2 (GraphPad Software, Inc.). Association between sex hormones or SOFA and SAPS II scores and cytokine/chemokines were determined using linear regression and correlation analysis (Pearson). Statistical significance of cytokine and chemokine levels in male and female COVID-19 patients was assessed by Student's *t*-test. Statistical significance was defined as $P < 0.05$ (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

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Role of the funding source

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.

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Results

We here report on the first 45 COVID-19 patients who were admitted to the Intensive Care Units (ICU) of the University Medical Center Hamburg-Eppendorf (UKE). In total, 7 (16%) patients were treated with extracorporeal membrane oxygenation and 33 patients (73%) were mechanically ventilated.

COVID-19 patient demographics.

Among the first 45 patients requiring intensive care, 35 were males and 10 were females (**Table 1**). The median age within males and females was comparable with 62 and 67.5 years, respectively. All patients presented at least one comorbidity. Hypertension was detected in most patients (45.7% in males and 40% in females), followed by cancer (35% in males and 40% in females), obesity (34.3% in males and 30% in females), type II diabetes (25.7% in males and 20% in females) and chronic heart diseases (8.6% in males and 0% in females). Acute Respiratory Distress Syndrome (ARDS) detected was classified using the Berlin Definition[6] and most cases were moderate or severe in male (50% or 35%) and female (40% or 50%) COVID-19 patients. Sequential organ failure assessment scores (SOFA) were calculated in males and females Sequential organ failure assessment scores (SOFA)[7] were evaluated in males and females and were divided into three tiers according to their severity: low (2-3), high (4-7) or very high (8-11) presenting very low (2-3), low (4-7) or higher (8-11) scores in males (17.1%, 42.9% and 40%) and females (0%, 60% and 40%). These results represent the mono-organ failure of the lung but the absence of other organ failures such as liver and/or kidney failure. Moreover, most patients were at a good neurological status prior to intubation (data not shown). Simplified Acute Physiology Score (SAPS) II[8] includes age and admission type, estimating the probability of mortality with similar distributions between males and females and low scores. Only 5 males (14.3%) and 3 females (30%) achieved values above 51 points and thus have a predicted mortality of 50%. In contrast to these low scores and thus predicted mortality, only 12 (27%) of the 45 patients died. 9 of the deceased patients were male. 22 (49%) of the treated patients could be discharged from the intensive care unit already stabilized. 11 (24%) of the patients analyzed in this study, continue to be treated in the intensive care unit of the UKE after April 29th , 2020.

Low testosterone levels in the majority of male COVID-19 patients on admission

Due to the strong sex bias of males-to-females with a ratio of 3.5:1 in this study, we measured sex hormones known to play a key role not only in fertility but also in key innate and adaptive immune pathways [9,10]. Importantly, sex hormones were measured on the day of patient admission to exclude potential unspecific effects due to invasive procedures at the ICU. In 68.6% of male COVID-19

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patients, testosterone levels were strongly reduced (**Table 1**). Herein, 14.3% of males showed low, 28.6% very low and 25.7% extremely low testosterone levels. Importantly, of the 9 male COVID-19 patients who died, 7 had low testosterone levels, 1 patient had normal testosterone levels at the lowest percentile and 1 patient presented testosterone levels within normal range (data not shown). In contrast, 60% of females showed elevated testosterone levels. Among the 3 female patients who died, one had elevated testosterone levels. In general, the majority of male COVID-19 patients (68.6%) present reduced testosterone levels, while the majority of female patients (60%) show elevated testosterone levels (**Figure 1a**). We then measured levels of the sex hormone-binding globulin (SHBG) in male and female patients. 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. Most female patients (60%) presented elevated testosterone levels. In contrast, 28.5% of male COVID-19 patients presented elevated SHBG levels. Interestingly, in 7 of these 10 patients, elevated SHBG was not combined with low testosterone levels (data not shown). This suggests that an additional 7 patients with "normal" appearing testosterone levels might indeed suffer from masked testosterone deficiencies. Taken together, these data suggest that the vast majority of male COVID-19 patients present low testosterone levels. Moreover, male COVID-19 patients with low testosterone levels seem to have an elevated risk to die than those with normal testosterone levels.

Low dihydrotestosterone levels in the majority of male COVID-19 patients on admission

The actions of androgens such as testosterone and dihydrotestosterone are mediated via the androgen receptor (AR), a ligand-dependent nuclear transcription factor and member of the steroid hormone nuclear receptor family. AR bound to testosterone or with higher affinity to its more potent form, dihydrotestosterone, facilitates the translocation of this complex into the nucleus, where they bind to androgen-response elements on DNA and thereby act as transcription factors of various cellular genes that play key roles in cell survival [11]. Thus, we analyzed the levels of dihydrotestosterone in male and female COVID-19 patients. In males, dihydrotestosterone levels were reduced in the majority of cases with 48.6% (**Table 1**). In contrast, dihydrotestosterone levels were within normal range in females. Interestingly, low dihydrotestosterone levels in male patients correlated with low testosterone and/or SHBG levels. This suggests that low dihydrotestosterone levels in male COVID-19 patients are likely due to low testosterone levels in general.

Primary and secondary hypogonadism in male COVID-19 patients

The vast majority (95%) of testosterone is produced in the Leydig cells of the testis pending on stimulation by luteinizing hormone (LH). Only small amounts (5%) are produced in the adrenal glands.

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Low levels of testosterone (also called hypogonadism) may either be of hypothalamic-pituitary origin (secondary hypogonadism), of testicular origin (primary hypogonadism) or a combination of both, which is predominantly found in the aging male population as late onset hypogonadism [12,13]. To shed light on the origin of the partially very severe testosterone deficiency in male COVID-19 patients, we further analyzed related hormones. Levels of the luteinizing hormone (LH) were elevated in 31.4% of male COVID-19 patients, while being within the normal range in all female patients (**Table 1**). Interestingly, 6 out of the 35 male patients with low testosterone levels presented elevated levels of the luteinizing hormone (LH) at the same time (data not shown), suggesting impairment of Leydig cell steroidogenesis in 17.1% of the male patients. Follicle stimulating hormone (FSH) levels were elevated in 4 out of 35 male patients (11.4%). Elevated FSH levels in these male patients were combined with elevated LH levels. In 40% of female patients, FSH levels were reduced, which may indicate loss of ovarian function. Other hormones, such as thyroid stimulating hormone (TSH), T3 and T4 were within normal ranges in almost all male patients (91.4% (TSH), 91.4% (T3), 97.1% (T4)) and the majority of female patients (70% (TSH), 70% (T3), 100% (T4)). This does not indicate major defects in the general metabolism, indicating no impairment of thyreotrope axis or other dysregulations due to critical illness. Cortisol levels were elevated in 51.4% of male and 80% of female COVID-19 patients. These findings suggest that a distinct proportion of male patients (6 out of 35; 17.1%) show primary hypogonadism. The remaining proportion on the other hand (18 out of 35; 51.4%) of male COVID-19 patients present low testosterone levels that is not of testicular but likely of hypothalamic origin.

Obese male COVID-19 patients are more likely to have elevated estradiol levels

Estradiol levels were elevated in 45.7% of male COVID-19 patients and reduced in 22.9% (**Table 1**). Interestingly, 10 of the 16 male patients (62.5%) with elevated estradiol levels were classified as obese with a BMI ≥30 (data not shown). Estradiol levels were also elevated in 40% of female patients and none of them was obese (data not shown). These findings suggest that both, female and male patients present elevated estradiol levels (**Figure 1b**). Moreover, elevated estradiol levels seem to be coupled with obesity in most male but not in female patients.

In male COVID-19 patients, level of inflammation correlates with level of disease severity

Several independent studies reported that cytokine storm is a major hallmark of severe COVID-19 in humans [14]. Therefore, we sought to analyze whether the severe testosterone deficiency detected in male COVID-19 patients might be associated with altered cytokine and chemokine levels. First, we analyzed levels of 27 cytokines and chemokines in female versus male patients (**Figure S1**). In 26 out of 27

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analyzed cytokines and chemokines, no significant differences were observed between males and females. Only levels of eotaxin, which is produced e.g. by IFN-γ stimulated endothelial cells and TNF-activated monocytes, were slightly reduced in female patients. Thus, cytokine and chemokine responses do not seem to differ between male and female COVID-19 patients simply due to their sex. However, cytokines and chemokines play a key role in antiviral immune responses upon acute infections. Therefore, we performed linear regression analysis to correlate a potential link of cytokine and chemokine levels to disease severity in males and females. Among all cytokines and chemokines assessed, in males, a series of cytokine and chemokine levels (FGF, IL-1rα, MCP-1, MIP-1α, IL-6, IL-10, IL-1ß, IL-5, PDGF-BB, IFN-γ and IL-7), except for VEGF, were increased in male patients with increasing disease severity as defined by the SOFA score (Figure 2 a-l). Interestingly, most of the cytokines and chemokines altered in male COVID-19 patients are involved in inflammation (IL-6, IL-1ß, IFN-γ), immune activation and migration (MCP-1, MIP-1α, IL-5, IL-7), anti-inflammatory pathways (IL-10), angiogenesis and endothelial function (FGF, VEGF, IFN-γ), wound healing (PDGF-BB). VEGF, which negatively correlated with disease severity, plays a key role in vascular homeostasis, suggesting it is disturbed in severely ill male COVID-19 patients. In female patients, among all 27 cytokines and chemokines analyzed, only IL-2 levels positively correlated with disease severity (Figure 2m). IL-2 is mainly produced by activated T-cells. High IL-2 levels are associated with the vascular leak syndrome [15,16]. Lung endothelial cells, which express high-affinity IL-2R, the receptor of IL-2, are believed to induce via IL-2 binding, vascular permeability that in turn leads to life-threatening pulmonary oedema. These findings show that differential cytokines and chemokines correlate with disease severity in males and females. In males, an increase of a series of inflammatory cytokines correlates with disease severity. Herein, particularly factors involved in angiogenesis and endothelial function seem to be altered in their homeostasis. In females, only IL-2, which is also involved in vascular homeostasis, seems to be a correlate of disease severity.

In male COVID-19 patients, low testosterone levels correlate with elevated inflammation

Next, we wanted to see whether there is a potential mechanistic link between inflammation and the lack of testosterone. Sex hormones exert key functions on many cellular targets via binding to sex hormone receptors expressed on various immune cells [17]. The androgen receptor (AR), which is encoded by the X chromosome, is expressed in many cells of the immune system, including hematopoietic stem cells, monocytes, macrophages, neutrophils, CD4+ and CD8+ T-cells [17]. Linear regression analysis of all 27 measured cytokines and chemokines in relation to testosterone or estradiol levels revealed distinct correlations in males and females. In male COVID-19 patients, high-level expression of VEGF, IL-2 and IFN-γ was more likely to be detected in patients with low testosterone

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levels (**Figure 3a-c**). Conversely, most male patients with high testosterone levels presented low-level expression of VEGF, IL-2 and IFN-γ, all factors involved in angiogenesis and endothelial cell function. Correlation of estradiol levels, which were elevated in 45.7% of all male COVID-19 patients, to all 27 cytokines and chemokines assessed, revealed that only IL-6 and VEGF positively correlate with estradiol. IL-6 is a poor prognostic marker in many viral infections, including COVID-19 as we also show in this study (**Figure 2e**). It seems to be further elevated in male patients with elevated estradiol levels. Conversely, in female COVID-19 patients, inflammatory IL-6 levels as well as GM-CSF, IL-12, IL-1ß and IL-15 seem to increase with elevated testosterone levels that were present in 60% of the female COVID-19 patients. In general, low testosterone levels in male COVID-19 patients correlate with inflammatory cytokine expression. In contrast, high testosterone levels in female COVID-19 patients correlate with elevated expression of inflammatory cytokines. Thus, testosterone exerts a protective immune response in men, while accelerating inflammation in women.

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Discussion

The androgen receptor (AR), encoded on the X chromosome, is widely expressed in many cells and tissues. AR has a diverse range of biological actions including important roles in the development and maintenance of the reproductive, musculoskeletal, cardiovascular, immune, neural and hematopoietic systems [18]. AR is a nuclear transcription factor that binds to testosterone and to higher affinity to dihydrotestosterone, which is converted by the enzyme 5-α reductase from the prohormone testosterone. Then, the AR-testosterone/dihydrotestosterone complex is imported into the nucleus, where it binds to androgen-response elements on DNA and initiates the transcription of various genes, including those involved in reproduction but also those required to mount antiviral immune responses [11]. While the AR is present on female and male immune cells, its function is mainly driven by the amount of testosterone and dihydrotestosterone present that is different in men and women.

Herein, we did not observe major differences in the induction of cytokines and chemokines between male and female COVID-19 patients. However, analyzing major sex hormones, we found that the vast majority of male COVID-19 patients suffer from severe testosterone (68.6%) and dihydrotestosterone (48.6%) deficiencies upon hospital admission. Thus, it seems rather unlikely that patients with such a lack in key AR activating factors will be able to mount immune responses to combat viral infections. Reduced dihydrotestosterone levels were observed in patients with low testosterone and/or high SHBG levels suggesting that it is a consequence of lacking sufficient testosterone pro-hormone than for other causes. Indeed, low testosterone levels particularly correlated with high levels of inflammatory cytokines, such as IL-2 and IFN-γ. Conversely, most female COVID-19 patients (60%) presented elevated testosterone levels that correlated with elevated inflammatory cytokine expression, such as IL-6 and IL-1ß. Thus, low testosterone levels in men and high testosterone levels in women correlate with inflammatory cytokines responses in a sex-specific manner. It was reported before that testosterone has a sex specific and protective effect on vascular aging by mitigating oxidative stress an inflammation [19]. Both factors play key roles in COVID-19 disease outcome.

Moreover, a distinct proportion of male patients showed elevated estradiol levels that was coupled with obesity in some of them. This is in line with previous reports that obese patients may present shifted testosterone-to-estradiol levels due to high-level aromatase activity, an enzyme expressed in adipose tissues [20]. Interestingly, elevated estradiol levels in male patients correlated with elevated IL-6 levels that is a poor prognostic marker in COVID-19 patients [14]. This is in line with reports on the inflammatory role of estrogens in men [21,22]. An increase in serum estrogen levels was also observed in

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other patient cohorts with acute critical illness and was proposed to be due to increased peripheral aromatization but only after several days of hospitalization [23]. This was not the case in our cohort, where we analyzed patients´ samples on the day of admission.

Other comorbidities present in this cohort are also associated with altered sex hormones. Patients with type II diabetes have a higher risk for low testosterone but not estradiol levels [24,25]. Another hit might be presented by increasing age, since testosterone levels decrease with increasing age and male COVID-19 patients presented a median age of 62 years.

While writing this manuscript, colleagues from Wuhan uploaded a study on MedRxive reporting on slightly reduced testosterone but not estradiol levels in a COVID-19 male cohort with a median age of 39 years, where the majority of cases (>86%) underwent mild infections [26]. It should be noted that testosterone-producing Leydig cells express the SARS-CoV-2 receptor ACE-2 to very high levels even higher than in respiratory tissues [27,28]. However, it remains yet unclear whether SARS-CoV-2 replication directly in Leydig cells may present a testosterone level reducing hit.

Another testosterone level reducing hit could be the induction of "cytokine storm" as recently reported in H7N9 influenza infected individuals (submitted to MedRxive). Avian H7N9 influenza also shows a strong male bias and is a known strong inducer of inflammatory cytokines defined as "cytokine storm" comparable to SARS-CoV-2 infection. In both infections, high IL-6 levels present a poor prognostic marker [14]. Indeed, it was reported before that "cytokine storm", including high TNF-α levels may suppresses steroidogenesis in the testis by inhibiting defined pathways [29,30]. Conversely, gonadal steroids were also shown to inhibit IL-6 secretion in turn [31]. This is in line with our findings here that low testosterone levesl correlate with high levels of inflammatory cytokines. Moreover, in the avian H7N9 human cohort, low testosterone levels in male H7N9 patients strongly correlated with lethal outcome.

In summary, it seems that several conditions are combined in male COVID-19 patients that are associated with low testosterone levels (increasing age, obesity, type II diabetes, cytokine storm). Thus, these multiple testosterone-reducing hits eventually lead to severe deficiencies in testosterone and dihydrotestosterone levels in male COVID-19 patients preventing them to mount sufficient immune responses to combat the deadly infection. These findings might help to discuss personalized intervention strategies for male COVID-19 patients taking e.g. established protocols for treatment of male hypogonadism into consideration.

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Author contributions

GG conceived the study. SK, MS, DJ and AN were responsible for COVID-19 patient management and recruitment. GG, SK, MS and BT designed and overviewed the study design. BT, HJ, MZ and SSB conducted the cytokine and chemokine assays. GG, BT and MS analyzed all data and developed the figures. JA, AM, TB critically reviewed the manuscript and were involved in study design. GG wrote the manuscript. All authors revised the manuscript.

Declaration of Interests

All authors declare no competing interests.

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Figure 1

Testosterone and estradiol levels in male and female COVID-19 patients.

Testosterone (a) and estradiol (b) levels in serum or plasma of male (*n* = 35) and female (*n* = 10) COVID-19 patients were measured and classified according to the reference range.

Figure 2

Correlation of cytokine and chemokine levels in male and female COVID-19 patients to disease severity (SOFA Score).

SOFA scores from SARS-CoV-2 positive male (a-l) and female (m) patients were plotted over the expression levels of all assessed cytokines and chemokines. Linear regression was performed and shown are the respective *r* (correlation coefficient) and P values of the regression lines. A P value < 0.05 was considered statistically significant, i.e. strong positive or negative correlation between disease severity/sex hormone levels and cytokine/chemokine expression. Here, only those showing significant differences in linear regression are shown: FGF (a), IL-r1α (b), MCP-1(c), MIP-1α (d), IL-6 (e), IL-10 (f), IL-1β (g), IL-5 (h), PDGF-BB (i), IFN-γ (j), IL-7 (k), VEGF (l), and IL-2 (m).

Figure 3

Correlation of cytokines and chemokine levels in male and female COVID-19 patients to sex hormone levels (estradiol and testosterone).

Testosterone and estrogen levels measured in the sera/plasma from SARS-CoV-2 positive male (a-e) and female (f-k) patients were plotted over the expression levels of all assessed cytokines and chemokines. Linear regression was performed and shown are the respective *r* (correlation coefficient) and P values of the regression lines. A P value < 0.05 was considered statistically significant, i.e. strong positive or negative correlation between disease severity/sex hormone levels and cytokine/chemokine expression. Here, only those showing significant differences in linear regression are shown: VEGF (a,e), IL-2 (b), IFN-γ (c), IL-6 (d,f), GM-CSF (g), IL-12 (h), IL-1β (i) and IL-15 (j).

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Table 1.

Demographic, clinical and laboratory findings of COVID-19 patients on admission.

Characteristics	Males (n=35)	Females (n=10)
Median age (range) – yr	62 (31-80)	67.5 (54-84)
Comorbidities		
Hypertension	16 (45.7%)	4 (40%)
Cancer	10 (35%)	4 (40%)
Obesity (BMI ≥30)	12 (34.3%)	3 (30%)
Diabetes type II	9 (25.7%)	2 (20%)
Chronic Heart Diseases	3 (8.6%)	0 (0%)
ARDS		
mild	4 (11.4%)	0 (0%)
moderate	14 (40%)	4 (40%)
severe	10 (35%)	5 (50%)
SOFA		
2-3	6 (17.1%)	0 (0%)
4-7	15 (42.9%)	6 (60%)
8-11	14 (40%)	4 (40%)
SAPS II		
15-30	6 (17.1%)	0 (0%)
31-40	16 (45.7%)	4 (40%)
41-50	8 (22.9%)	3 (30%)
≥ 51	5 (14.3%)	3 (30%)
Total testosterone levels		
Normal males 18-50 yr: 8.69-29 nMol/l ≥51 yr: 6.68-25.8 nMol/l	11 (31.4%)	
Low: 5-6.67/8.68 nMol/l	5 (14.3%)	
Very low: 3-4.9 nMol/l	10 (28.6%)	
Extremely low: <3 nMol/l	9 (25.7%)	
Normal females postmeno.: 0.09-1.5 nMol/l		4 (40%)
High: 1.6-4 nMol/l		5 (50%)
Very high: 5-8 nMol/l		1 (10%)
Dihydrotestosterone levels		
Normal males: 10-90 ng/dl	18 (51.4%)	
Low: <10 ng/dl	17 (48.6%)	
Normal females: ≤30 ng/dl		10 (100%)
Estradiol levels		
Normal males: 27.1-52.2 pg/ml	11 (31.4%)	
High: 52.2-90 pg/ml	12 (34.3%)	

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Very high: 90-185 pg/ml	4 (11.4%)	
Low <27.1	8 (22.9%)	
Normal females postmeno.: 5-49.9 pg/ml		5 (50%)
High: 50-100 pg/ml		2 (20%)
Very high: 101-130 pg/ml		2 (20%)
Sex hormone-binding globulin		
Normal males: 10-40 nMol/l	24 (68.6%)	
High: 41-100 nMol/l	6 (17.1%)	
Very High: ≥101 nMol/l	4 (11.4%)	
Normal females: 26-110 nMol/l		9 (90%)
High: ≥101 nMol/l		1 (10%)
Thyroid stimulating hormone		
Normal: 0.27-4.2 µU/ml	32 (91.4%)	7 (70%)
Free T3		
Normal: 1.8-4.6 ng/dl	32 (91.4%)	7 (70%)
Free T4		
Normal: 8-17 ng/dl	34 (97.1%)	10 (100%)
Luteinizing hormone		
Normal males: 0-8.6 mIU/ml	22 (62.9%)	
High: ≥8.7 mIU/ml	11 (31.4%)	
Normal females: <58.5 mIU/ml		10 (100%)
Follicle stimulating hormone		
Normal males: 1.5-12.4 mIU/ml	30 (85.7%)	
High: 12.5-25 mIU/ml	4 (11.4%)	
Normal females: 25.8-134.8 mIU/ml		6 (60%)
Low: 10-25.7 mIU/ml		4 (40%)
Cortisol		
Normal: 2.5-19.5 µg/dl	16 (45.7%)	2 (20%)
High: 19.6 µg/-30µg/dl	18 (51.4%)	8 (80%)

ARDS: acute respiratory distress syndrome; SOFA: Sequential Organ Failure Assessment; SAPS II: Simplified Acute Physiology Score II.

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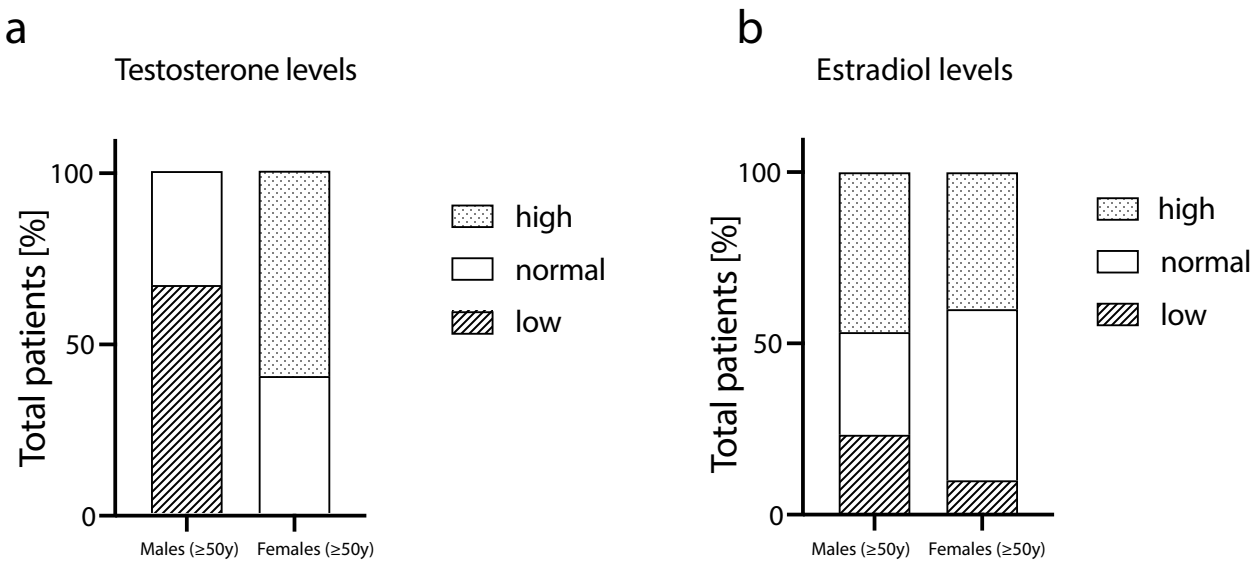


Figure 1

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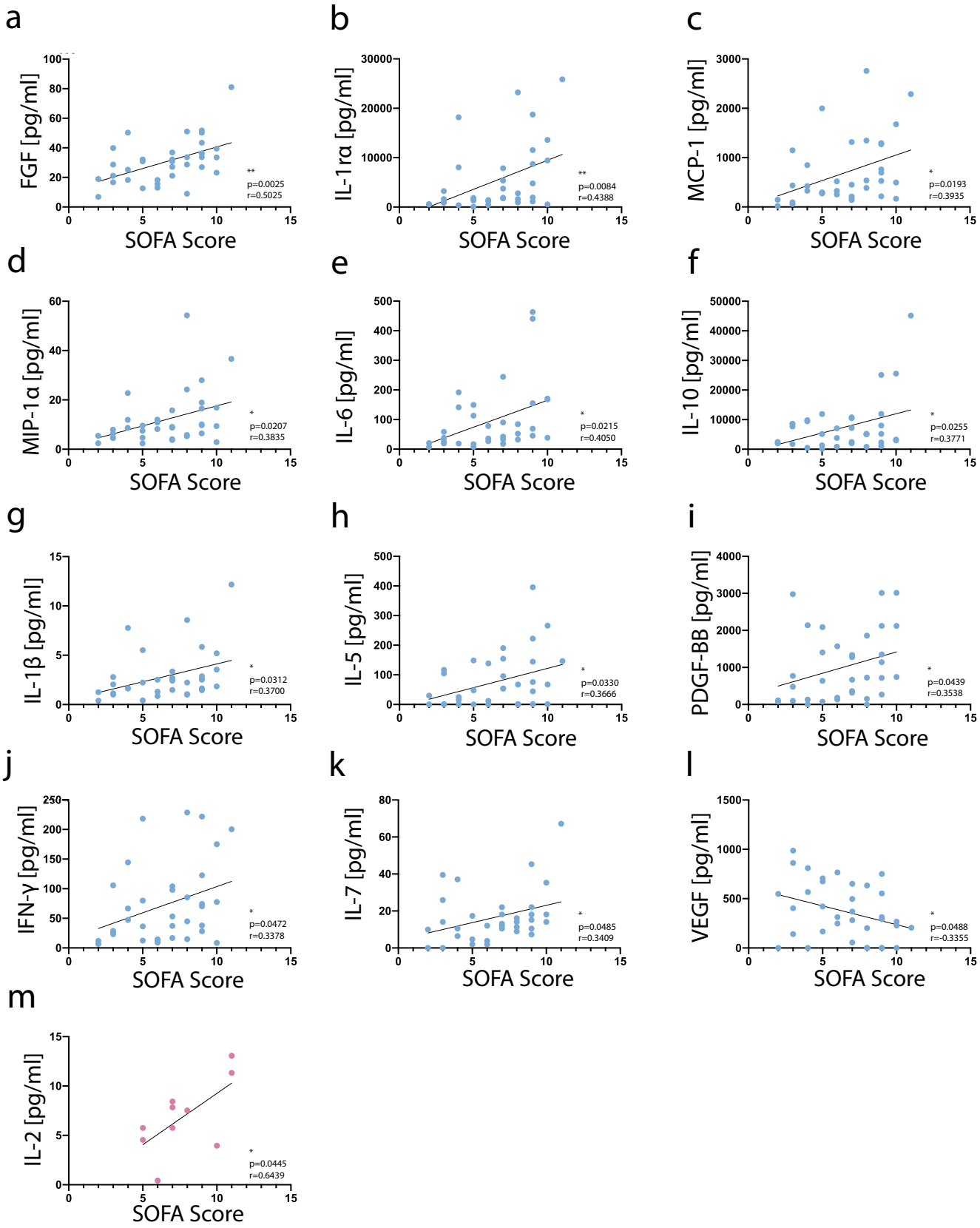


Figure 2

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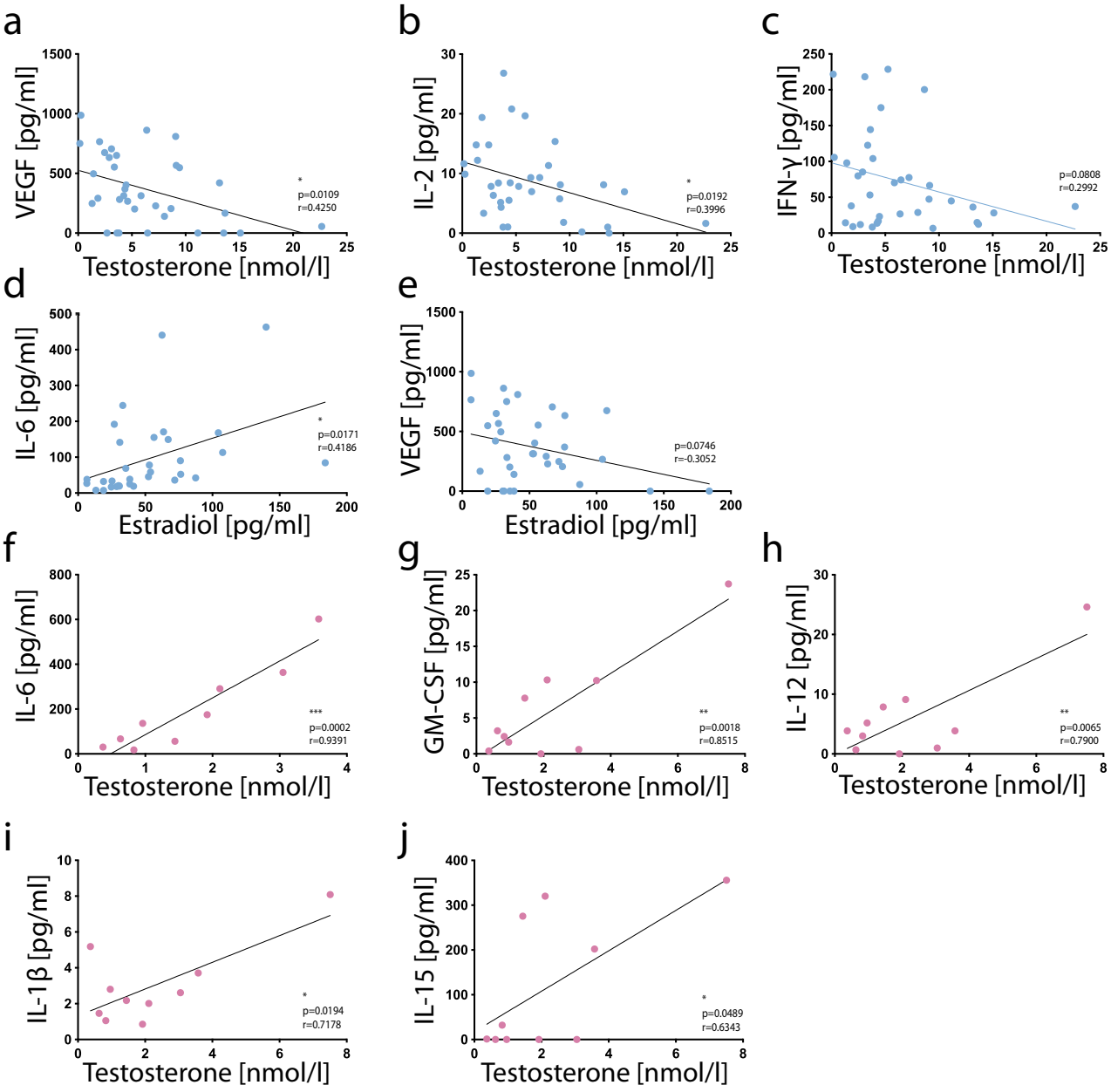


Figure 3