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EXPERT OPINION

The Role of Sex Hormones in the Disparity of COVID-19 Outcomes Based on Gender

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GENDER DISPARITY IN COVID-19

Since the earliest phase of the outbreak of Coronavirus Disease 19 (COVID-19), gender disparities in clinical course and adverse outcomes of the disease became evident.

The Global Health 5050 website provides updated sex-disaggregated data for COVID-19 indicators (http://globalhealth5050.org/covid19), which include at August, 24, 2021, 134,343,423 cases, 5,332,290 hospitalizations, 242,107 Intensive Care Unit admissions, and 3,217,722 deaths. As reported by this website, men are more represented among COVID-19 patients requiring hospitalization (56.6% vs 43.4%; http://globalhealth5050.org/covid19). The gap is even more evident if patients admitted to ICU are considered, of whom 67.3% are men (http://globalhealth5050.org/covid19). With the exception of a very limited number of countries, also mortality for COVID-19 is unbalanced towards men who represent 58.8% of deaths attributed to SARS-CoV-2 infection, irrespective of age (http://globalhealth5050.org/covid19).

The underlying reasons for these differences are not completely understood. Several hypotheses have been advanced and it is most likely that multiple factors participate. Clinical and preclinical evidence has been accrued to explain the role of sex hormones in the gender discrepancy of COVID-19 outcomes. Most of the reviews and literature summaries on this topic tend to bring more out the role of estradiol as a putative protective factor. In this Expert Opinion, we aimed to highlight the elements that lead to question estradiol as the key hormone and to take testosterone (T) in deeper consideration as a modulator of COVID-19 outcomes in both genders.

LIFESTYLE AS A POSSIBLE SOURCE OF GENDER DISPARITY

The different exposition to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection due to lifestyle, including job position, may be a possible factor, which explain the gender disparity. Globally, men lead a life characterized by more intensive and various interpersonal exchanges, both for recreational activities and professional ones. Moreover, a study evaluating the attitudes and behaviors towards the pandemic in more than 21,000 subjects showed that women have a greater perception regarding the seriousness of COVID-19.[1] Accordingly, they showed a closer agreement with the strategies and policies for activity restraining and reported a higher compliance with public health and distancing rules.[1]

Based on this, one could expect that worse outcomes in men are secondary to a greater infection rate. This may be apparently the case for several African, Western, South and Southeast Asian countries where, however, this excess is likely due to different opportunity of access to healthcare of men and women. For most countries, where studies on gender discrepancy in COVID-19 were mainly conducted, there is substantial similarity in the male to female ratio among the infected subjects (https://globalhealth5050.org/the-sex-gender-and-covid-19-project/). Moreover, when infections among healthcare workers are considered, 2 out of 3 are females (https://globalhealth5050.org/the-sex-gender-and-covid-19-project/). Overall, these proportions may suggest that women are even more prone to be infected by SARS-CoV-2 if exposed to risky environments. In light of this, the greater severity of the disease in men appears even more dramatic.

COMORBIDITIES AS POSSIBLE SOURCE OF GENDER DISPARITY

Among the putative conditions affecting the increased severity in men, different prevalence in risk factors and morbidities known to worsen the COVID-19 course may be hypothesized.

A recent meta-analysis provided a comprehensive summary of the clinical characteristics of COVID-19 hospitalized patients and assessed the risk factors for mortality.[2] Male gender was confirmed as a risk factor for death. Hypertension, diabetes mellitus (DM), and cardiovascular diseases (CVD), were the most prevalent morbidities[2] with frequencies among hospitalized COVID-19 patients (40.8%, 22.3% and 18.5%, respectively) that exceeded 2-to-3 fold that observed in general population. Studies with higher representation of subjects with hypertension, DM, CVD, chronic obstructive pulmonary disease, chronic kidney disease, and active cancer reported higher mortality rates.[2] Since all the aforementioned morbidities recognize male gender as a nonmodifiable risk factor, this may partly explain the excess in male hospitalization and mortality for COVID-19. However, the comparison of COVID-19 patients in the different waves of outbreaks showed a change in their characteristics with a

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progressive decrease in age and prevalence of some comorbidities[3] but a roughly similar male to female ratio among subjects admitted to hospital.[3–5] This suggests that there are further factors, besides comorbidities, which may explain the adverse outcomes in men.

SEX HORMONES AS POSSIBLE SOURCE OF GENDER DISPARITIES

In each field of medicine and biology, when gender disparities are recognized, sex hormones are the major candidates for being the responsible. In fact, sex hormones circulate in men and women at different concentrations.

Evidence on Estrogens

Present literature has greatly underlined the data, which suggest a beneficial effect of estrogens and a detrimental one for androgens in COVID-19 patients. This is based on the assumption that estrogens are "female" and androgens are "male" hormones. Indeed, preclinical data on animal models of SARS and Middle East Respiratory Syndrome (MERS) caused by coronaviruses similar to SARS-CoV-2, in line with present epidemiological data on COVID-19, showed that female mice have better disease course and lower mortality than males[6] and that ovariectomy or tamoxifen were associated with increased mortality.[6] This may suggest that estrogens are protective towards coronavirus infection but it should not be forgotten that ovariectomy causes not only estrogen but also androgen deprivation. Moreover, selective estrogen receptor inhibitors (SERMs), such as tamoxifen, have also tissue-specific pro-estrogenic effects and one of the most notorious adverse event with SERMs is venous thromboembolism, a life-threatening event in COVID-19. Hence, the role of sex hormones in COVID-19 is likely to be far more complicate than "estrogens is the good and androgens the bad."

As an effect of estrogen levels, females mount a stronger immune response with higher levels of cytokines and antibodies that provides better protection from pathogens.[7] However, in COVID-19, the clinical worsening and life-threatening phase rely right in an exaggerate inflammatory response with the so called "cytokine storm" for which a hyperactive immune system could be even detrimental. In addition, some,[8] although not all,[9] studies show that estrogens upregulate angiotensin-converting enzyme 2 (ACE2) that, on one hand, promotes the most favorable effects of renin-angiotensin system but, on the other hand, is the key enzyme that allow SARS-CoV-2 entering the pneumocytes and starting lung tissue damage and subsequent inflammatory events. The putative beneficial effects of estrogens on COVID-19 are also disproven by the observation that, in postmenopausal women, the lower risk of worse COVID-19 outcomes is still maintained on age-matched men (https://globalhealth5050.org/the-sex-gender-and-covid-19-project/). In addition, a study on 286 hospitalized COVID-19 male patients and 281 healthy controls have shown that patients have slightly but significantly higher estradiol levels and that higher estradiol is associated with worse, rather than better, COVID-19 clinical outcomes.[10] Estradiol *per se* has not been confirmed a risk factor in another recent study (which used mass-spectrometry rather than immunoassays for sex hormone measurement),[11] although estradiol to T ratio was found higher among men with more severe COVID-19. These data do not allow attributing definitely a detrimental effect of estradiol in men with COVID-19 because they could be the reflection of severely decreased T levels; nonetheless, they allow excluding that estradiol may be beneficial for men with COVID-19.

Focus on Testosterone

In the dichotomy of estrogens = female and androgens = male, it is important to consider that, in premenopausal women, serum estradiol fluctuates considerably peaking at the mid-cycle up to 1,000 pmol/L and declining progressively through the luteal phase by the lowest values around 50 pmol/L that are achieved in early follicular phase. In menopausal women, estradiol is commonly below 30 pmol/L. Adult men have stable estradiol levels ranging 30 to 150 pmol/L and, with ageing, only a slight decline is detected. Although there is, of course, substantial difference in estradiol levels between men and women, the abovementioned values show that its magnitude is smaller than it could be thought, at least for a considerable part of life. Conversely, the differences in testosterone (T) levels are more pronounced. Healthy premenopausal women have serum T levels around 0.5-1.0 nmol/L, which gradually decline with ageing. On the other hand, in healthy young men, total T has concentrations in the order of 15-25 nmol/L and, despite T declines with ageing, only in surgically or drug-induced castration, levels as low as in women are achieved. In this view, it is rationale to consider the role of T as a modulating factor of COVID-19 severity, responsible for the gender discrepancy.

The transmembrane protease/serine subfamily member 2 (TMPRSS2) is, besides ACE2, another key step of the SARS-CoV-2 infection. After the spike protein binds ACE2, it allows the fusion of viral and cell membranes thus promoting the internalization of SARS-CoV-2. TMPRSS2 is expressed not only in the airways but also in several other tissues, including the endothelium of micro- and macro-vessels where it favors clotting and, therefore, predisposes to the most frightening complications of COVID-19, namely the disseminated intravascular coagulation and pulmonary embolism.

Before being considered in the pathogenesis of COVID-19 and its complications, TMPRSS2 has been extensively studied in the context of prostate cancer, where the androgen-dependence of its expression has been demonstrated. Therefore, it could be hypothesized that lower T levels could protect from SARS-CoV-2 infection and be the responsible for better outcomes in women.

In an earlier phase of the pandemic, a retrospective study performed in Veneto, one of the most severely affected regions in

Italy and in the world at that time, provided data supporting the protective role of low T. By matching the data of regional registries of cancer patients with those on COVID-19 infected subjects, men with prostate cancer undergoing androgen deprivation therapy (ADT) were shown to have 3 to 4-fold higher risk of SARS-CoV-2 infection and of worse outcomes from COVID-19, as compared with men with cancer not upon ADT.[12] It should be however recognized that, due to the limited number of SARS-CoV-2 infections in the study population, the confidence intervals for the odds ratio were quite wide with borderline statistical significance in some cases. Later on, 2 Brazilian studies investigated the effect of antiandrogens in patients with mild to moderate COVID-19, showing a beneficial effect in terms of viral clearance, reduction of inflammatory markers and clinical remission, as compared with placebo.[13,14]

The putative detrimental role of T is in contrast with the physiologic decline of its levels in ageing men. In fact, the gap in adverse outcomes between men and women is particularly evident after the age of 60 years (http://globalhealth5050.org/covid19 last updated on August 24, 2021), when men commonly have T levels significantly lower than healthy younger subjects and in 15-20% of cases achieve values consistent with hypogonadism. In ageing men, the drop of T in the hypogonadal range is favored by the presence of comorbidities, including obesity, CVD, DM and other chronic conditions that are highly prevalent among COVID-19 patients with worse outcomes. In this view, the role of low T levels, rather than high, should be considered as a risk factor in COVID-19.

At the beginning of the first wave of SARS-CoV-2 infection in Italy, we collected data on hospitalized men with COVID-19 with the aim of studying their gonadal function and the possible relationship with their clinical outcomes.[15] Our study was the first one to demonstrate that in men admitted to a sub-intensive care unit for COVID-19, serum T was severely low with high luteinizing hormone (LH). This hormone pattern denotes a primary testicular damage, which is not common to critical patients that more often have a down-regulation of the hypothalamus-pituitary-testicular axis with both low T and LH. Noteworthy, ACE2 and TMPRSS2 are both expressed in the testis with ACE2 strongly represented in Leydig cells, which may explain the testicular damage from SARS-CoV-2.[16] In our study, we also showed that low T is a predictive factor for adverse outcomes. In particular, men with total T <5 nmol/L have 20-fold higher risk of transfer to intensive care unit and 30 fold higher to die. Afterwards, other studies with similar design confirmed our results.[10,17–19]

The association of low T with adverse COVID-19 outcomes in men may have different explanations. Indeed, it could be the reflection of heavier comorbidity burden and be a simple marker of frailty. However, T has known effects on the immune system. In particular, in contrast with estrogens, T has immunosuppressive effects that target several arms of the immune system, including the cells effectors of the innate response and those of the adaptive response. Among its effects, T is able to downregulate the production and release of pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, interferon (IFN)γ, IL-12, and induce the production of anti-inflammatory cytokines, such as IL-10 and IL-4.[20] The overall anti-inflammatory effect of androgens is proven in experimental models of inflammatory and autoimmune diseases in which T treatment is able to dampen the disease progression.[20] Recently, these findings have been confirmed a larger prospective study, which showed that lower T and increased estradiol to T ratio were associated more severe COVID-19 status and course and increased cytokine concentrations.[11]

Conclusions on the clinical meaning of low T in men with COVID-19 cannot be drawn based on the current data. Low T may represent a marker of frailty. Indeed, it is known that T commonly declines in subjects with chronic morbidities[21] and, despite studies in are scanty, this is reported also in acute critical illnesses.[22] However, due to the aforementioned role of T on immune response, a direct pathogenic effect of T in the COVID-19 clinical course cannot be excluded. The hypothesis that maintaining normal T levels in men affected by COVID-19 is beneficial should be corroborated by adequately designed randomized placebo-controlled clinical trials evaluating the efficacy of T replacement therapy. Such studies are not available so far. However, data exist on experimental animal models of infection from influenza viruses showing that orchiectomized mice replaced with T have better disease course and lower inflammatory features in lung tissue than the nonhormone replaced controls.[23]

According to these lines of evidence, eugonadal status in men infected by SARS-CoV-2 is a protective status and severely decreased T during the infection must be regarded as a risky condition rather than protective. But what about T in women with COVID-19? As an effect of the aforementioned dichotomy estrogens=female and androgens=male, the research in the field of the effects of T in women is in its infancy and its specific role in COVID-19 has been scarcely investigated. In a German study on 10 women (mean age 67.5 years; range 54-84 years) hospitalized in ICU for COVID-19, circulating T was higher than normal values of menopausal women in 60% of cases.[18] Interestingly, higher serum T was positively associated with circulating pro-inflammatory cytokine levels.[18] We recently replicated and confirmed these results in a similar cohort of 17 women aged 69.0 [57.5-74.0] years admitted in a sub intensive care unit for COVID-19. Interestingly, in this cohort, higher T at the admission was associated with increased circulating makers of inflammation with a threshold identifiable at T>1 nmol/L, above which procalcitonin, lactate dehydrogenase and ferritin steeply increased.[24] Curiously, T levels in these women were not associated with LH. This is apparently paradoxical because, in women, most of T is produced by the ovary upon LH stimulation. However, in menopausal women, it is expected that the contribution from the ovary to T production decrease. The other

steroidogenic gland that has the potential to produce T is the adrenal although, normally, its contribution to circulating T is limited. In our cohort, T levels were positively correlated with cortisol thus supporting the adrenal origin of the hormone.[24] This is an intriguing finding because it suggests that T in women may be considered a marker of adrenal activation upon stressful conditions, such as SARS in COVID-19. Indeed, it is reported that females, either animal models or humans, have a more pronounced response of the hypothalamus-pituitary-adrenal axis to stressors and they experience a slower habituation to stress than males.[25] This could explain the lively response of T increase observed in women hospitalized in sub-intensive care unit for COVID-19. Nonphysiologically high (because higher than expected in menopausal women) T levels should be considered a marker of greater effort of the immune system to face a critical condition. Whether this translates into a successful or unsuccessful effort with better or poor prognosis is still to be studied but it represents an interesting new field of research for women health.

CONCLUSIONS

In conclusion, the gender disparity concerning COVID-19 outcomes is still to be fully explained. Several factors may contribute. Among these, sex hormones are candidate prognostic factors. Although detrimental and beneficial role for T and estradiol, respectively, have been hypothesized, this explanation seems simplistic. In men, low T have been associated with worse COVID-19 course, whereas in women, higher T is associated with a heavier immune response. There is still much research work to do for corroborating these results and to understand is this pathophysiologic observation has diagnostic or even therapeutic relevance in COVID-19.

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