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SARS-CoV-2, TESTOSTERONE AND FRAILTY IN MALES (PROTEGGIMI): A MULTIDIMENSIONAL RESEARCH PROJECT

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ABSTRACT

Preliminary published data depicts a much greater prevalence of malewith laboratory-confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) referred for intensive care unit admission and severe sequelae in several countries. In this context, males seem to not only be more susceptible to the infection compared to female subjects, at least in western countries, but their case fatality rate attributable to SARS-CoV-2 infection is also highest. Therefore, we may speculate that the different hormonal milieu could have a more profound pathophysiological role in association with SARS-CoV-2, with endogenous testosterone leaving men more prone to develop more serious complications related to the SARS-CoV-2 infection. Another option is that SARS-CoV-2 infection per se causes an acute stage of male hypogonadism, the depletion of androgenic action triggering serious or an even fatal course of the disease. Therefore, we stongly advocate the development of a prospective multidimensional andrological translational research project in men, which we called the PROTEGGIMI study. In this *Opinion Article* we will not only highlight novel research activity in this area but also invite other researchers and learned scientific societies to join us in our efforts to understand an important and very newly discovered gap in knowledge, which may have serious implications for the lives of millions of men.

INTRODUCTION

The new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), previously provisionally named 2019 novel coronavirus or 2019-nCoV disease (COVID-19), has been associated with acute respiratory distress syndrome[1] and infected patients have a relatively high risk of death. Recently it has been also hypothesized that SARS-CoV-2-induced infection may lead to a more complex clinical picture termed microCLOTS syndrome (i.e., microvascular SARS-CoV-2-associated lung vessels obstructive thromboinflammatory syndrome)[2]. Further data has suggested that the human lung, liver, gastrointestinal tract, cardiovascular and the urinary systems are potential targets of SARS-CoV-2 infection[3]. In this context, the first retrospective case series published on a large cohort of consecutive patients with laboratory-confirmed SARS-CoV-2 referred for intensive care unit (ICU) admission to the coordination center of the SARS-CoV-2 in the ICU Network of the Lombardy region in Italy clearly depicted a dramatic snapshot: of 1591 patients, extraordinarily 1304 (82%) were males at the final follow-up at March 25, 2020[4]. Similar trends have also been reported in other countries[5].

ANDROLOGICAL RELEVANCE

Not only has this terrible pandemic infection had a devastating effect on the everyday life of the population globally, it has also further highlighted a prevailing gender gap in health; indeed, it seems that male individuals are not only more susceptible to the infection, at least in the western countries, but their death rate attributable to SARS-CoV-2 is also highest[6], thus confirming the pre-exisiting gender inequality in life expectancy[7]. The reasons for this gender disparity in disease severity are unclear, but may be related to host factors. If these observations are extrapolated to the real-life setting of both our daily clinical practice and that of andrological translational research to which we have dedicated ourselves over the past 20 years and more, this may mean that the hormonal milieu could have a much greater pathophysiological role in association with SARS-CoV-2[8,9].

In other words, testosterone - a hormone in many ways consubstantial with the existence of males - is not only important for their quality of life[10], but may lead to men being more susceptible to the development of other serious complications related to infection of SARS-CoV-2 compared to women. Another option is that SARS-CoV-2[8] infection causes an acute stage of male hypogonadism, the

depletion of androgenic action triggering serious or an even fatal course of the disease. Recent preliminary data would also support this hypothesis[11]. Indeed, comparing sex-related hormones of a cohort of men of reproductive age with SARS-CoV-2 infection and those of age-matched healthy men, Ma et al. reported the first direct evidence of a potential alteration of the androgenic hormonal milieu, as a consequence of the complex influence of the medical condition toward sex hormones; this being true at least in reproductive-aged SARS-CoV-2-infected males[11].

However, we can only speculate for the time being, as a potential pathophysiological interaction of sex hormones (and, mostly, testosterone), SARS-CoV-2[8] and its receptor angiotensin-converting enzyme 2 (ACE2) – the "entrance" into target host cells[12,13] – has yet only been minimally investigated[8,14].

On the whole, coronavirus entry into host cells is mediated by the trans-membrane spike (S) glycoprotein that forms homotrimers protruding from the viral surface[13]. S comprises two functional subunits which are responsible for binding to the host cell receptor (S_1 subunit) and fusion of the viral and cellular membranes (S_2 subunit). S_1 and S_2 subunits interact each other within a complex system that leads to an extensive and irreversible set of conformational changes. As a result, coronavirus entry into susceptible cells is a highly complex process that eventually requires the concerted action of receptor-binding and the proteolytic processing of the S protein to promote virus-cell fusion. In addiction, different coronaviruses have been demonstrated to use distinct domains within the S_1 subunit to recognize a varity of attachments to entry receptors; SARS-CoV viruses interact directly with ACE2 via their S domain B (S^B) to enter target cells[13].

Recently, ACE2 expression levels have been demonstrated to be higher in males than in females, at least in the lungs[15]. Furthermore, a more detailed analysis of the expression pattern of ACE2 in adult human testes, utilising uniform manifold approximation and projection (UMAP) and marker gene analysis, also indicates that ACE2 is predominantly enriched in spermatogonia, Leydig and Sertoli cells[16]; therefore, it has been speculated that the human testis may be a further potential route of SARS-CoV-2 infection. Conversely, early spermatocytes, late spermatocytes, spermatids and somatic cells express very low levels of ACE2[16]. Of relevance, the enrichment of ACE2 in Leydig and Sertoli cells demonstrated a 3-fold higher percentage compared to ACE2-expressing cells among type II alveolar epithelial cells (AT2) in human lungs[16]. The previously described high ACE2 expression in

testicular cells – and in contrast, low levels of observed expression ACE2 in ovarian tissue[17,18] – supports the concept of the testes as specific repositories, supporting the hypothesis that male subjects have even delayed viral clearance of SARS-CoV-2 compared to their female counterparts[18].

As a consequence, ACE2 expression in human testes – which has been also found at the protein levels[18] – suggests that SARS-CoV-2 could eventually infect the male testis, with a consequential risk in terms of male reproductive dysfunction, because of a disruption of spermatogenesis if spermatogonia were infected by the virus[16].

Furthermore, SARS-CoV-2 was found to utilise transmembrane serine protease 2 (TMPRSS2) for viral S protein priming[19]. TMPRSS2 expression has been found to be concentrated in spermatogonia and spermatids[16]. Interstingly: TMPRSS2 is an androgen-regulated cell surface protease, which had been previously found to be predominantly expressed in prostate epithelium[20]. Moreover, the connection is even more definitive since i) the development of the prostate gland is finely regulated by androgens which modulate its growth and function; and, ii) androgens exert a major role in prostate cancer development and progression and, among others, one of the hypotheses relies on a chromosomal rearrangement of the androgen regulated gene TMPRSS2 with the ETS transcription factor ERG[20,21]. Thus, to substantiate this hypothesis one must experimentally confirm it.

ACTION IS NEEDED

Whereas we are still at the stage where the whole world focuses on limiting the spread of the SARS-CoV-2 and saving lives of severely ill SARS-CoV-2 patients, as scientists we also have responsibility for elucidating the underlying cause of the wide gender difference in sensitivity to the harmful effects of SARS-CoV-2 and to explore the possible adverse long-term effects on male reproductive function.

Therefore, these questions have facilitated the development of a prospective multidimensional andrological translational research project, which we have called **PROTEGGIMI** (**PRO**getto **TE**stosterone e fra**GI**lità negli uo**MI**ni) study.

PROTEGGIMI (**TE**stosterone and frailty in **M**ales: a multidimensional research **PRO**ject) will aim to:

1) develop an international (European) data Registry providing information on demographic, epidemiological and pathological/functional outcomes of laboratory-confirmed SARS-CoV-2 in males and of the same-ethnicity and age-matched healthy controls, taking into consideration that case fatality rate – and, their severe sequelae – may vary geographically, between age groups and temporarily;

2) assess both cross-sectionally and with a case-control analysis the circulating hormonal milieu in patients (according to age; ethnicity; comorbidities; BMI; symptoms; therapeutic approaches, and outcomes), and in age-matched healthy controls;

3) assess the genomic profile of specific subsets of confirmed SARS-CoV-2 males at higher susceptibility for severe sequaele and greater risk of death, in association with a specific androgenic profiles;

4) develop in vitro culture systems to investigate the impact of testosterone on different tissues, including human ACE2 in the testis and the reproductive tract[16], including epithelial and endothelial lung cells[22];

5) develop an animal model which may recapitulate the differences in terms of overall outcomes according to sex (females vs. males) and the associated hormonal milieu[23].

Moreover, it is clear that to elucidate the underlying pathophysiological mechanisms of SARS-CoV-2 action in the acute phase, there is also the need to evaluate long-term effects of the virus and in this respect we have agreed to develop a longitudinal study in terms of androgenic profile and monitoring possible SARS-CoV-2 infection-related changes in terms of male accessory glands physiology as well as semen quality as biological markers of overall male health and, more specifically, male reproductive potential. Indeed, very recent preliminary case-control findings have demonstrated that the process of spermatogenesis could potentially be damaged after SARS-CoV-2 recovery, especially in men of reproductive age[11].

This can and, we believe, should be our commitment to translational research as andrologists in this dramatic era of SARS-CoV-2.

WHY THIS OPINION ARTICLE?

In this *Opinion Article* we have highlighted the urgent need for novel research activities in this area and invite collaboration among Scientific Societies involved in the field of andrology, including other scientific researchers to join us in our efforts bridge a very newly-discovered gap in knowledge, which may have serious short- and long-term implications for the lives of millions of men.

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