Opinion Paper

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Do sex-specific immunobiological factors and differences in angiotensin converting enzyme 2 (ACE2) expression explain increased severity and mortality of COVID-19 in males?

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Abstract: Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2), shares similarities with the former SARS outbreak, which was caused by SARS-CoV-1. SARS was characterized by severe lung injury due to virus-induced cytopathic effects and dysregulated hyperinflammatory state. COVID-19 has a higher mortality rate in men both inside and outside China. In this opinion paper, we describe how sex-specific immunobiological factors and differences in angiotensin converting enzyme 2 (ACE2) expression may explain the increased severity and mortality of COVID-19 in males. We highlight that immunomodulatory treatment must be tailored to the underlying immunobiology at different stages of disease. Moreover, by investigating sex-based immunobiological differences, we may enhance our understanding of COVID-19 pathophysiology and facilitate improved immunomodulatory strategies.

Keywords: ACE2; coronavirus; COVID-19; gender; immunology.

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2), shares similarities with the former SARS outbreak, which was caused by SARS-CoV-1. [1] SARS was characterized by severe lung injury due to virus-induced cytopathic effects and dysregulated hyperinflammatory state [2]. COVID-19 has a higher mortality rate in men both inside and

outside China. [3, 4] This has been attributed to sex differences in comorbidities, smoking, cultural and psychosocial gender factors. [5] However, sex-specific immunobiological differences may also be involved, which may influence efficacy of COVID-19 immunomodulatory therapies.

RNA viruses like SARS-CoV-2 can be recognized by endosomal Toll-like receptor (TLR) 3 and TLR7, leading to production of type I interferons (IFN- α and IFN- β) which suppress viral replication and augment the host antiviral response. [6] SARS-CoV-1 inhibits proteins involved in TLR3 and TLR7 signaling pathways, [7, 8] thereby attenuating type I IFN response. In mice models, timing of type I IFN-response impacted SARS-CoV-1 disease severity. [2] Pretreatment of IFN- α inhibited SARS-CoV-1 replication *in vivo*, and an early type I IFN response (through administration of recombinant IFN- β 6 h post infection) protected mice from clinical lung disease. [2, 9] On the contrary, delayed and persistent type I IFN-response was associated with dysregulated hyperinflammatory state and severe lung disease characterized by excessive pulmonary inflammation, alveolar edema, increased pro-inflammatory cytokines (including interleukin-6) and enhanced T-cell apoptosis. [2]

The TLR7 gene is located on X chromosome, escapes X chromosome inactivation and has higher expression in females. [10] TLR7 ligands induce greater release of IFN- α from peripheral blood mononuclear cells from females *in vitro*. [11] Furthermore, female sex hormones have immunomodulatory effects. [12] Estradiol augment TLR7-induced type I IFN-response, [13] and SARS-CoV-1 had higher mortality in gonadectomized female mice compared to non-gonadectomized female counterparts. [14]

Additionally, females have higher CD4+ lymphocyte counts, [12] a subtype of blood cells essential for maintaining an effective and balanced immune response, with loss of CD4+ cells resulting in potentially increased immune-mediated pneumonitis and delayed viral clearance. [15] SARS-CoV-2 infection results in significant lymphopenia, which is associated with higher mortality. [16] Greater CD4+

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lymphocyte reserve may potentially decrease the risk of severe COVID-19 in women.

Finally, alterations in tissue expression of primary host receptor for SARS-CoV-2, angiotensin converting enzyme 2 (ACE2), may play a role in these sex differences. In three different RNA expression databases, (Human Protein Atlas, FAMTOM5 and GETx), ACE2 was found to be highly expressed in testicular cells at the protein levels, while little ACE2 expression was seen in ovarian tissue. [17] Evidence of COVID-19 infection of testicles is suggested by reported significant alterations in testosterone to luteinizing hormone (T to LH) ratio, with a low ratio in those infected with the virus (0.74) as compared to healthy controls (1.31). [18] Moreover, autopsy reports from SARS-CoV-1 demonstrated orchitis. [19] Importantly, the testes are a site of immune privilege, protected by the blood-testes barrier. As such, we proffer that the high ACE2 expression combined with immune privilege that limit T lymphocyte destruction of virally infected cells, may enable testes to serve as viral reservoir for COVID-19, leading to delayed viral clearance, potentially higher viral loads, and prolonged accumulative lung and systemic tissue damage.

We hypothesize that sex-specific immunobiological differences, including timing of type I IFN-response, may contribute at least in part to the observed sex differences in COVID-19 severity and mortality. Clinical trials using IFN- α are currently underway. We advise caution as type I IFN response may be dynamic and pleiotropic. Immunomodulatory treatment must be tailored to the underlying immunobiology at different stages of disease. Investigating sex-based immunobiological differences may enhance our understanding of COVID-19 pathophysiology and facilitate improved immunomodulatory strategies.

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