

EDITORIAL

**CORONAVIRUS COV-19/SARS-CoV-2 AFFECTS WOMEN LESS THAN MEN:
CLINICAL RESPONSE TO VIRAL INFECTION**P. CONTI¹ and A. YOUNES²¹*Postgraduate Medical School, University of Chieti, Chieti, Italy;*²*Medical Center “Mai più Dolore”, Pescara, Italy**Received March 31, 2020 – Accepted April 6, 2020*

CoV-19/SARS-CoV-2 is a highly pathogenic virus that causes coronavirus-19 disease (COVID-19) an acute respiratory distress syndrome which provokes serious problems for global health. Studies suggest that there are many differences between men and women in the immune response to CoV-19 infection and inflammatory diseases. Women, compared to men, are less susceptible to viral infections based on a different innate immunity, steroid hormones and factors related to sex chromosomes. The presence of two X chromosomes in women emphasize the immune system even if one is inactive. The immune regulatory genes encoded by X chromosome in female gender causes lower viral load levels, and less inflammation than in man, while CD4⁺ T cells are higher with better immune response. In addition, women generally produce higher levels of antibodies which remain in the circulation longer. The levels of activation of the immune cells are higher in women than in men, and it is correlated with the trigger of TLR7 and the production of IFN. TLR7 is higher in women than in men and its biallelic expression leads to higher immune responses and increases the resistance to viral infections. TLR7 is expressed in innate immune cells which recognizes single strand RNA virus by promoting the production of antibodies against the virus and the generation of pro-inflammatory cytokines including IL-6 and IL-1 family members. Moreover, in women the production of inflammatory IL-6 after viral infection is lower than in males and is often correlated with a better longevity. In addition, on the X chromosome there are loci that code for the genes involved in the regulation of immune cells such as FOXP3, and transcription factor for Treg involved in virus pathogenesis. The X chromosome influences the immune system by acting on many other proteins, including TLR8, CD40L and CXCR3 which can be over-expressed in women, and influence the response to viral infections and vaccinations. However, the biallelic expression of the X-linked genes can promote harmful autoimmune and inflammatory responses. Cardiovascular diseases are more frequent in males and subjects without cardiovascular dysfunctions infected by CoV-19 have a better prognosis, but these effects are still under study. It is hoped that certain drugs, such as CoV-19 receptor blockers, anti-inflammatories (against rheumatic diseases), monoclonal antibodies, anti-IL-1 and anti-IL-6, the remdesivir drug (analogue adenosine, effective against ebola), hydroxychloroquine (for the treatment of malaria) and vaccines, will open up new strategies and new therapeutic ways to combat this terrible virus.

The living beings around us, including the plant world, all coexist with microorganisms, including viruses, which normally host their species

and do not attack man. This also happens with fertilization, the human species can reproduce only with individuals of the same species, but when a

Key words: coronavirus 19; CoV-19, gender; virus; immunity; cytokine

Corresponding Author:

Prof. Pio Conti,
Postgraduate Medical School,
University of Chieti-Pescara,
Chieti 66013, Chieti, Italy
Tel./Fax: +39 08711 574136
e-mail: pconti@unich.it

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

species jumps (for example from animal to man, zoonotic disease, a fortunately infrequent event), a biological revolution takes place which leads to the formation of “monsters” or lethal pathologies. This is what happened with SARS-CoV-2 (CoV-19). Wild animals, such as snakes and pangolis, slaughtered by humans, can transmit CoV-19 to humans, who do not have an efficient immune system for this unknown microorganism which can cause COVID-19.

A number of studies suggest that there are many differences between men and women in the immune response to infectious and inflammatory diseases such as tuberculosis, malaria, hepatitis, HIV-1, mumps, measles, adenovirus, flu, etc. (1). Data from hospitals around the world tell us that males suffer more than women (25%) from respiratory system diseases, including those caused by acute viral infections. Women, compared to men, are less susceptible to viral infections based on a different innate and adaptive immunity, steroid hormone production by the gonads and factors related to sex chromosomes. Sex hormones are known to modulate innate immune responses in various infectious diseases including viral infections (2). In CoV-19 infections, sexual differences can affect several aspects, such as anti-viral immune response, morbidity, transmission and pathogenesis. Estrogen treatment prevents osteoporosis by inhibiting the cytokine pathway necessary for the differentiation and activation of osteoclasts. Estrogens modulate receptor response and pro-inflammatory cytokine production which, when released in excess can be very harmful and may lead to death (3). Therefore, the immune system can be modulated by estrogens that bind to the ER (estrogen receptor) alpha or beta receptor. ER-alpha is expressed by all immune cells and is involved in their maturation and regulation, and also is immune protective, since it is responsible for the production of IFN type I and the activation of NK cells. ER β has opposite effects to ER α and is involved in pro-inflammatory phenomena (4). ER α loss in older women is correlated with immunosuppression, demonstrating that estrogen can protect against COVID-19.

Peripheral blood mononuclear cells (PBMCs) treated with estrogen respond better to the antigen and

express Toll-like 7 (TLR7) with greater efficiency. CoV-19 binds to TLRs (key molecules in triggering innate immunity) of macrophages, dendritic cells and mast cells, causing an inflammatory cytokine storm with respiratory distress syndrome. Activation of TLR in the lung due to CoV-19 can promote vascularization and hyperemia with the production of inflammatory cytokines and chemokines that aggravate the state of the patient with COVID-19 (5). The presence of two X chromosomes in women affects the immune system even if one is inactive. The X chromosome acts on various elements of the immune system such as FOXP3, TLR7, TLR8, CD40L and CXCR3 which can be over-expressed in women and influence the response to viral infections and vaccinations (6). In CoV-19 infection, plasma viral load levels are lower in women than in men, while CD4⁺ T cells are higher, demonstrating higher response of the immune system in women. In addition, after vaccination, women generally produce in the circulation higher levels of antibodies, which have longer life than in men. The immune regulatory genes encoded by the X chromosome in the female gender cause lower viral load levels, inflammation and death after CoV-19 infection (7). Furthermore, the levels of activation of the immune cells influenced by sex hormone are higher in women than in males, and this is correlated with the activation of TLR7 and the production of IFN. Immune cells respond to CoV-19 infection with the production of IFNs which are cytokines that modulate the immune response, activate the host's cytotoxic cells and have anti-viral activity, even if it is highly pro-inflammatory (8). Low levels of IFNs control CoV-19 replication in absence of inflammation. In the immune cells of female individuals, the biallelic expression of the X-linked genes can promote harmful autoimmune and inflammatory responses; on the other hand it can be useful in the case of immune depression, as occurs in COVID-19. Hence, related to the X chromosomes, TLR7 is higher in women than in men and its expression leads to higher immune responses, although these reactions can cause autoimmune phenomena (9). Therefore, the female has a greater predisposition to autoimmunity, due to the overexpression of the endosomal TLR7 receptor

gene located on the X chromosome and the amount of TLR7 is crucial. It has been noted that TLR7 over-expression in women increases resistance to viral infections, as happens in CoV-19 infection. TLRs are important in controlling virus replication which can be inhibited by specific ligands for TLRs. Furthermore, TLR7 is expressed in dendritic cells, circulating monocyte, macrophages, and B cells which recognize single strand RNA CoV-19 by promoting the production of anti-CoV-19 antibodies and the generation of pro-inflammatory cytokines including IL-6 and IL-1 family members. On the X chromosome there are loci that code for the genes involved in the regulation of immune cells such as FOXP3, transcription factor of Treg (T regulatory) cells, TLR7 and TLR8 that bind the virus. TLR7 allows women to have a lower mortality rate, in most acute viral diseases with severe inflammation. In fact, in women, the production of inflammatory IL-6 in viral infections is lower than in males, and is often correlated with better health, even if sometimes the data are contradictory.

CoV-19 binds to immune cells, causing the antibody response in both COVID-19 and healthy non-symptomatic carrier patients (10). Early viral detection occurs through pattern recognition receptors (PRRs) expressed by APC cells and lung fibroblasts. These receptors activated by CoV-19 trigger NF- κ B which overproduce pro-inflammatory cytokines and chemokines, which aggravate the pathological state and lung functions. The PRRs are TLRs such as TLR-4, TLR3, TLR2/6, TLR7/8, which activate IL-4 as a protector cytokine and can trigger pro-inflammatory IL-6 production, which increases inflammation.

Damage-associated molecular pattern (DAMP) as high-mobility group box (HMGB) -1 (that activates the NF- κ B), and adenosine triphosphate (ATP) proteins are mainly associated with virus inflammation and other pathologies. CoV-19 infection induces stress and cellular inflammation and also the production of DAMP which in physiological conditions is not detected by the immune system. DAMP is involved in inflammation of the upper airways infected with CoV-19, mediates muscle weakness in several diseases and plays a crucial role

in the pathogenesis of fever. NLRP inflammasome protein is associated with DAMP secretion and is passively secreted in non-apoptotic cell death or actively in CoV-19-induced lung damage (11). The TLR2 receptor is involved in the secretion of DAMP, while the TLR4 acts as an inflammatory signal and can be activated by DAMP molecules. DAMPs have also been associated with nociceptive signaling and with increased virus spread and replication. After CoV-19 infection, DAMP molecules, such as ATP protein, can be released in stress and bind to P2X receptors (P2XRs) or P2Y receptors (P2YRs) which are involved in inflammation. ATP also plays a role in the activation of NLRP3 inflammasome linked with the generation of caspase-1, resulting in the formation of IL-1 which produces fever, inflammation and pain, and induces other pro-inflammatory cytokines. Antibodies against DAMP molecules confer significant protection and therapeutic target against damage inflammation and infection by CoV-19.

In innate immunity, CoV-19 stimulates immune cells, such as lymphocytes and monocytes, to produce IFN, important cytokines of the immune system; on the other hand the virus binds to the target cells, such as lung macrophages, pneumocytes and endothelial cells, and infects them (12). Activated natural killer (NK) cells recognize the infected CoV-19 cells as not-self and they kill them by fueling inflammation. In specific immunity, CoV-19 stimulates B lymphocytes and plasma cells to produce CoV-19 neutralizing IgG antibodies. IgG located on the infected cell are recognized by the Fc γ RIII receptor (CD16) of killer cells that enhance phagocytes, lysis and cell killing (antibody-dependent cell-mediated cytotoxicity) with consequent production of pro-inflammatory cytokines. CoV-19 infection can cause interstitial pneumonia with the formation of alveolar exudates, fibrosis and respiratory failure. In addition to this clinical picture, patients can develop *Staphylococcus* bacteria super-infection, a necrosis of the alveolar coatings and hemorrhagic exudation, severe respiratory syndrome, with a very high mortality rate (13). In CoV-19-infected lung, hyperemic and emphysematous areas are found with highly dilated vessels, micro-thrombus, diffuse alveolar damage, jalinous tissue formation and fibrosis. The other

organs of affected patients by the virus are also altered, such as heart, which is hypertrophic (with ventricular hypertrophy in hypertensive patients), and liver, which appears enlarged (hepatomegaly). The peripheral blood presents a marked neutrophilia with thrombocytopenia and lymphopenia. If the alveolar bronchial lavage fluid is analyzed, an overproduction of some major pro-inflammatory cytokines, such as IL-6, IL-1, and TNF, can be found, as well as a high concentration of chemokines including CXCL10 (IP-10), CXCL8 (IL-8), CCL2 (MCP1), CCL3 (MIP-1 α), and CCL5 (RANTES). Cytokines IL-1 and TNF are produced to limit the pathogenic action of CoV-19, but when they are released in excess in the surrounding tissues produce acute inflammation and may lead to death (14).

In clinical tests where testosterone levels were high, increased predisposition and susceptibility have been noted to cardiovascular inflammation, that is more frequent in males who are more vulnerable to CoV-19. Patients without cardiovascular disease infected by CoV-19 have a better prognosis compared to those with cardiac dysfunction and hypertension.

The angiotensin converting enzyme divides angiotensin I to generate angiotensin II and is a peptide that has various biological functions. A homologue of angiotensin-converting enzyme, termed angiotensin-converting enzyme 2 (ACE2), which protects murine lungs from acute respiratory distress syndrome (ARDS), has been recently identified as a receptor for CoV-19. CoV-19 binds to the ACE2 receptor, which is in various animal species, such as cats, pigs, but probably also in dogs and mice, which can develop COVID-19. CoV-19 and its Spike protein binds to ACE2 by weakening lung protection against the 'flu. Men compared to women expose more ACE2 receptor, located on the endothelium of the pulmonary vessels, that binds the virus and allows it to enter the cell. Several organs express ACE2 including heart, lung and kidney, therefore, the blockade or preventing the expression of this receptor could open new therapeutic ways to combat COVID-19. Inhibitors of the angiotensin converting enzyme (ACEI) and/or angiotensin II receptor type 1 are used in some clinical cases to stem the devastating effects of CoV-19 (15). These

inhibitors alleviate COVID-19 disease by reducing the viral load and also IL-6 levels that control viral replication via NF- κ B. They also have an effect on the immune system by increasing the number of CD3⁺ and CD8⁺ T cells in the peripheral blood of patients affected by CoV-19.

Many studies describe the influence of sex hormones on autoimmunity, and there are several autoimmune diseases more common to women than men, such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis, dermatomyositis, Sjogren syndrome, Hashimoto's thyroiditis, scleroderma, etc. For example, one of the most common autoimmune diseases in the female gender compared to the male is chronic autoimmune thyroiditis (or Hashimoto's thyroiditis) which in Western population affects 5-15% of women, while in men it is present in 1-5%. In this disease, the immune system produces anti-thyroglobulin antibodies by preventing the binding to iodine. An anti-thyroid stimulating hormone antibody is also generated which inhibits the pathway that leads to the production of T3 and T4. In both cases, follicular cells undergo apoptosis by reducing hormone production (16).

It is hoped that certain drugs, such as CoV-19 receptor blockers, anti-inflammatories (against rheumatic diseases), monoclonal antibodies, anti-IL-1 and anti-IL-6, the remdesivir drug (analogue adenosine, effective against ebola), hydroxychloroquine (for the treatment of malaria), and vaccines, will open up new strategies and new therapeutic ways to combat this terrible virus.

REFERENCES

1. Fung TS, Huang M, Liu DX. Coronavirus-induced ER stress response and its involvement in regulation of coronavirus-host interactions. *Virus Res* 2014; 194:110-23.
2. Kadel S, Kovats S. Sex hormones regulate innate immune cells and promote sex differences in respiratory virus infection. *Front Immunol* 2018; 9:1653.
3. Angele MK, Pratschke S, Hubbard WJ, Chaudry IH. Gender differences in sepsis: cardiovascular and immunological aspects. *Virulence* 2014;5(1):12-19.

4. Huang Z, Fang F, Wang J, Wong CW. Structural activity relationship of flavonoids with estrogen-related receptor gamma. *FEBS Lett* 2010; 584(1):22-26.
5. Hayashi K, Hooper LC, Detrick B, Hooks JJ. HSV immune complex (HSV-IgG: IC) and HSV-DNA elicit the production of angiogenic factor VEGF and MMP-9. *Arch Virol* 2009; 154(2):219-26.
6. Quakkelaar ED, Melief CJ. Experience with synthetic vaccines for cancer and persistent virus infections in nonhuman primates and patients. *Adv Immunol* 2012; 114:77-106.
7. Haque A, Akçeşme FB, Pant AB. A review of Zika virus: hurdles toward vaccine development and the way forward. *Antivir Ther* 2018; 23(4):285-93.
8. Qureshi S, Medzhitov R. Toll-like receptors and their role in experimental models of microbial infection. *Genes Immun* 2003; 4(2):87-94.
9. Souyris M, Mejía JE, Chaumeil J, Guéry JC. Female predisposition to TLR7-driven autoimmunity: gene dosage and the escape from X chromosome inactivation. *Semin Immunopathol* 2019; 41(2):153-164.
10. Conti P, Gallenga CE, Tetè G, et al. How to reduce the likelihood of coronavirus-19 (CoV-19 or SARS-CoV-2) infection and lung inflammation mediated by IL-1. *J Biol Regul Homeost Agents* 2020; 34(2). doi: 10.23812/Editorial-Conti-2. [Epub ahead of print].
11. Pandolfi F, Altamura S, Frosali S, Conti P. Key Role of DAMP in inflammation, cancer, and tissue repair. *Clin Ther* 2016; 38(5):1017-28.
12. Conti P, Ronconi G, Caraffa A, et al. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. *J Biol Regul Homeost Agents* 2020; 34(2). pii: 1. doi: 10.23812/CONTI-E. [Epub ahead of print].
13. Shifrin N, Raulet DH, Ardolino M. NK cell self tolerance, responsiveness and missing self recognition. *Semin Immunol* 2014;26(2):138-44.
14. Seki M, Kohno S, Newstead MW, et al. Critical role of IL-1 receptor-associated kinase-M in regulating chemokine-dependent deleterious inflammation in murine influenza pneumonia. *J Immunol* 2010; 184(3):1410-18.
15. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; 203(2):631-37.