Review

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Immune system and COVID-19 by sex differences and age

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Abstract

In COVID-19 disease, are reported gender differences in relation to severity and death. The aim of this review is to highlight gender differences in the immune response to COVID-19. The included studies were identified using PubMed, until 30 October 2020. The search included the following keywords: SARS-CoV-2, COVID-19, gender, age, sex, and immune system. Literature described that females compared to males have greater inflammatory, antiviral, and humoral immune responses. In female, estrogen is a potential ally to alleviate SARS-COV-2 disease. In male, testosterone reduces vaccination response and depresses the cytokine response. In the older patients, and in particular, in female older patients, it has been reported a progressive functional decline in the immune systems. Differences by gender were reported in infection diseases, including SARS-CoV-2. These data should be confirmed by the other epidemiological studies.

Keywords

age, aging, COVID-19, gender, immune system, SARS-CoV-2, sex, vaccine

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Introduction

The novel coronavirus COVID-19 was reported, as a pandemic, by the World Health Organization (WHO), on 11 March 2020. The ages of infected people range from a 4-week-old to >90 years and very few cases are observed in children and infants.[1] Male patients represent 59%–68% of all reported cases[2] with higher mortality rates. In over 75-year subjects, this population, in fact, represent a poor immune function, and they are most susceptible to infection and to complications.[3,4] In particular, the death rate in COVID-19 patients is 2.8% for female and 4.7% for male.[5] In Italy, male patients represent 65% of all deaths[6] with death ratio male/female: 1.79.[7] For each female died for COVID-19, 1.5 to 2 males died for this virus.[8] The quality and magnitude of immune response may, therefore, vary between men and women, pre- and post-menopausal women, or adults compared to children. Pre-menopausal adult women generally have stronger immune responses than children, men, or women during the post-menopause.[9] Different are the factor for gender differences in COVID-19 infection. In particular, there are multifactorial reasons (such as genetics, lifestyle differences, comorbidities, hormones, immune system, and aging) for these differences.[10,11] The aim of this review is to highlight gender differences in the immune response to COVID-19.

Methods

The included studies were identified using PubMed, until 30 October 2020. The search included the following keywords: SARS-CoV-2, COVID-19, gender, age, sex, immune system, and aging. Clinical trials, and

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retrospective and prospective studies were included. Studies written in languages other than English were excluded. Two authors (T.C. and O.P.) reviewed all study abstracts. Studies were included if gender differences in immune system were reported. All selected studies were qualitatively analyzed.

Immune response differences by gender

Sex hormones, for example, testosterone and estrogen, play diverse roles in immune responses.[12] Estrogen receptors (ERα and ERβ) are expressed in a diverse array of immune cells (T, B, natural killer cells, macrophages, DCs, and neutrophils). It is well known that estrogen suppresses T and B cell lymphopoiesis, activates B cell function, and influences T cell development. Moreover, estrogen regulates a number of cytokines (such as interleukin (IL)-1, IL-10, and interferon γ (INFγ)) that modulate the immune response. While estrogen has immune-stimulatory roles, progesterone and androgens are immune-suppressive and counteract the pathways affected by estrogen.[13] In particular, progesterone increases IL-4, reduces IFN-γ T helper cell type 1 (Th1) responses, and reduces T cell proliferation and T cell dependent antibody responses. However, in CD8 T cells, progesterone reduces IFN-γ and cytotoxicity.[14] The androgens also have immune-suppressive effects on the immune response. Testosterone in comparison to estrogen may predispose men to a widespread COVID-19 infection. Low serum levels of T, which should be supposed to characterize the hormonal milieu in seriously ill individuals, may predispose men, especially elderly men, to poor prognosis or death.[15] Furthermore, the effect of sex hormones on immune cell functions are proposed to be dose-dependent.[12] However, it has been reported[16] that immune profiles by gender diverge as age increases, even though the hormone levels decrease with age.

Immune response differences by age

The age is factor that induces the modification of immune response. One of the crucial attributes of an aging immune system is a chronic low-grade pro-inflammatory state. This state is greater in female than in male subjects.[17] To this regard, Ilardi et al.[18] have reported that gender and aging are a risk factor for COVID-19 infection.

In particular, it has been reported that immune-aging is an important factor in the increased susceptibility of older adults to infection[19] as well as their poor vaccination responses.[20] Immune-aging is strongly linked with a suboptimal innate immune response to viral pathogens. Physiological aging is accompanied, in fact, by a sub-clinical chronic low-grade state of systemic inflammation, *inflamm-aging*, characterized by elevated serum levels of acute phase proteins (e.g. C-reactive protein) and pro-inflammatory cytokines (e.g. TNF-α, IL-6, and IL-8).[21,22] It has been reported that *inflamm-aging* predisposes older adults to severe COVID-19 by suppressing the immune response to SARS-CoV-2.[23]

However, with aging, we observe both changes in cellular composition and in function.[18,24] In particular, in older patients (>65 years old), females have increased frequency of T and B cells, while blood B cells (numbers and percentages) were lower in older male.[25,26] To this regard, aging is accompanied by a reduction in the size of the peripheral B cell pool, with $CD19^+$ B cells significantly lower in older adults.27 Some authors have reported an age-related increase in the percentage or number of circulating CD^{27+} memory B cells.[28] Other study reported that the frequency of immunoglobulin M (IgM) memory B cells is decreased[29] or unchanged with age,[30] but aging is associated with secretion of antibodies that are weaker and of lower affinity.[31] In addition, $CD8^+$ T cells are particularly efficient in clearing virus-infected cells[32] and promote the production of virus-specific antibodies[33] that block the entrance of extracellular virus.[34]

However, naïve T cell frequencies decreased with age, particularly in $CD8^+$ T cells in both male and female subjects.[35] This is associated with gradual replacement of functional epithelial cells with fat and fibrous tissue,[36] thymic involution, and T cell immune-senescence.[37] In female subjects are reported elevated thymic function compared to male at all ages.[38] Another important factor of immune-aging is reduced natural killer cell cytotoxicity (NKCC), decline in lytic activity, and reduction in cytokine and chemokine production.[39] Beyer et al.[40] reported that female (18–64 years of age) generate a more robust protective antibody response and that the response of female to a half dose of the influenza vaccine is equivalent to the antibody response of male to the full dose. Moreover, Wang et al.[41] evidenced that in older female, influenza vaccination has been associated with lower hospitalization and mortality rates compared to male. However, the progressive reduction in testosterone levels observed in males, with aging can promote cell damage and inflammation.11 Therefore, aging has been linked with such chronic activation of innate immunity, with low-grade inflammation.[42]

Immune system in COVID-19 infection

The deleterious clinical manifestation of COVID-19 could be from a combination of exuberant innate immune responses and virus-induced direct cytopathic effects when the adaptive immune response fails to eliminate the virus. Severe COVID-19 patients exhibit significantly higher plasma levels of pro-inflammatory cytokine/chemokine, accompanied by impaired T cell and antibody response.[43] The elevation of these cytokines, including IL-6, has led to the hypothesis that an innate "cytokine storm" is the main cause for the toxicity, respiratory depression, and end-organ damage, seen in the severe COVID-19 cases.[44] However, it has been reported

that dysregulated immune response was associated with the severity of COVID-19.[45] In particular, disease severity was associated with reduced numbers of T cells in the blood[45] and male subjects experience a stronger "inflamm-aging" syndrome than female. It has been suggested that an accumulation of senescent cells may be responsible for this increase in lung inflammation with aging.[46]

The COVID-19 contributes to the damage to various organs, and it can infect endothelial cells and circulate through the body.[47] In fact, severe endothelial injury has been found in the lungs from COVID-19 patients.[48,49]

Thus, an increased presence of senescent cells may predispose to the development of severe COVID-19 by the following two conditions:

- Reduced immune cell clearance.[50]
- Increasing viral load.

Females and viral infections

Female are disproportionately affected by autoimmune disorders, whereas male is more susceptible to infectious diseases.[11] These differences are observed, both in terms of their intensity and prevalence.[51] Probably, the reduced susceptibility of females to viral infections could be attributed to the protection from X chromosome and sex hormones.[51] To this regard, it has been reported that the response to viruses differs between females and males.[11] Several studies have described that, during viral infections, females have greater inflammatory, antiviral, and humoral immune responses compare to males.[52] Sex steroids can have a crucial role on the function of inflammatory cells and regulation of the immune response.[53] In fact, it has been reported that they exert the following actions:

- Suppressive role in immune functions, acting on the androgen receptors.54
- Suppresses immune cell activity by reducing inflammatory and promoting anti-inflammatory mediators.[54]

These responses contribute to better clearance of viruses, including SARS-CoV-2 in female subjects. These data are in according with other studies that described higher antibody levels in adult females compared to males.[55] However, testosterone reduces vaccination response, while estrogen is a potential ally to alleviate SARS-COV-2 infection.[56–58]

ACE-2 and gender differences

Angiotensin-converting enzyme 2 (ACE-2) represents the primary route of infection of COVID-19. It is located on X chromosome, and female may have higher levels of this enzyme. It is expressed in lungs, kidneys, myocardium, gastrointestinal system, and reproductive organs. Although it remains unclear how a greater expression of ACE-2 in female patients seems not linked to worst rates of infection and worst outcomes in COVID-19 pandemic, it is clear that ACE-2, that represents the route of infection, also exerts several immunomodulating effects that may explain less severe clinical outcomes. Actions exerted by this enzyme consist not only in the conversion of angiotensin I, but also in immunomodulation and prevention of lung injury, with a protective effect in female subjects.[59]

What is known for Vaccine?

An effective vaccine is the best long-term therapy to the COVID-19 pandemic. The two most primary endpoints for defining the effectiveness of a COVID vaccine are as follows:

- Protection from infection as defined by seroconversion.
- Prevention of clinically symptomatic disease.

Effective vaccines are essential for interrupting the chain of transmission from animal reservoirs and infected humans to susceptible hosts. They are often complementary to antiviral treatment in the control of epidemics caused by emerging viruses. For rational COVID-19 vaccine, it is critical to understand the fundamental host–coronavirus interaction and protective immune mechanism.[60] The benefit of a cocktail vaccine strategy could induce immunity that can protect the host against not only the S-ACE2 interaction and viral entry to the host cells, but also protect against the accessary non-structural adhesin proteins (e.g. nsp3), which might also be vital to the viral entry and replication.[61] However, in relation to vaccine response by gender, a study of 331 Chinese patients, with confirmed SARS-CoV-2 infection, reported that the anti-SARS-CoV-2 immunoglobulin G (IgG) responses were related to the severity of the disease. The sex distribution of recovering cases was 36% and 65% for male and female, respectively.[52] Interestingly, the anti-SARS-CoV-2 IgG titers were similar by gender in patients with mild COVID-19 disease. In patients with severe disease, the female subjects exhibited a higher antibody response, than male, with the production of antibodies at earlier phases of the disease. These results were also consistent with other studies describing higher antibody levels (including more functional antibodies) in adult females compared to adult males.[62] The more intense immune responses in female predispose these subjects to autoimmune diseases, and to experience more adverse reactions to the vaccine.[57] Some of these differences can be linked to hormonal differences, such as estrogen and testosterone levels.[56] It has been reported that testosterone reduces vaccination response.[58]

Conclusion

It has been reported that an age-related increase in viral-induced inflammation has associated with exaggerated response with significant lung damage, leading to the increased morbidity and mortality rates in older adults. The current literature about COVID-19 indicates that the immune system plays a crucial role in setting the severity of the disease. To prevent progression of the severe forms of the disease, the immune system needs to be targeted. Moreover, higher titers of anti-S and anti-N IgG and IgM correlate with worse clinical readouts and older age,[63] suggesting potentially detrimental effects of antibodies in some patients. Even if they are not adopted in the current race for safe and effective COVID-19 vaccines, it is not too early for governmental agencies and not-for-profit organizations around the world to support the commercialization of these technologies to prepare for the need of rapid mass vaccination for the whole world in the next pandemic.

Key messages

- Females have increased resistance to viral, bacterial, fungal, and parasitic organisms than males.
- Females are less susceptible to microbial infections.
- Females have a higher innate immune response than males.
- Females produce lower levels of inflammatory mediators and increase the production of immunosuppressive molecules to reduce systemic inflammation.
- The protection of females to microbial and viral affections is attributed to the protection provided by the X chromosome and sex hormones, which modulate the innate and adaptive immunity.
- Elevated estrogen levels in female COVID-19 patients may reduce the severity and mortality of COVID-19 deaths through an elevation in the innate and humoral response.
- Men tend to have a higher risk of severe infection and mortality related to COVID-19.
- The severity of COVID-19 is related to the level of the pro-inflammatory cytokines and subsets of immune cells.

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References

1. Dong E, Du H and Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis* 2020; 20(5): 533–553.
2. Yu Y, Shi Q and Chen H. Clinical trial analysis of 2019-nCoV therapy registered in China. *J Med Virol* 2020; 92(7): 711–712.
3. Guzik TJ, Mohiddin SA, Dimarco A, et al. COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. *Cardiovasc Res* 2020; 116(10): 1666–1687.
4. Li X, Geng M, Peng Y, et al. Molecular immune pathogenesis and diagnosis of COVID-19. *J Pharm Anal* 2020; 10(2): 102–108.
5. https://www.worldometers.info/coronavirus/coronavirus-age-sex-demographics
6. https://www.epicentro.iss.it/coronavirus/
7. Gebhard C, Regitz-Zagrosek VR, Neuhauser HK, et al. Impact of sex gender on Covid-19 outcomes in Europe. *Biol Sex Diff* 2020; 11: 29.
8. https://www.nytimes.com/2020/04/07/health/coronavirus-new-york-men.html
9. Giefing-Kroll C, Berger P, Lepperdinger G, et al. How sex and age affect immune responses, susceptibility to infections, and response to vaccination. *Aging Cell* 2015; 14(3): 309–321.
10. Bartz D, Chitnis T, Kaiser UB, et al. Clinical advances in sex-and gender-informed medicine to improve the health of all: A review. *JAMA Int Med* 2020; 180: 574–583.
11. Klein SL and Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* 2016; 16(10): 626–638.
12. Klein SL, Marriott I and Fish EN. Sex-based differences in immune function and responses to vaccination. *Trans R Soc Trop Med Hyg* 2015; 109(1): 9–15.
13. Moulton VR. Sex hormones in acquired immunity and autoimmune disease. *Frontiers Immun* 2018; 9: 2279.
14. Ortona E, Pierdominici M and Rider V. Editorial: Sex hormones and Gender differences in immune responses. *Front Immunol* 2019; 10: 1076.
15. Giagulli VA, Guastamacchia E, Magrone T, et al. Worse progression of COVID-19 in men: Is testosterone a key factor? *Andrology* 2021; 9(1): 53–64.
16. Márquez EJ, Chung CH, Marches R, et al. Sexual-dimorphism in human immune system aging. *Nat Commun* 2020; 11(1): 751.
17. Fink AL and Klein SL. Sex and gender impact immune responses to vaccines among the elderly. *Physiology* 2015; 30(6): 408–416.
18. Ilardi A, Politi C and Ciarambino T. COVID-19: Could sex and age be a risk factor? Minerva Med. *Epub ahead of print* 20 July 2020. DOI: 10.23736/S0026-4806.20.06705-1.

19. Molony RD, Malawista A and Montgomery RR. Reduced dynamic range of antiviral innate immune responses in aging. *Exp Gerontol* 2018; 107: 1305.
20. Lord JM. The effect of ageing of the immune system on vaccination responses. *Hum Vaccin Immunother* 2013; 9(6): 1364–1367.
21. Bartlett DB, Firth CM, Phillips AC, et al. The age-related increase in low-grade systemic inflammation (Inflamm-aging) is not driven by cytomegalovirus infection. *Aging Cell* 2012; 11(5): 912–915.
22. Franceschi C, Bonafe M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci* 2000; 908: 244–254.
23. Hazeldine J and Lord JM. Immunesenescence: a predisposing risk factor for the development of COVID-19? *Front Immunol* 2020; 11: 573662.
24. Ucar D, Marquez EJ, Chung CH, et al. The chromatin accessibility signature of human immune aging stems from CD8(+) T cells. *J Exp Med* 2017; 214(10): 3123–3144.
25. Bienvenu LA, Noonan J, Wang X, et al. Higher mortality of COVID-19 in males: sex differences in immune response and cardiovascular comorbidities. *Cardiovas Res* 2000; 116: 2197–2206.
26. Hirokawa K, Utsuyama M, Hayashi Y, et al. Slower immune system aging in women versus men in the Japanese population. *Immun Ageing* 2013; 10(1): 19.
27. Ademokun A, Wu YC and Dunn-Walters D. The ageing B cell population: composition and function. *Biogerontology* 2010; 11(2): 125–137.
28. Veneri D, Ortolani R, Franchini M, et al. Expression of CD27 and CD23 on peripheral blood B lymphocytes in humans of different ages. *Blood Transfus* 2009; 7(1): 29–34.
29. Shi Y, Yamazaki T, Okubo Y, et al. Regulation of aged humoral immune defense against pneumococcal bacteria by IgM memory B cell. *J Immunol* 2005; 175: 3262–3267.
30. Frasca D, Landin AM, Lechner SC, et al. Aging down-regulates the transcription factor E2A, activation-induced cytidine deaminase, and Ig class switch in human B cells. *J Immunol* 2008; 180: 5283–5290.
31. Frasca D, Nguyen D, Riley RL, et al. Effects of aging on proliferation and E47 transcription factor activity induced by different stimuli in murine splenic B cells. *Mech Ageing Dev* 2003; 124(4): 361–369.
32. Channappanavar R, Fett C, Zhao J, et al. Virus-specific memory CD8 T cells provide substantial protection from lethal severe acute respiratory syndrome coronavirus infection. *J Virol* 2014; 88: 11034–11044.
33. Channappanavar R, Zhao J and Perlman S. T cell-mediated immune response to respiratory coronaviruses. *Immunol Res* 2014; 59: 118–128.
34. Sok D and Burton DR. Recent progress in broadly neutralizing antibodies to HIV. *Nat Immunol* 2018; 19(11): 1179–1188.
35. Olson NC, Doyle MF, Jenny NS, et al. Decreased naive and increased memory CD4(+) T cells are associated with subclinical atherosclerosis: the multi-ethnic study of atherosclerosis. *PLoS ONE* 2013; 8(8): e71498.
36. Yang H, Youm YH, Sun Y, et al. Axin expression in thymic stromal cells contributes to an age-related increase in thymic adiposity and is associated with reduced thymopoiesis independently of ghrelin signaling. *J Leukoc Biol* 2009; 85(6): 928–938.
37. Fagnoni FF, Vescovini R, Passeri G, et al. Shortage of circulating naive CD8(+) T cells provides new insights on immunodeficiency in aging. *Blood* 2000; 95: 2860–2868.
38. Clave E, et al. Human thymopoiesis is influenced by a common genetic variant within the TCRA-TCRD locus. *Science Translational Med* 2018; 10(457): eaao296.
39. Mariani E, Pulsatelli L, Meneghetti A, et al. Different IL-8 production by T and NK lymphocytes in elderly subjects. *Mech Ageing Dev* 2001; 122: 1383–1395.
40. Beyer WE, Palache AM, Kerstens R, et al. Gender differences in local and systemic reactions to inactivated influenza vaccine, established by a meta-analysis of fourteen independent studies. *Eur J Clin Microbiol Infect Dis* 1996; 15(1): 65–70.
41. Wang CS, Wang ST and Chou P. Efficacy and cost-effectiveness of influenza vaccination of the elderly in a densely populated and unvaccinated community. *Vaccine* 2002; 20: 2494–2499.
42. Márquez EJ, Trowbridge J, Kuchel GA, et al. The lethal sex gap: COVID-19. *Immun Age* 2020; 17: 13.
43. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395: 497–506.
44. Mehta P, McAuley DF, Brown M, et al. COVID-19: Consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; 395: 1033–1103.
45. Qin C, Zhou L, Hu Z, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin Infect Dis* 2020; 71: 762–768.
46. Parikh P, Wicher S, Khandalavala K, et al. Cellular senescence in the lung across the age spectrum. *Am J Physiol Lung Cell Mol Physiol* 2019; 316: L826–L842.
47. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020; 395: 1417–1141.
48. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* 2020; 383: 120–112.
49. Beacon TH, Su RC, Lakowski TM, et al. SARS-CoV-2 multifaceted interaction with the human host. Part II: Innate immunity response, immunopathology, and epigenetics. *IUBMB Life* 2020; 72: 2331–2354.
50. Vukmanovic-Stejic M, Chambers ES, Suarez-Farinas M, et al. Enhancement of cutaneous immunity during aging by blocking p38 mitogen-activated protein (MAP) kinase-induced inflammation. *J Allergy Clin Immunol* 2018; 142(3): 844–856.
51. vom Steeg LG and Klein SL. SeXX matters in infectious disease pathogenesis. *PLoS Pathog* 2016; 12(2): e1005374.
52. Zeng F, Dai C, Cai P, et al. A comparison study of SARS-CoV-2 IgG antibody between male and female COVID-19 patients: a possible reason underlying different outcome between gender. *medRxiv preprint*, 2020, https://www.medrxiv.org/content/10.1101/2020.03.26.20040709v1
53. Vom Steeg LG, Attreed SE, Zirkin B, et al. Testosterone treatment of aged male mice improves some but not all aspects of age-associated increases in influenza severity. *Cell Immunol* 2019; 345: 103988.

54. Bereshchenko O, Bruscoli S and Riccardi C. Glucocorticoids, sex hormones, and immunity. *Front Immunol* 2018; 9: 1332.
55. Klein SL, Jedlicka A and Pekosz A. The Xs and Y of immune responses to viral vaccines. *Lancet Infect Dis* 2010; 10(5): 338–349.
56. Ruggieri A, Anticoli S, D'Ambrosio A, et al. The influence of sex and gender on immunity, infection and vaccination. *Ann Ist Super Sanita* 2016; 52(2): 198–204.
57. Fischinger S, Boudreau CM, Butler AL, et al. Sex differences in vaccine-induced humoral immunity. *Semin Immunopathol* 2019; 41(2): 239–249.
58. Trigunaite A. Suppressive effects of androgens on the immune system. *Cell Immunol* 2015; 294: 87–94.
59. Bhatia K, Zimmerman MA and Sullivan JC. Sex differences in angiotensin-converting enzyme modulation of Ang (1–7) levels in normotensive WKY rats. *Am J Hypertens* 2013; 26(5): 591–598.
60. Roper RL and Rehm KE. SARS vaccines: where are we? *Expert Rev Vaccines* 2009; 8: 887–898.
61. Ong E, Wong MU, Huffman A, et al. COVID-19 coronavirus vaccine design using reverse vaccinology and machine learning, 2020, https://www.biorxiv.org/content/10.1101/2020.03.20.000141v2
62. Potluri T, Fink AL, Sylvia KE, et al. associated changes in the impact of sex steroids on influenza vaccine responses in males and females. *NPJ Vaccines* 2019; 4: 29.
63. Jiang HW, Li Y, Zhang HN, et al. Global profiling of SARS-CoV-2 specific IgG/IgM responses of convalescents using a proteome microarray. *medRxi preprint*, 2020, https://www.medrxiv.org/content/10.1101/2020.03.20.20039495v1