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Pivonello R. Auriemma R.S. Pivonello C. Isidori A.M. Corona G. Colao A. Millar R.P.

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ROSARIO PIVONELLO[a,b], RENATA SIMONA AURIEMMA[c], CLAUDIA PIVONELLO[c], ANDREA M. ISIDORI[d], GIOVANNI CORONA[e], ANNAMARIA COLAO[b,c*] AND ROBERT P. MILLAR[f,g*]

[a]DIPARTIMENTO DI MEDICINA CLINICA E CHIRURGIA, SEZIONE DI ENDOCRINOLOGIA, UNITÀ DI ANDROLOGIA E MEDICINA DELLA RIPRODUZIONE E DELLA SESSUALITÀ MASCHILE E FEMMINILE (FERTISEXCARES), UNIVERSITÀ FEDERICO II DI NAPOLI, NAPLES, ITALY; [b]UNESCO CHAIR FOR HEALTH EDUCATION AND SUSTAINABLE DEVELOPMENT, FEDERICO II UNIVERSITY, NAPLES, ITALY; [c]DIPARTIMENTO DI MEDICINA CLINICA E CHIRURGIA, SEZIONE DI ENDOCRINOLOGIA, UNIVERSITÀ FEDERICO II DI NAPOLI, NAPLES, ITALY; [d]DEPARTMENT OF EXPERIMENTAL MEDICINE, "SAPIENZA" UNIVERSITY OF ROME, ROME, ITALY; [e]ENDOCRINOLOGY UNIT MEDICAL DEPARTMENT, MAGGIORE-BELLARIA HOSPITAL, BOLOGNA, ITALY; [f]CENTRE FOR NEUROENDOCRINOLOGY, DEPARTMENT OF IMMUNOLOGY, FACULTY OF HEALTH SCIENCES, UNIVERSITY OF PRETORIA, GEZINA, PRETORIA, SOUTH AFRICA; [g]NEUROSCIENCES INSTITUTE AND INSTITUTE OF INFECTIOUS DISEASES AND MOLECULAR MEDICINE, UNIVERSITY OF CAPE TOWN, CAPE TOWN, SOUTH AFRICA.

*EQUALLY CONTRIBUTED AS SENIOR AUTHORS

Short title: Sex disparities in COVID-19 severity and outcome

CORRESPONDENCE

Rosario Pivonello, MD, PhD

Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Unità di Andrologia e Medicina della Riproduzione e della Sessualità Maschile e Femminile (FERTISEXCARES), Università "Federico II" di Napoli, Naples, Italy

Staff of UNESCO Chair for Health Education and Sustainable Development, Section of Andrology and Reproductive and Sexual Medicine

Phone: +39 0817464983

E-mail: rosario.pivonello@unina.it

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Abstract

The COVID-19 outbreak is a global public health issue, having profound effects on most aspects of societal well-being, including physical and mental health. A plethora of studies, globally, have suggested the existence of a sex disparity in the outcome of COVID-19 patients, that is mainly due to mechanisms of viral infection, immune response to the virus, development of a hyperinflammation, and development of systemic complications, particularly thromboembolism. These differences appear to be more pronounced in elderly COVID-19 patients. Epidemiological data report a sex difference in the severity and outcome of COVID-19 disease with a more favourable course of the disease in women compared to men, regardless of age range although the rate of SARS-CoV-2 infection seems to be similar in both sexes. Sex hormones, including androgens and estrogens, may not only impact viral entry and load, but also shape the clinical manifestations, complications and, ultimately, the outcome of COVID-19 disease. The current review comprehensively summarizes current literature on sex disparities in susceptibility and outcomes of COVID-19 disease as well as the literature underpinning the pathophysiological and molecular mechanisms, which may provide a rationale to a sex disparity. These include sex hormone influences on molecules that facilitate virus entry and priming, as well as the immune and inflammatory response, as well as coagulation and thrombosis diathesis. Based on present evidence, women appear to be relatively protected from COVID-19 because of a more effective immune response and a less pronounced systemic inflammation, with consequent moderate clinical manifestations of the disease, together with a lesser predisposition to thromboembolism. Conversely, men appear to be particularly susceptible to COVID-19 disease because of a less effective immune response with consequent increased susceptibility to infections, together with a greater predisposition to thromboembolism. In elderlies, sex disparities in overall mortality following SARS-CoV-2 infection is even more palpable, as elderly men appear more prone to severe COVID-19 because of a greater predisposition to infections, a weaker immune defence and an enhanced thrombotic state compared to women. The information revealed from the review highlights potential novel therapeutic approaches employing the administration of hormonal or anti-hormonal therapy in combination with antiviral drugs in COVID-19 patients.

Background

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in December 2019 in Wuhan, China, and rapidly spread globally, reaching pandemic dimensions [1]. At the latest update of the 14th of October 2020, more than 38 million cases have been reported from 214 countries and territories, resulting in more than 1 million deaths against a background of more than 29 million recoveries (https://www.worldometer.info/coronavirus). **Table 1** shows epidemiological data for COVID-19 from across the world.

The clinical syndrome of COVID-19 typically includes fever and dry cough, less commonly associated with dyspnoea and fatigue, with muscle and joint pain, headache and dizziness, and gastrointestinal disturbances, together with loss of smell and taste [1]. The wide spectrum of clinical manifestation range from a silent (asymptomatic disease) or mild (common cold-type disease) clinical syndrome, in the majority of cases, to a severe disease in a minority of cases, progressing toward acute respiratory distress syndrome (ARDS), followed by thromboembolism, septic shock and multi organ injury, likely in dependence of viral load and host conditions [1]. The dramatic lethality of COVID-19 is related to the relevant SARS-CoV-2 capacity to infect the population, and the relevant ability of the infection to induce serious and life-threatening clinical complications in subsets of the population, especially elderly people and subjects with cardiometabolic diseases and/or conditions of immunosuppression [2].

SARS-CoV-2 is a coronavirus belonging to the aggressive beta-CoV type, such as SARS-CoV and MERS-CoV, formed by a single-strand positive RNA covered by an envelope that contains the spike proteins, responsible for binding to receptors and for fusion with membrane of host cells [1, 3]. SARS-CoV-2 binds angiotensin-converting enzyme 2 (ACE2), a regulator of the renin-angiotensin-system (RAS) involved in the biotransformation of the vasoconstrictor angiotensin II (AngII) to the vasodilator angiotensin 1-7 (Ang(1-7)), abundantly expressed on the cell surface of human alveolar epithelial cells, or pneumocytes, but also present on respiratory tract epithelial cells, intestine enterocytes, kidney proximal tubule cells, vessel endothelial cells and cardiomyocytes as well as hematopoietic and immune cells, and used by the virus as an entry receptor into the host cells [1, 4-7]; SARS-CoV-2 uses different protease enzymes, especially transmembrane protease serine 2 (TMPRSS2), a specific protease expressed on the cell surface of human pneumocytes, but also present in intestine enterocytes, kidney cells and vessel endothelial cells, as priming factor,

through the favouring of the internalization of the virus into the host cell, by means of the cleavage of the spike proteins, which facilitate the fusion of envelope of the virus with the membrane of the host cells, and, consequently, the viral priming [5]. Importantly, TMPRSS2 and ACE2 are co-expressed not only in respiratory system, particularly in lung, but also in intestine and kidney and vascular system [1, 4-7], which are main targets of SARS-CoV-2 and mostly damaged organs in COVID-19.

The main conduit of infection is the respiratory system, where ACE2 expression progressively decreases from the nose to the bronchioalveolar tree of the lung [8]. The first relevant host cells for virus are represented by pneumocytes; inside the pneumocytes, the virus replicates, inducing an increase of viral load, with progeny viruses spreading and infecting surrounding receptive cells [3, 6, 9]. The infected host cells undergo pyroptosis, a dramatic inflammatory form of programmed cell death occurring frequently as consequence of infection from cytopathic viruses [6, 9]. During pyroptosis, the activation and assembly of inflammasomes, the typical cytosolic multiprotein complexes responsible for the activation of inflammatory responses, triggers the maturation, and secretion, through the formation of pores in the cell membrane, of proinflammatory cytokines, especially interleukin-1 (IL-1). Ultimately, this phenomenon induces cell destruction with generation and release of damage-associated molecular patterns, which together with proinflammatory cytokines, are recognized by local cells of innate immune system, such as alveolar macrophages, triggering the generation and secretion of chemokines and cytokines, mainly including interleukin 6 (IL-6). Moreover, cells of innate immune system, expressing the toll like receptors (TLRs), including TLR4 and TLR7, able to identify various pathogens, recognize spike protein and/or single-strand RNA of SARS-CoV-2 and locally attract additional innate response immune cells, including not only macrophages but also monocytes, dendritic cells, natural killer (NK) cells and neutrophils, together with immune cells of adaptive immune response, especially T cells, therefore promoting further inflammation, also emphasized by the secretion of T cell produced interferon-γ (IFN-γ), and establishing an inflammatory feedback loop [6, 9].

The degree of COVID-19 is the result of the interplay between virus virulence and host resistance, with the transition between innate and adaptive immune response, still not fully elucidated, playing a crucial role in the clinical progress of the SARS-CoV-2 infection from a mild to a severe disease [6, 9]. In subjects with healthy immune response, the initial inflammation induces by the innate immune system attracts adaptive immune cells, such as virus-specific T cells and B cells, to the site of the infection; T cells, CD4+ T cells (T helper), CD8+ T cells (T suppressor) and T

regulatory, cooperate for eliminating the infected cells before the virus spread, whereas B cells produce virus-specific neutralizing antibodies, which block the virus and permit macrophages to recognize and phagocyte neutralized viruses and death cells, whose elimination associates with minimal lung damage, resulting in absent or mild clinical syndrome and following recovery [6, 9]. Conversely, in subjects with pre-existent or virus-induced dysfunctional immune response and/or an intense viral load, the immune response is not effective and T cells and macrophages are not able to inhibit viral proliferation and to eliminate the infected cells, with B cells eventually producing non-neutralizing antibodies. This condition may enhance virus infection and consequent exaggerated production of cytokines, which include not only IL-1 and IFN-y but also macrophage inflammatory protein 1 (MIP1), monocyte chemoattractant protein-1 (MCP1), granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF), and various different interleukins, such as IL-2, IL-7, IL-8, IL-10, IL-17, IL-18, and especially IL-6, resulting in an excessive inflammatory reaction, or hyperinflammation, characterized by the so called cytokine storm, and responsible for the typical interstitial pneumonia [6, 9]. Concomitantly, the accumulation of inflammatory cells and cytokines and the viral infection of endothelial cells lead to endothelial cell injury, or endothelitis, and degradation of extracellular matrix, inducing the loosening of interendothelial junctions, and promoting increased vascular permeability and vascular leakage [10]. Moreover, neutrophil extracellular traps, structures of nucleotides and proteins expelled by neutrophils to ensnare pathogens, activate platelets that, together with the hypercoagulability, resulting from the inflammatory-induced stimulation of procoagulant factors, such as fibrinogen, tissue factor, factors (F) VIII, FVIIa, FXa, and FIIa and Von Willebrand and mainly from the inhibition of anticoagulant factors, such as tissue factor pathway inhibitor, antithrombin III and activated C protein, lead to the development of microvascular thrombosis [11]. The status of hyperinflammation and hypercoagulability act together in inducing thromboembolism in the lungs, thus leading to respiratory failure [12]. Notably, the hyperinflammation, with recruitment of immune cells from the blood to the lungs and infiltration of lymphocytes to the airways, and the consequent hypercoagulability, may explain the main laboratory findings of the COVID-19-related interstitial pneumonia, biochemically characterized by lymphopenia, increase of inflammatory markers (erythrocyte sedimentation rate, C-reactive-protein, ferritin), alteration of coagulation parameters (prolonged thrombin and prothrombin time, prolonged international normalized ratio, thrombocytopenia, increase of fibrinogen and D-dimer), together with a dramatic increase in cytokines, such as TNF, and,

especially, IL-1 and IL-6 [1, 2]. The hyperinflammation, with the cytokine storm, because of endotheliopathy and vascular permeability, circulates to different organs, with resulting systemic inflammation, whereas hypercoagulability leads to disseminated coagulopathy, resulting in diffuse thrombosis. The consequent cardiovascular collapse and reduction of peripheral perfusion lead to septic shock and multiorgan injury, with consequent liver and kidney failure that is likely the major cause of death in COVID-19 [9, 12].

Figure 1 shows the dysfunctional immune response and the exaggerated inflammatory response to SARS-CoV-2 infection in case of severe COVID-19, which promotes systemic inflammation, endotheliopathy, disseminated coagulopathy and thromboembolism, leading to septic shock, multiorgan injury and eventual death.

Sex disparities in severity and outcome of COVID-19

Although global epidemiological data suggest a similar prevalence in virus infection between men and women, a clear sex-related difference in the severity of the disease, with a more favourable outcome in women compared to men has been described [13-17]. Indeed, according to the last update of the global situation on 14th October 2020, the overall prevalence of contagion in men and women was 53.3% and 46.7%, whereas the prevalence of death in men and women was 58.3% and 41.7%, respectively. In Italy, which is one of first country, after China, to be involved in the world, and one of the countries with the highest contagion and lethality in Europe, the National Institute of Health (NIH) confirmed a difference in mortality between men and women. At the update on the 30th of March 2020, corresponding to the week following the first Italian epidemiological peak of the infection, the NIH reported in the entire Italian population an infection rate slightly higher in men (52206, 55.7%) than in women (41549, 44.3%), but a lethality rate in men (6930, 13.3%), which almost doubled that recorded in women (3083, 7.4%), with a clearly higher relative prevalence of death in men (69.1%) than in women (30.8%). This finding was confirmed at all age groups, and is strengthened by the evidence of a median age of deceased patients lower in men (79 years) than in women (85 years) (http://www.epicentro.iss.it/coronavirus). Noteworthy, during the first phases of pandemic the infection rate was calculated predominantly on the symptomatic population, which was generally tested for the presence of the virus. Conversely, during the second phase of the pandemic, a wider population was tested for the presence of the virus, including predominantly asymptomatic subjects, which were in contact with

symptomatic and/or positive subjects in a population prevention strategy, with an expected change of the infection rate in men and woman. Interestingly, a sex-related difference has been confirmed at the latest update on 14th October 2020, when the NIH reported in the entire Italian population a slightly lower infection rate in men (1730180, 48.3%) than in women (185300, 51.7%), but a lethality rate in men (20759, 12%) persistently higher, although at lesser extent, than in women (15474, 8.4%), with a higher relative prevalence of death in men (57.3%) than in women (42.7%), nevertheless testifying the persistence of the sex-related difference in disease severity and outcome. This finding was confirmed at all age groups including in the elderly age, where lethality rate was still higher in men as compared to women. The progressive reduction of the sex-related difference in lethality might reflect a more timely and accurate diagnosis, consequence of a wider research for the presence of the virus in symptomatic and asymptomatic subjects, and a more effective treatment, consequence of the increasing knowledge of the infection mechanisms and body reactions, also considering the typical trend of Italian population, where elderly people, especially people after the age of 80 years, is composed by more women than men.

A similar epidemiological disparity between sexes was previously reported in patients with SARS and MERS with mortality rate being higher in elderly men, especially those with critical illness [18].

Table 1 shows the overall prevalence of infections and deaths in men and women worldwide. **Table 2** shows sex disparities of mortality data in COVID-19 available from literature.

Hypotheses underlying sex disparities in Covid-19 outcome

Several hypotheses have been postulated to explain the greater severity and the less favourable outcome of COVID-19 in men compared to women. Differences in cultural and social behaviours in men and women have been claimed [19, 20], together with the presence of comorbidities, such as cardiovascular and respiratory diseases, as well as smoking habits and alcohol intake, which are generally more prevalent in men compared to women [20]. Furthermore, the disparities between sexes has been also attributed to the evidence that men adhere to hygiene practices, including simple handwashing behaviour, less rigorously and assiduously than women [20, 21], with consequent easier infection and burden of the disease in men compared to women.

Besides these considerations, an increasing body of evidence has indicated that the sex-related difference in the severity and outcome in COVID-19 patients is mainly ascribable to mechanisms of

viral infection, immune response to the virus, development of a hyperinflammation and hypercoagulability and/or systemic inflammation and thromboembolism.

The current reviews described the present evidence focusing on data derived by human studies and including animal studies in sections with scarce, conflicting, or absent human studies. However, some of the investigations reporting on these mechanisms are not always robustly evidence-based, permitting exclusively an iconoclastic but balanced assessment of current available information on the issue.

Sex disparities in the mechanisms of virus infection

Similarly to SARS-CoV, but differently to MERS-CoV, SARS-CoV-2 uses the cell surface enzyme ACE2 and protease TMPRSS2 that provide virus cell entry and priming. Different genetic and endocrine mechanisms, including sex hormones actions, might influence the mechanisms of SARS-CoV-2 virus infection.

Genetic mechanisms seem to regulate ACE2 and TMPRSS2 expression in humans differently in men and women. ACE2 appears to be differently expressed in men and women, due to the gene localization on X chromosome, but the differential expression seems to occur in a tissue specific manner. Although ACE2 is an X-linked gene escaping from X inactivation, suggesting a greater generalized expression in women compared to men, data from postmortem donors displayed a significant male-biased ACE2 expression in the majority of tissues, including lung and gastrointestinal system and blood vessels, and a female-biased ACE2 expression in a minority of tissues, including subcutaneous adipose tissue, cardiac left ventricle and pancreas [22]. However, ACE2 expression in bronchial epithelial cells has been supposed to be higher in women than in men, since ACE2 gene has been demonstrated to be epigenetically regulated through DNA methylation with evidence of hypomethylation in women compared to men [23]. Interestingly, ACE2 was expressed in male reproductive system, especially in spermatogonia, Leydig and Sertoli cells [24], and in female reproductive system, especially in the ovary [25], although the impact of SARS-CoV-2 infection on ACE2 gonadal function is not fully investigated [26]. In contrast to ACE2, TMPRSS2 is apparently not associated with a significant sex-related differential expression in humans, likely because the gene is not localized on sex chromosomes. Despite bioinformatic reports of a slightly increased TMPRSS2 in the bronchial epithelial cells in men compared to women, a non-significant sex-biased TMPRSS2 expression has been demonstrated in lung [27].

Noteworthy, the presence of different single nucleotide polymorphisms (SNPs) in TMPRSS2 gene leads to the formation of two specific haplotypes; one of these haplotypes, characterized by the presence of three specific SNPs, and more frequently expressed in Italian than East Asian populations, is predicted to be associated with upregulation of TMPRSS2 expression and has been found to be also associated with increased susceptibility to H7N9 influenza A virus infection, whose incidence is double in men that in women; this evidence might also explain the high susceptibility to SARS-CoV-2 infection and lethality of COVID-19 in Italian men [27]. Interestingly, TMPRSS2 is expressed in male reproductive system, predominantly in the prostate, seminal vesicles and epididymis but not in the testes [28], and seems not to be expressed in female reproductive system [29], although the impact of SARS-CoV-2 infection on TMPRSS2 gonadal function is not fully investigated [26].

Endocrine mechanisms, including the action of sex hormones, seem to regulate ACE2 and TMPRSS2 expression and/or function, in a species-specific and tissue-specific manner, differently in males and females. Noteworthy, sex hormones have been found to modulate not only ACE2 expression, but also ACE2 activity, probably through post-transcriptional or post-translational mechanisms, although with conflicting results between animals and humans. In rodents, where renal ACE2 expression and activity are higher in males than females, gonadectomy, characterized by a suppression of sex hormone production, increased renal ACE2 activity in females, but not in males, whereas estradiol treatment reduced renal ACE2 activity in both gonadectomized males and females [30], suggesting a negative role of estrogens in modulating ACE2 activity and, consequently, cardiovascular homeostasis in animal models. Nevertheless, testosterone treatment was found to modulate the RAS pathway, in particular to upregulate the classical pressor pathway (ACE/AngII/AT1R axis), by increasing renal and hepatic angiotensinogen expression, as well as renal renin expression and activity and AT1R expression [31, 32], and downregulate the depressor pathway (ACE2/Ang(1-7)/AT2R axis), by decreasing myocardial ACE2 activity and aortic AT2R expression, in models of gonadectomized male and/or gonadectomized and non-gonadectomized female rodents [33, 34]. Notably, in non-gonadectomized male mice, treatment with the androgen receptor antagonist enzalutamide has been shown to decrease lung ACE2 expression, which become similar to the expression registered in female mice [35]. Moreover, the sex-determining region Y (SRY) gene, located on the Y chromosome, seems to be responsible for the upregulation of pressor pathway, particularly angiotensinogen, renin and ACE, and downregulation of ACE2, expression in experimental models of transfected Chinese hamster ovary (CHO-K1) cell line [36].

These controversial data on different tissue do not clarify whether androgens exert a potential positive effect in modulating ACE2 activity and RAS pathway and, consequently, cardiovascular homeostasis in animal models. Conversely, in humans, in an experimental model of cardiac atrial tissue derived from elderly men, estradiol treatment induced a shift from ACE to ACE2 expression and consequently from the classical pressor ACE/AngII/AT1R pathway to the alternative depressor ACE2/Ang(1-7)/AT2R pathway of the RAS axis [37, 38]. Moreover, in transgender women, estradiol treatment reduced blood pressure, by stimulation of ACE2 activity and increase of Ang-(1-7)/AngII balance [39]. These evidences suggested that estrogens stimulate ACE2 expression and activity, with a positive final effect on cardiovascular homeostasis in humans. Sex hormones, particularly androgens and to a smaller extent, estrogens, have been also found to modulate TMPRSS2 expression, although with conflicting results between animals and humans. In rodents, lung TMPRSS2 expression is unaffected by the use of the androgen receptor antagonist enzalutamide in male mice, suggesting the absence of androgen stimulation of TMPRSS2 expression [35]. Conversely, in humans, testosterone stimulates TMPRSS2 expression in human prostate cancer cells [40], whereas estradiol stimulates and inhibits TMPRSS2 expression in human prostate and breast cancer cells, respectively [41, 42], in experimental setting, suggesting a major role of androgens on enhancing TMPRSS2 expression. Interestingly, in humans, a second specific haplotype, including TMPRSS2 variants, and characterized by the expression of at least seven SNPs, has been denominated European haplotype because relatively frequent in European, including Italian, and totally absent in East Asian population, and has been functionally linked to an SNP, located in an androgen-responsive enhancer for TMPRSS2 gene, therefore suggested to be associated with an androgen-dependent upregulation of TMPRSS2 expression [27]; this evidence might also contribute to explain the high susceptibility of SARS-CoV-2 infection and severity of COVID-19 in Italian men.

Figure 2 summarizes the evidences of sex chromosome and sex hormone regulation of TMPRSS2 and ACE2 expression and activity and their implications in the RAS function.

On the basis of these data it can be hypothesized that, in men, the increased expression of ACE2 in the lung, together with the androgen stimulation of TMPRSS2 and the expression of TMPRSS2 variants associated with increased susceptibility to viral infection, might theoretically increase susceptibility to SARS-CoV-2 infection and contribute to a higher virus spread, and development of more severe disease with worse outcome. Conversely, in women, the reduced ACE2 expression in the lung and the estrogen inhibition of TMPRSS2 expression might theoretically reduce the

susceptibility to SARS-CoV-2 infection and contribute to a lower virus spread, and development of less severe disease with better outcome. Interestingly, the estrogen-dependent stimulation of ACE2 activity might play a crucial role in the cardiovascular protection and confer to women a more effective defense compared to men against the deterioration of the clinical course of the disease, at least during the reproductive age. Notably, this difference in the mechanisms of virus infection seem to have an impact on disease severity and outcome but apparently not on the prevalence of infection, since the rate of SARS-CoV-2 infection seems to be similar in men and women, become recently just slightly higher in men than in women, contrary to the severity and outcome of the disease, which was always clearly worse in men than in women.

Sex disparities in the immune and inflammatory response

Similarly to SARS and MERS, it is generally established that severe COVID-19 is characterized by a dramatic inflammatory state initially localized in the lung, due to the massive release of proinflammatory cytokines, produced by virus-infected cells, which attracts towards the site of infection innate immune cells, which are also infected by the virus [6, 9]. In case of pre-existing or virus-induced dysfunctional immune response, the enhanced release of proinflammatory cytokines and the recruitment of the immune cells from blood to the site of infection might damage the lung and establish a proinflammatory feedback loop, determining vascular permeability and a cytokine storm, which in turn may diffuse through the circulation into the various organs, promoting multi organ injury and leads to the development of systemic illness [3, 6, 9]. Nevertheless, a very recent study propose an intriguing theory, suggesting that SARS-CoV-2 infection might directly induce an immunological collapse, secondary to severe immunosuppression, with reduction of CD4+ T cells, CD8+ T cells, B cells and NK cells, leading to a profound defect in host immunity, and consequent failure to control unrestrained virus replication and dissemination with direct host cytotoxicity, rather than to the cytokine storm-induced multi organ failure [43].

Different genetic and endocrine mechanisms, including sex hormones actions, might influence the mechanisms of immune and inflammatory response to COVID-19.

Genetic mechanisms seem to regulate susceptibility to infections and directly impact immune response between sexes, with distinctions in innate and adaptive immune responses that remain constant from birth to old age [46]. Sexually dimorphism in animal and humans contributes to a

stronger immune response in females compared with males. The sex-related difference in immune response is mainly due to the evidence that a relevant number of genes involved in the positive regulation of innate and adaptive immune response are located on sex chromosomes, especially on X chromosome. Indeed, males have only one X chromosome, condition that from an evolutionary point of view represents a disadvantage, as every newly arisen recessive and deleterious mutation on the X-linked genes, including the immune-related genes, results in the functional loss in the entire cohort of cells and is manifested phenotypically. Conversely, females are generally protected by this phenomenon because one of the X chromosomes is randomly inactivated, resulting in cell mosaicism, where only the half of cells results in the functional loss following the eventual deleterious mutation, including functional loss of immune function [44]. Moreover, cell mosaicism also confers an additive immunological advantage to females compared to males in case of X-linked genes escape inactivation; indeed, the X-linked immune-related genes extensively and generally positively involved in innate and adaptive immune response, might result in a relatively increased levels of functional immune factors, likely protecting females more than males from infections, including virus infections, or to mitigate the clinical manifestation of the infective disease in females more than in males, with a different impact on the prognosis [44]. In addition, genetic variations (SNPs) among genes in Y chromosome are suggested to negatively influence immune response, thus increasing susceptibility to infections in males compared to females [45].

Differences in susceptibility to respiratory viral infectious diseases between males and females have been shown in rodent models. Male mice infected with SARS-CoV-MA15, reproducing a clinical syndrome similar to SARS, are more susceptible to disease than females and the degree of sex-bias increases with advancing age; the enhanced susceptibility of male mice was associated with elevated virus titers, neutrophil infiltration and proinflammatory cytokine (IL-1β, IL-6 and TNF) levels in the lungs, suggesting a worse innate and adaptive immune response in males than in females, probably related to the different expression and function of immune-related gene on X chromosome [18]. In male mouse consomic strains, genetic variations in the Y chromosome predisposes males to H1N1 influenza A virus infection, confirming the potential negative role of Y chromosome in the immune response and in the susceptibility to specific viral infections [46]. These studies are consistent with the findings of an increased risk for a number of infectious diseases due to genetic background, and for a more severe clinical manifestation due to a more profound inflammatory response in men compared to women. Men are more likely to develop

severe respiratory inflammatory syndrome, as a consequence of lung neutrophil infiltration and exaggerated production of cytokines and chemokines, whereas females generally had more favorable outcome, particularly when virulent pathogens are provided of a high inflammatory potential [47]. These evidences are also likely to apply to different viral infection, including coronavirus and, particularly, SARS-CoV-2 infection and consequent COVID-19.

Studies on rodents demonstrated a difference in innate and adaptive immune response between males and females, with females possessing a greater activity of innate immune cells, including macrophages, dendritic cells and neutrophils, and a greater number and/or activity of adaptive immune cells, belonging either to humoral or cell-mediated responses, specifically with increase of B cell numbers and activity, as well as T helper1 (Th1) and T helper2 (Th2) responses, compared to males [48]. Studies in rodents investigating the sex-related difference in regulatory T cell response are contradictory [48].

In humans, men and women display differences in the innate immune response, with men possessing an increased number of NK cells and enhanced ability of macrophages to produce proinflammatory cytokines, and with women displaying an enhanced ability to activate dendritic cell with consequent increase of INFα production, and macrophages and neutrophils with consequent increase of phagocytotic activity [48]. Notably, TLR4, whose genomic localization is on chromosome 3, is an extracellular receptor binding lipopolysaccharide, which influences cytokine production and displays higher expression on immune cells derived from men than those derived from women [48], although it is difficult to draw a conclusion on sex differences in TLR4 expression and responsiveness. Conversely, TLR7, whose genomic localization is on X chromosome, is an intracellular receptor that binds single-stranded nuclei acids and, due to escape from X chromosome inactivation, results in greater expression in immune cells derived from women than in those derived from men [49]. Similarly, men and women also display differences in the adaptive immune response with men possessing an increase in CD8+ T cell number, and a Th1 bias, and women possessing an increase in CD4+ T cell number with a Th2 bias, together with an increase of T cell activity in terms of proliferation, activation and cytotoxicity, and an increase of B cell number and activity, leading to an increased ability to produce antibodies [48]. **Table 3** shows sex difference in innate and adaptive immune response based on data from humans and rodents.

Endocrine mechanisms, including sex hormones actions, seem to influence immunological mechanisms, including inflammatory response, either in the innate of adaptive immune response,

differently in man and women throughout the course of life, as demonstrated by studies conducted on both rodents and humans [48]. Studies on rodents demonstrated that androgens reduce macrophage TLR4 expression in gonadectomized male mice [48]. Moreover, studies in rodents and humans reveal that androgens induce immunosuppressive and antinflammatory actions, by reducing NK and macrophage activity, CD8+ T cell number and activity, Th1 and Th2 cell activity and B cells number and activity [48]. On the other hand, studies on rodents demonstrated that estrogens induce macrophage TLR4 expression and enhance dendritic cell TLR7 signaling in gonadectomized female mice [48]. Interestingly, the administration of high doses of estradiol in mice exposed to SARS-CoV protects against the damage of hyperinflammatory response in the lung through recruitment of monocyte, macrophages and neutrophils that enhance the CD8+ T cell response [18]. Moreover, studies on humans, demonstrated that estrogens administration increase the activation of TLR7 signaling in dendritic cell of postmenopausal women [48]. Interestingly, estrogens, whose levels increase in women during the follicular phase and decrease during luteal phase of menstrual cycle and are very high in pregnancy and very low in the menopause, differentially modulate the innate and adaptive immune system in a concentration-dependent fashion. Indeed, low concentrations of estrogens are proinflammatory inducing IL-1β, IL-6 and TNF production and inducing the activity of Th1 cells, whereas high concentrations of estrogens are antinflammatory reducing IL-1β, IL-6 and TNF production and inducing the Th2 activity [48]. Therefore, the totality of these evidences suggests that susceptibility and response to SARS-CoV-2 in COVID-19 may vary depending on sex, and in women throughout the course of life during the different phases of the reproductive cycle. **Table 4** shows the impact of sex hormones in innate and adaptive immune responses based on data from humans and rodents.

Additionally, sex hormones interact with the hypothalamic-pituitary-adrenal (HPA) axis, particularly with the systemic effectors glucocorticoids (GCs), and cooperate to the regulation of the immune and inflammatory response. GCs have an immunomodulatory action, with predominant immunosuppression, specifically a suppressive effects on innate and adaptive immune cells and on inflammatory reaction [50]. Moreover, GCs inhibit the hypothalamus-pituitary-gonadal axes in both sexes, blocking the secretion of testosterone from the testes, and of estradiol from the ovaries [51]. In turn, estradiol has a direct stimulatory effect, whereas androgens have a mild suppressive effect on HPA axis [51]. These data contribute in explaining the generally superior ability of women to be protected from infections and to respond to infectious disease.

Taken together these findings suggest that men and women differ in their genetic predisposition to infectious disease. In men the single X chromosome and cluster of genes polymorphisms located on Y chromosome confer immunological disadvantage and, consequently, a greater frailty against infections. Moreover, the androgen-mediated immunosuppressive and proinflammatory effects, leading to a less effective innate and adaptive immune response, together with the inhibitory effect of androgens on HPA axis, contribute to a more severe disease with worse outcome. Conversely, in women the double X chromosomes, with the possibility of escape from X-linked gene inactivation, and the lack of Y chromosome confer immunological advantage, and, consequently, a greater protection from infections. Moreover, the estrogen-mediated immunostimulatory and antinflammatory effects, leading to a more effective innate and adaptive immune response, together with the stimulatory effect of HPA axis, contribute to a less severe disease with better outcome, especially during the reproductive age.

Sex disparities and coagulopathy and thrombosis

Similarly to SARS and MERS, it is generally established that severe COVID-19 is characterized, concomitantly to dysfunctional immune response and systemic inflammation, by a disseminated coagulopathy and thromboembolism, which is a major contributor of death [52]. The hemostatic changes, represented by the increase of procoagulant and decrease of anticoagulant pathways, appear to be supported by a direct virus-induced damage, systemic inflammation and endotheliopathy, together with liver dysfunction [12]. Presently, the coagulopathy associated with COVID-19 is denominated thromboinflammation, since it appears to result most likely from the systemic inflammation, secondary to virus infection, favoured by endotheliopathy and complicated by the prolonged stasis, rather than to an intrinsic thrombotic virus effect [12, 53-55]. Indeed, the dramatic increase in IL-6, the main marker of the hyperinflammation, is correlated with fibrinogen, marker of hypercoagulability, therefore confirming the strong correlation between systemic inflammation and coagulopathy in COVID-19 [56]. Additionally, the virus infection of endothelial cell induces inflammatory infiltration and consequent cell apoptosis, which generate the peculiar endotheliopathy, with consequent platelet activation and thrombus formation with development of either microvascular or macrovascular thrombosis [12, 53, 55]. Microvascular thrombosis was directly demonstrated by recent evidence at autopsy in patients died from COVID-19 demonstrating widespread microangiopathy and thrombosis into the lung, as well as in different organs and districts of the body [7, 57], whereas macrovascular thrombosis has

been demonstrated by the occurrence of ischemic stroke and/or acute coronary syndrome and myocardial infarction in patients with COVID-19 [53, 58, 59].

Different genetic and endocrine mechanisms, including sex hormones actions, might influence the mechanisms of hypercoagulation and thrombosis diathesis to COVID-19; however, it is noteworthy that these evidences are assumed by the report of hemostasis, but not in specific reports dedicated to COVID-19.

Genetic mechanisms seem to regulate hemostasis in humans without difference in men and women. Indeed, some genetic variants, such as factor V Leiden, prothrombin G20210A, and blood group non-0, are known to induce a 2-5-fold increase in the risk of thrombosis, but none of the genes coding for these factors are on sex chromosomes, and no difference between men and women has been found in the relative risk of venous thrombosis related to genetic risk factors [60].

Endocrine mechanisms, including sex hormones actions, seem to regulate hemostasis differently in males and females. Indeed, sex hormones directly act on platelet response and coagulation cascade [61]. Evidence emerging from rodents demonstrated distinct effect of androgens and estrogens on hemostasis. Indeed, treatment with estrogens reduced platelet aggregation and sensitivity only in female rats [62] and thrombus formation in female and male rats [63]. In both male and female rats, treatment with testosterone markedly increased mortality and thrombus size, which was doubled in males compared to females at baseline, whereas treatment with estradiol decreased mortality in both sexes and thrombus size in male but not in female rats [64]. Flutamide decreased mortality rate in both sexes compared with testosterone treatment alone [64]. Moreover, gonadectomy reduced in male and enhanced in female rats platelet aggregability. Treatment with testosterone increased and estradiol decreased platelet aggregability in both male and female gonadectomized rats. In male gonadectomized rats, flutamide and estradiol antagonized the testosterone enhanced platelet aggregability [65].

These evidences collected in animal models suggest that estrogens reduce platelet response in both males and females, thus displaying a protective effect against thrombosis, whereas androgens enhance platelet response and ultimately thrombosis-related mortality, finally suggesting that males are more susceptible to thromboembolism as compared to females. These evidences seem to be generally confirmed in humans with exceptions of specific conditions.

In humans, the risk of thromboembolism is reported to be at least 3-fold higher in men than in women at any age, with higher frequency in men during elderly age, and lower frequency in

women during fertile age [61, 66-68], suggesting also in humans a possible positive role of estrogens and a negative role of androgens on hemostasis at least in standard conditions of exposure to normal levels of sex hormone. Noteworthy, differently from results in animal models, in women conditions of exposure to supraphysiologic estrogen levels, such as during the use of estrogen-containing oral contraceptives, or extremely high estrogen levels, such as in pregnancy, represent two exceptions given that these conditions increase the procoagulant factors FVII, FIX, FX, FXII and FXIII levels, and decrease the anticoagulant factors protein S and antithrombin levels thus altering the hemostatic balance towards a prothrombotic state [69, 70]. Interestingly, in transgender women, the use of estrogens is independently associated with a 3-fold increase in cardiovascular mortality as it is known to induce the increase in body and visceral fat, weight and triglyceride, to reduce lean mass, and to promote prothrombotic blood changes [71, 72]. In transgender men the use of androgens does not increase cardiovascular mortality despite its negative impact not only on hematocrit but also on lipid profile [71, 72]. Notably, in men, the condition of testosterone deficiency, such as in hypogonadism, is associated with increase of procoagulant factor, including FV, FX and C-reactive-protein, and decrease of anticoagulant factors, such as antithrombin III [73]; this evidence suggest that the condition of androgen deficiency further worsen the susceptibility of men for thrombosis, explaining the exaggerated increase of incidence of thrombosis in old compared to young men. On the other hand, in women in condition of estrogen deficiency such as in menopause, genetic and environmental factors, including inappropriate diet, smoking habit, and reduction of physical exercise, can induce changes in the vascular endothelium, platelet activity, blood coagulation and fibrinolysis, therefore promoting thrombosis [74]; the estrogen deficiency likely contributes to explain the higher incidence of thrombosis observed in old compared with young women [75].

These evidences suggest that man and women differ in their predisposition to thromboembolism, since in men thrombotic risk is higher compared to women at any age, and even increased in older compared to younger patients. Despite testosterone may not exert a direct negative impact on cardiovascular mortality, testosterone deficiency appears to promote thrombosis, thus contributing to increase mortality risk in men. Conversely, women take advantage from a less frequent occurrence of thromboembolism during fertile age, but conditions of estrogen deficiency treated with hormonal replacement as well as menopause and the use of estrogen-containing oral contraceptives increase thrombotic risk and related mortality in women.

Additionally, exogenous systemic corticosteroids may exert influence on thromboembolism, acting on hemostasis directly and, indirectly, through changes in sex hormones, with a dichotomous response between sexes [76]. In healthy conditions, GC treatment has been shown to increase FVII, FVIII and FXI procoagulant factors, whereas in conditions of inflammation status, GC therapy inhibits vWF and fibrinogen secretion, but increase the antifibrinolytic plasminogen activator inhibitor type 1 (PAI-1) levels, contributing to the occurrence of a hypercoagulability state [77], as reported also in patients with endogenous GC excess such as in Cushing's syndrome, which are associated with a thrombosis diathesis [78]. In addition, excess of endogenous GC lowers testosterone-to-estradiol ratio in men, while increasing it in women [78], thus inducing a sex hormone deficiency status in both sexes, which contribute to enhance thrombotic diathesis.

Taken together these findings suggest that COVID-19, by promoting peculiar endotheliopathy and thromboinflammation, facilitates the occurrence of microangiopathy and macroangiopathy leading to fatal thromboembolism and exacerbates the greater thromboembolic risk of men compared to women. Indeed, COVID-19 induces a hypercoagulability state, which adds up to the different predisposition to thromboembolism between men and women. Despite not fully elucidated, the sum of a pathologic condition to a physiologic predisposition might help in explaining the greater severity and the worse outcome of COVID-19 in men compared to women.

Role of hypogonadism in men and women

An increasing amount of evidence seems to indicate the condition of sex hormone deficiency as a deleterious factor for immune and inflammatory response or thrombosis diathesis and, consequently, as a negative prognostic factor for the severity and outcome of COVID-19. At confirmation of this hypothesis, an association between male hypogonadism and poor outcome of COVID-19 has been reported. Indeed, in 31 male patients with COVID-19 admitted to the respiratory intensive care unit, low total and calculated free testosterone levels were significantly associated with the presence of negative prognostic factors, including increased serum LDH, ferritin, procalcitonin as well as increased neutrophil with decreased lymphocyte count, and with increased mortality [79]. Therefore, low testosterone levels have been suggested as predictors of poorer clinical outcome and mortality in COVID-19 [79, 80]. Whether male hypogonadism is an associated comorbidity or a consequence of SARS-CoV-2 infection and development of COVID-19 is still unclear. Smell loss reported by COVID-19 patients might result from the injury of olfactory

bulbs and the impairment in GnRH secretion (a “Kallman-like” condition), suggesting a secondary origin for male hypogonadism in COVID-19. This hypothesis is in line with experimental studies with SARS-CoV, responsible for SARS, demonstrating that the olfactory bulbs may be a primary conduit for virus entry into the brain, followed by transneuronal spread of the virus [81]. Recently, SARS-CoV-2 infection has been found to induce an increase in TNF-α and IL-1β in olfactory epithelium of COVID-19 patients with anosmia, therefore suggesting an inflammatory-mediated damage of the olfactory bulbs, probably responsible for the anosmia associated with COVID-19 [82]. On the other hand, the documented expression of ACE2 on Leydig cells [24], despite the lack of the evidence demonstrating the TMPRSS2 co-expression [5], but not excluding the possible co-expression of a different virus priming factor, might induce a damage of testis function, leading to a primary hypogonadism. According with this hypothesis, recent findings have found functional hypogonadism with increased luteinizing hormone (LH) levels in 31% and a clear primary hypogonadism in 17% of male COVID-19 patients admitted to intensive care units [83]. Conversely, the role of the “physiologic hypogonadism” seen in menopausal women is yet to be clarified. Interestingly, LH levels are within normal range but follicle-stimulating hormone (FSH) levels reduced in 10 COVID-19 middle aged to elderly women [83], likely suggesting the occurrence of a secondary hypogonadism. Interestingly, aging is characterized by a physiological sex hormone decline, which is progressive in men during life and sharp in women with the transition from reproductive age to menopause. Such physiological hypogonadism could play a direct or indirect role in the development of systemic inflammation, endotheliopathy and thromboembolism in COVID-19 patients. Indeed, in men aging is accompanied by a physiological decline of testosterone levels [84], or frequently a pathological condition of a real hypogonadism, denominated late-onset hypogonadism [85], which contributes to the progressive increase in the risk cardiometabolic disease, cardiovascular events and cardiovascular mortality [86]. Similarly, in women aging is accompanied by a physiological decline of estrogens and progesterone levels leading to menopause, which contributes to the progressive deterioration of cardiovascular risk factors and increased risk of cardiovascular disease and mortality [87]. Indeed, sex hormones are known to directly impact cardiovascular health. Particularly, androgens decline may predispose to endotheliopathy and thrombosis, associated with a defective immune response, leading to impaired virus clearance and systemic inflammation [88]. On the other side, estrogen decline exerts a negative impact on endothelial function and contribute to the development of thrombotic diathesis, because the sudden reduction in estrogen levels increases the oxidative stress, through

the downregulation of the expression of genes related to antioxidants [89], to reduce nitric oxide and to increase reactive oxygen species (ROS) [90], together with an enhancement of the cellular endothelin-1 system and a negative impact on RAS pathway activity [91]. These evidences may partly explain the high mortality rate seen in elderly patients with SARS-CoV-2.

COVID-19 in elderly: role of inflammaging

Based on current available evidence, epidemiological data collected in elderly might reflect the effects of age-dependent physiological fall in sex hormones in both sexes. Moreover, not only a sex-biased, but also an age-biased immune and inflammatory response and thrombotic diathesis has been reported in the general population and in patients with COVID-19. Global data provide unquestionable evidence that elderly subjects, especially those with pre-existing comorbidities, are at higher risk for development of severe disease with poor outcome of COVID-19 [2]. In healthy elderly individuals the composition and quality of immune response is profoundly reduced by aging, because of a physiological reduction in B cell number and activity, CD8+ T cells and CD4+ T cells activity and ability to respond to antigens [92]. Aging is also characterized by chronic low-grade inflammation, not caused by a pathogen, designated as inflammaging [89, 93], which predispose to higher frailty and earlier mortality and may therefore be responsible for the higher susceptibility to SARS-CoV-2 infection and for the worse outcome of COVID-19. Healthy individuals older than 60 years exhibit high baseline serum concentrations of IL-6, IL-8 and C-reactive-protein together with increased ROS levels [93]. Inflammaging appears to promote the accumulation of senescent cells in the respiratory tract, so initiating an inflammatory cascade that could inhibit T cell responses to viral infected cells [93]. In elderly COVID-19 patients, the defective immune response and the increased proinflammatory IL-6 concentration and decreased INFα/β production can promote lung inflammation and injury [94] and foster virus replication [95]. Indeed, innate immune response is reduced in elderly COVID-19 patients as demonstrated by the decreased activity of neutrophils, monocytes and macrophages leading to a limited phagocytosis, reduced nitric oxide and superoxide production and lower migration to infected tissues [96]. Considering the elevated basal inflammatory state in elderly, SARS-CoV-2 infection has been recently hypothesized to induce a particularly robust and dramatic inflammatory cytokine and chemokine expression responsible for an exaggerated and dysregulated host inflammatory response [96]. Moreover, despite high titers of neutralizing and antigen binding antibodies have been found in

elderly COVID-19 patients, it is still unknown whether such antibody response is protective, or pathogenic or expression of a severe disease [96]. Moreover, the increased oxidative stress of elderly may contribute to the endotheliopathy and potentially to thromboembolism. Noteworthy, the increased risk of thromboembolism reportedly seen in elderly men as compared to elderly women might mirror the effects of the physiological decline in testosterone, typically experienced with aging, which can even worsen this immune defective, proinflammatory and prothrombotic state [90]. This evidence seems to explain the vulnerability of elderly people, particularly elderly men more than elderly women, to COVID-19.

In Italy, at the update on the 30th of March 2020, the NIH reported in patients aged over 60 years an overall higher lethality rate (18.4%) as compared to younger patients (1.13%). Particularly, in elderly, infection rate was higher in men (21980, 65.2%) as compared to women (11726, 34.8%), and a lethality rate in men (3520, 16%) almost doubled that recorded in women (1090, 9.3%). Interestingly, at the latest update on 14th October 2020, the NIH reported in the same age groups a similar trend: overall lethality rate was higher in patients aged over 60 years (8.3%) compared to younger patients (0.4%), infection rate was 47% in men and 53% in women, and the lethality rate was 12.9% in men and 10.1% in women.

All together these findings suggest that elderly men might be more susceptible to severe COVID-19 because of a greater predisposition to infections, a weaker immune defence and an enhanced thrombotic state.

Interestingly, the age-related changes in glucocorticoids could either increase vulnerability to infections [97] and prime the response of the immune system [98]. On the other hand, the severe immune and inflammatory responses induced by SARS-CoV-2 provided the basis to suggest the use of antinflammatory therapy with exogenous systemic corticosteroids to prevent further injury in patients with COVID-10, although the use of such drugs is still debated, as they may delay virus elimination and increase the risk of secondary infection, especially in patients with pre-existing deterioration of the immune system [99].

Concluding remarks

The impact of sex on SARS-CoV-2 infection, severity and outcome of COVID-19 is nowadays an established evidence. Men and women differ in COVID-19 clinical severity and outcome, as suggested by several evidences. Social habits and comorbidities differ between the two genders,

since hygiene practices are more frequently adopted by women than men, whereas smoking and comorbidities (diabetes mellitus, hypertension and cardiovascular diseases) are more prevalent in men than women. Viral entry is facilitated in men, which display a genetically-based higher lung expression of ACE2 and a testosterone-enhanced TMPRSS2 expression than women, whereas estradiol increases ACE2 activity in women, thus contributing in protecting women from cardiovascular deterioration. Similarly, men display a genetically-based higher predisposition to infections than women, which in turn take advantage from a more effective immune system regulation as compared to men. Moreover, the direct proinflammatory effects of testosterone and antinflammatory effects of estradiol lead to an enhanced inflammatory system response in men as compared to women. Finally, although men and women do not display any difference in genetic predisposition to thromboembolism, men at any age have a higher thrombotic risk than women, in whom thrombotic risk is lower at fertile age and increases at menopausal age and/or under estroprogestin therapy. In elderly patients inflammaging leads to an immune defective, proinflammatory and prothrombotic state.

However, some limitations should be considered. Co-expression of ACE2 and TMPRSS2 on all tissues acting as potential conduits for viral entry is yet to be completely elucidated. While differential effects of testosterone and estradiol on RAS system have been clearly documented, less is known about consequences of androgen or estrogen deficiency in both sexes. Particularly, men are known to display a greater cardiovascular risk as compared to women. However, while the protective effect of endogenus estrogen in eugonadic women is well documented, scant evidence is available about the mechanisms underlying the greater predisposition of men to cardiovascular events and thromboembolism at any age, independently on gonadic state. Lastly, whether sex hormones differently impact immune, inflammatory and thrombotic state and whether differential decline in sex hormones in both sexes influences clinical severity and final outcome of COVID-19 is not fully understood. Therefore, sex disparities could assist in interpreting epidemiological findings as they contribute to the increased mortality seen worldwide in men in comparison to women. **Figure 3** summarizes the sex difference in susceptibility to SARS-CoV-2 infection and sex hormone influences on innate and adaptive immune response, inflammation and coagulation state.

Perspectives

Given that currently no specific medical treatment has been demonstrated to be effective in patients with COVID-19, the potential use of hormonal treatment alone or in conjunction with proposed therapeutics should receive serious consideration in COVIOD-19 treatment. According to the hypothesis of a negative role of androgens on the severity and outcome of COVID-19, the employment of anti-androgens has been proposed. Indeed, the inhibition of androgens might potentially antagonize TMPRSS2 actions, thus limiting virus internalization into the cells and consequent diffusion in the body [100]. Moreover, spironolactone, a mineralocortocoid receptor blocker with anti-androgenic properties, has been proposed to provide therapeutic benefit in COVID-19 patients by inhibiting the androgen-dependent expression of TMPRSS2, thus preventing SARS-CoV-2 infection [101, 102]. Noteworthy, the opposite evidence of hypogonadism as negative factor for the development of a severe COVID-19 with poor outcome, a paradoxical potential beneficial effect of androgen replacement therapy cannot be excluded [86]. On the other hand, according to the evidence of a positive role of estrogens in the development of a severe COVID-19 with poor outcome, estrogen treatment has been proposed, since estrogen treatment might notably impact the balance between immune and inflammatory responses to SARS-CoV-2, also affecting the occurrence of thrombotic diathesis, therefore influencing mortality. Particularly, low dose natural estrogens, like isoflavones from soya, given as a local nasal spray, could directly activate the local nasal immune system by increasing the activity of phagocytes, dendritic cells and NK, thus destroying the virus and preventing its diffusion into the lower respiratory tract or reducing the virulence, as already demonstrated in animal studies [103]. A randomized clinical trial of tripartite combination with estradiol, the flavonoid quercetin and vitamin D, known to affect the expression of the majority of human genes encoding SARS-CoV-2 targets, has been proposed as potential therapeutic intervention to treat and prevent COVID-19 pandemics [104]. The hormonally induced reduction of inflammatory response to COVID-19 by selective SERMs might represent another potential therapeutic target in such patients, as SERMs are able to suppress the activity of proinflammatory cytokines and induce antinflammatory cytokine expression, as previously demonstrated in Ebola patients [105]. Added to antiviral treatment and introduction of a support care protocol immediately on SARS-CoV-2 confirmation, hormonal therapy might contribute to reduce patient hospitalization, with potential beneficial impact on recovery and reduced mortality rates. Additional hormonal treatments, mainly systemic corticosteroids, might help in reducing inflammation and lung fibrosis, and might be useful to counterbalance the corticosteroid insufficiency typically seen in critical illness [106]. On the other hand, the use of

systemic corticosteroids might be affected by the occurrence of adverse effects, mainly including suppression of immune response, promotion of thrombosis, avascular necrosis, psychosis, diabetes and delayed viral clearance [99]. Therefore, a WHO guideline has recommended the use of systemic corticosteroids only for the treatment of severe and critically ill COVID-19 patients, as systemic corticosteroids have been reported to reduce the risk of 28-day mortality of 8.7% and 6.7% in patients with COVID-19 who are critically or severely ill respectively, whereas they may even increase the risk of 28-day-mortality in patients with non-severe COVID-19 [107]. However, at present, efficacy and safety of corticosteroids in COVID-19 need to be further elucidated [107].

In the light of these considerations, the use of hormonal treatment might offer new therapeutic strategies for SARS-CoV-2 alone or in combination with other therapies.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

R.P. conceived the review study and supervised the manuscript drafting. RS.A. and C.P. performed literature search, contributed to the interpretation of the data and prepared the figures. R.P., RS.A. and C.P. wrote the manuscript. A.M.I. G.C. A.C. R.P.M. provided a significant expert contribution in the scientific content revision process and R.P.M. critically reviewed and revised it for important intellectual content. All authors read and approved the final manuscript.

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Captions to the Table and Figures.

Figure 1: Immune, inflammatory and thrombotic response to SARS-CoV-2 infection in case of severe COVID-19 disease. SARS-CoV-2 enters into cells expressing the surface ACE2 receptors and TMPRSS2 (1). The replication and release of the SARS-CoV-2 cause the pyroptosis of host cells and release of proinflammatory cytokines by inflammosome (mainly IL-1, IL-8 and IL-18) and cell debris that activate alveolar macrophages, which in turn further release proinflammatory cytokines (mainly IL-10, GM-CSF and MIP1) and chemokines (2). These proteins attract other innate and adaptive immune cells in the lungs, damaging the lung infrastructure and with the addiction of increasing release of INFγ by T cell promoting a proinflammatory feedback loop and cytokine storm (3). Moreover, the production of non-neutralizing antibodies by B cells may enhance SARS-CoV-2 infection further exacerbating organ damage. Concomitantly, the damage of endothelial tissue directly caused by SARS-CoV-2 entry and the local inflammation induce endotheliopathy characterized by injured endothelial tissue (4) with a consequent vascular leaking (5). The extracellular NETs induce the aggregation of platelets and fibrin deposition leading to blood clots formation and promoting disseminated hypercoagulability (6). This mechanism finally results in microvascular thrombosis and systemic thromboembolism (7). As a consequence, septic shock and multiorgan failure may develop and represent potential major death determinants in COVID-19. (Created with BioRender.com).

Figure 2: Sex chromosome and sex hormones regulation of TMPRSS2 and ACE2 expression and activity, and implications in the RAS function.

ACE2 and TMPRSS2 are stechiometrically contiguous and mediate the SARS-CoV-2 cell fusion and entry. ACE2 is a X-linked gene with higher expression in women (pink line with arrow). Subsequently to membrane fusion and virus entry into the host cell, SARS-CoV-2 infection leads to down-regulation of ACE2. As a result, the ACE2/Ang(1-7)/AT2R axis is markedly attenuated, with amplification of the pressor ACE/AngII/AT1R axis. Sex chromosome and sex hormones contribute to the RAS regulation. In males, sex hormones and genes in sex chromosomes contribute by differentially modulating the RAS. Specifically, testosterone upregulates expression of angiotensinogen and AT1R (blue line with arrow), reduces expression of AT2R (blue line with inhibitor) and concomitantly inhibits renin activity (blue broken line with arrow). Moreover, SRY genes upregulate angiotensinogen, renin and ACE expression (blue line with arrow) and

downregulate ACE2 expression (blue line with inhibitor). These effects upregulate the classical constrictor and proinflammatory pathway ACE/AngII/AT1R axis. Moreover, testosterone positively regulates TMPRSS2 expression (blue line with arrow). In contrast, estradiol changes the balance towards depressor and antinflammatory ACE2/Ang(1-7)/AT2R axis increasing ACE2 activity (pink dot line with arrow). Moreover, estradiol negatively regulates TMPRSS2 expression (pink line with arrow) (Created with BioRender.com).

Figure 3: Sex differences in susceptibility to SARS-CoV-2 infection and sex hormone influence on innate and adaptive immune response, inflammation and coagulatory state (Created with BioRender.com).

Table 1: Global epidemiological sex-disaggregated mortality data from dataset Global 5050 (https://globalhealth5050.org/covid19/sex-disaggregated-data-tracker).

Table 2: Sex disparities of mortality data in COVID-19 disease available from literature.

Table 3: Sex difference in innate and adaptive immune response based on data from humans and rodents.

Table 4: Sex hormone induced impairment in innate and adaptive immune response based on data from humans and rodents.

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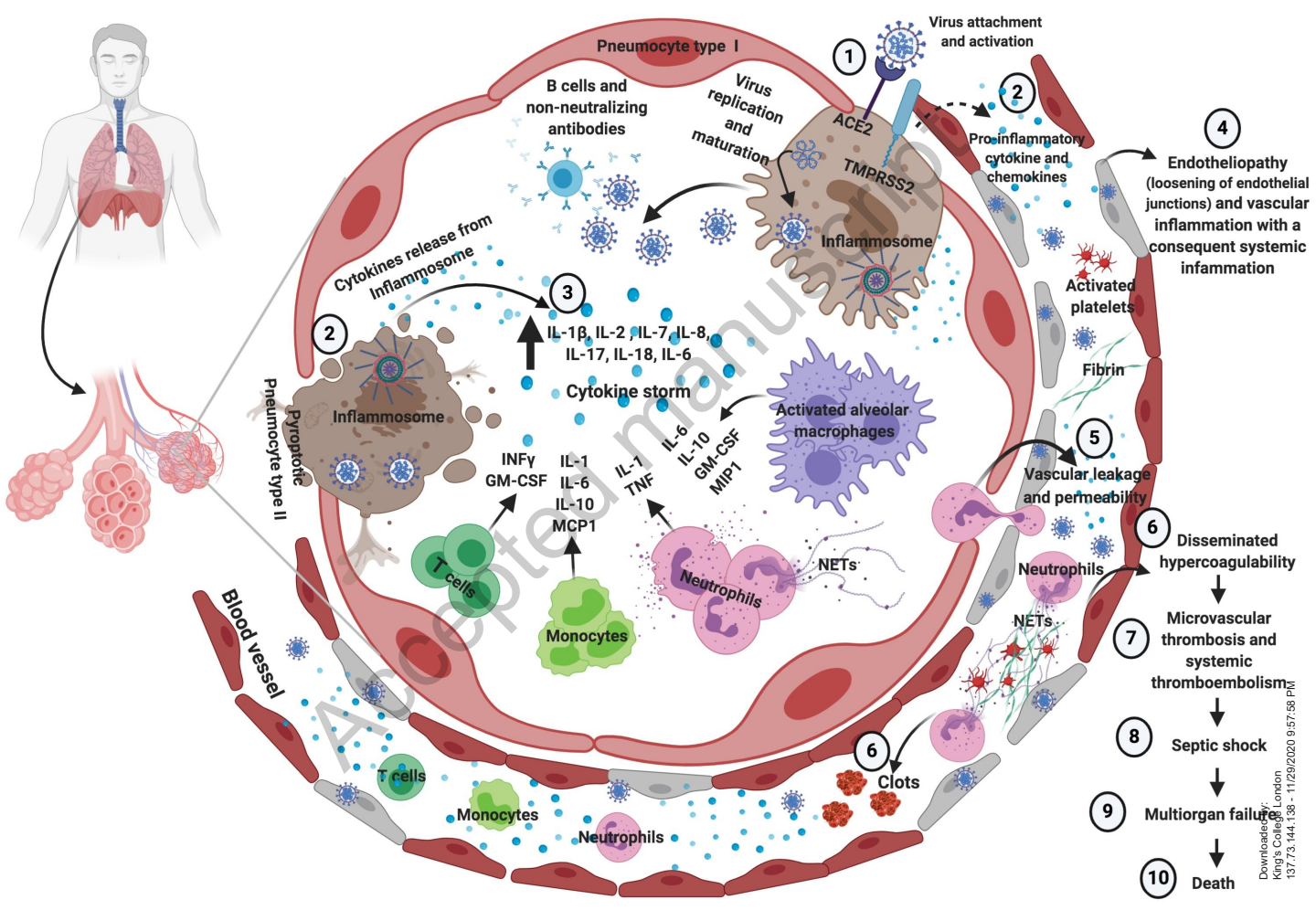

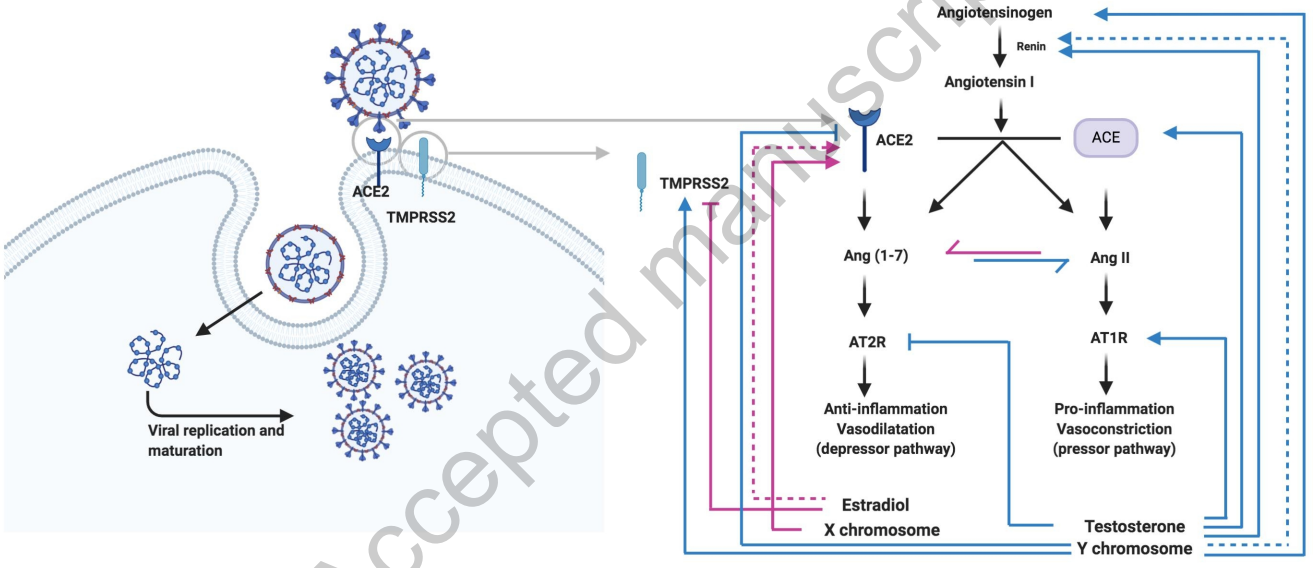

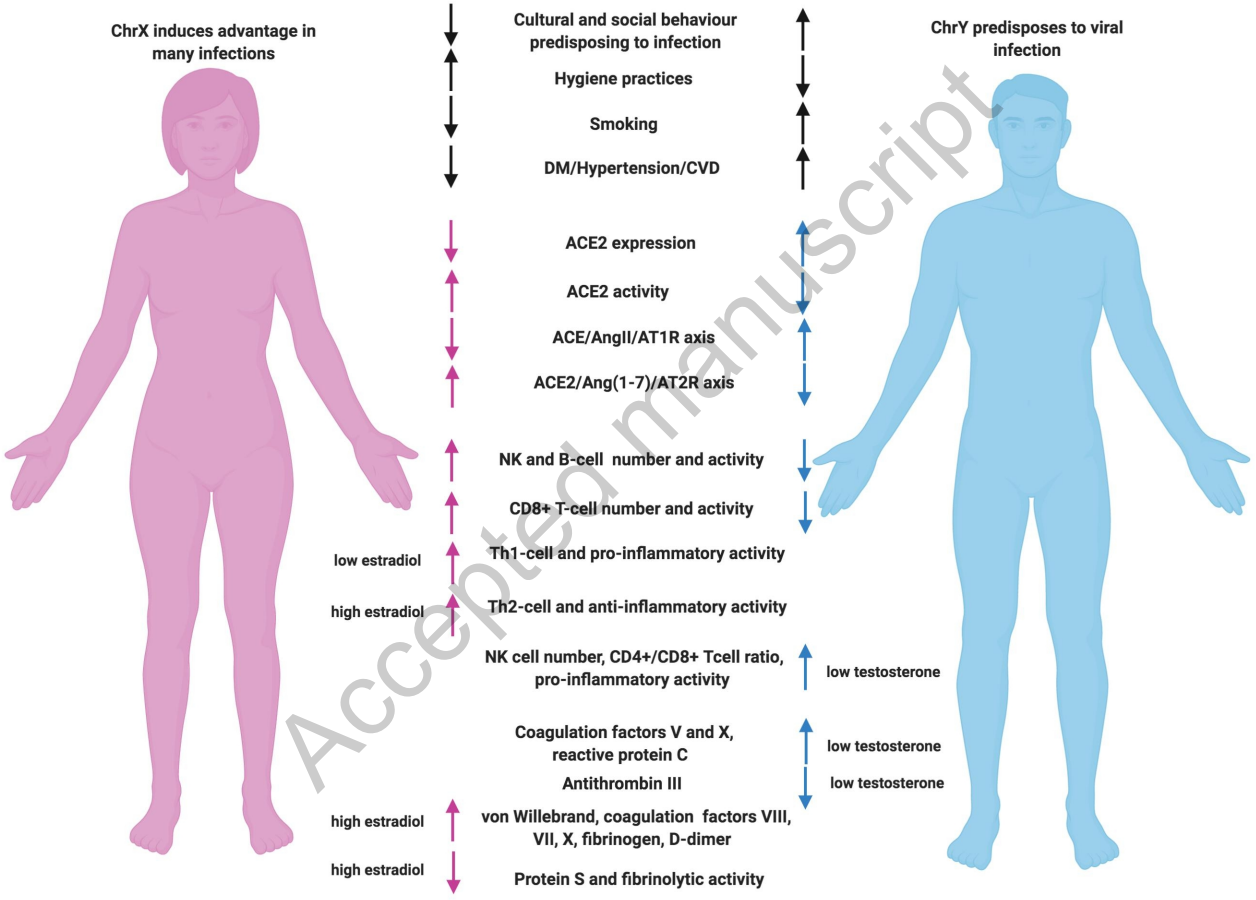

Table 1: Global epidemiological sex-disaggregated mortality data from dataset Global 5050 (https://globalhealth5050.org/covid19/sex-disaggregated-data-tracker). * Data available only for Australia

	TOTAL CASES	males (n)	females (n)	MALES (%)	FEMALES (%)	TOTAL DEATHS	males (n)	females (n)	MALES (%)	FEMALES (%)
Africa	1338268	745781	680332	55,7	44,3	12442	7619	4808	61,2	38,8
America	9781617	4852740	4928930	49,6	50,4	500010	293103	206907,0	58,6	41,4
Asia	5170908	3276910	1893834	63,4	36,6	39510	25591	13552	64,8	35,2
Europe	2797046	1290857	1506189	46,2	53,8	181402	101598	79804	56,0	44,0
Oceania	28829	13949	14880	48,4	51,6	860*	418*	442*	48,6*	51,4*
Total	**19116668**	**10180237,0**	**9024165,0**	**53,3**	**46,7**	**733364,0**	**427911,0**	**305071,0**	**58,3**	**41,7**

Table 2: Sexual disparities of mortality data in COVID-19 disease available from literature.

					MORTALITY RATE (%)			
AUTHOR	**Reference number**	**COUNTRY**	**PATIENT NUMBER**	**DEATH NUMBER**	**MEN**	**MEN %**	**WOMEN**	**WOMEN %**
Jin JM, et al	14	China	43	37	26	60,5	11	25,6
Yu C, et al	15	China	1464	212	150	70,7	62	29,3
Mikami, et al	16	USA	6493	2014	1128	56	886	44
Asfahan S, et al	17	China	44672	1023	653	63,8	370	36,2
Rivera-Izquierdo M, et al	18	Spain	238	61	38	29	23	21,5
Qin L, et al	19	China	548	90	62	68,9	28	31,1
Shah P, et al	20	Georgia	522	92	50	54,4	42	45,6
Du RH, et al	21	China	179	21	10	47,6	11	52,4
Posch M, et al	22	Austria	-	439	255	58	184	42
CovidSurg Collaborative	23	International	1128	268	172	64,2	94	35,1
Mani VR, et al	24	USA	184	32	23	71,9	9	28,1
Santorelli G, et al	25	UK	464	109	68	62,4	41	37,6
Nikpouraghdam M, et al	26	Iran	2964	239	167	69,9	72	30,1
Pan F, et al	27	China	124	89	67	75,3	22	24,7
Borobia AM, et al	28	Spain	2226	460	286	62,2	174	37,8
Wu Y, et al	29	China	402	21	15	71,4	6	25,6
Deiana G, et al	30	Italy	1223	97	52	53,6	45	46,4
TOTAL			**62874**	**5304**	**3222**	**60,70%**	**2080**	**39,30%**

Table 3: Sex difference in innate and adaptive immune response based on data from humans and rodents.

Innate immune system		
Immune cells	**Characteristic**	**Sex difference**
Dendritic cells	IFN activity	Higher in females
Macrophages	Activation and phagocytic ability	Higher in females
	Pro-inflammatory cytokine production	Higher in males
Neutrophils	Phagocytic ability	Higher in females
NK cells	NK cell number	Higher in males
Adaptive immune system		
Immune cells	**Characteristic**	**Sex difference**
T cells	CD4+ T cell number	Higher in females
	CD8+ T cell number	Higher in males
	CD4+/CD8+ T cell ratio	Higher in females
	T_H1 and T_H2 cell	T_H2 cell bias in females
		T_H1 cell bias in males
B cells	B cell number and activity	Higher in females
Immunoglobulins	Antibody production	Higher in females

Abbreviations: NK, Natural killer cells; IFN, interferon; Th, T-helper cells

Table 4: Sex hormone impairment induced in innate and adaptive immune response based on data from humans and rodents.

Male	Female	
↓ NK number	↑ NK number	
↓ IFN-γ	↑ IFN-γ	
↓ IL-4	↑ IL-4	
↓ IL-5	↑ IL-5	
↑ IL-10	↑ IL-10	
↑ IL-1	*Low-E* ↑ IL-1	*High-E* ↓ IL-1
↑ IL-6	↑ IL-6	↓ IL-6
↓ TNF	↑ TNF	↓ TNF
↓ Th1 and Th2 activity	↑ Th1 activity	↑ Th2 activity
↓ B cells number and activity	↑ B cells activity	
↓ CD8+ number and activity	↑ CD8+ activity	
↓ Antibody response	↑ Antibody response	

Abbreviations: NK, Natural killer cells; IFN, interferon; IL, interleukin; Low-E, low estrogens (follicular phase of reproductive cycle and menopause); High-E, high estrogens (luteal phase of reproductive cycle and pregnancy); TNF, tumor necrosis factor; Th, T-helper cells