MAYO CLINIC

REVIEW

COVID-19 and Sex Differences: Mechanisms and Biomarkers

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Abstract

Men are consistently overrepresented in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and coronavirus disease 2019 (COVID-19) severe outcomes, including higher fatality rates. These differences are likely due to gender-specific behaviors, genetic and hormonal factors, and sex differences in biological pathways related to SARS-CoV-2 infection. Several social, behavioral, and comorbid factors are implicated in the generally worse outcomes in men compared with women. Underlying biological sex differences and their effects on COVID-19 outcomes, however, have received less attention. The present review summarizes the available literature regarding proposed molecular and cellular markers of COVID-19 infection, their associations with health outcomes, and any reported modification by sex. Biological sex differences characterized by such biomarkers exist within healthy populations and also differ with age- and sex-specific conditions, such as pregnancy and menopause. In the context of COVID-19, descriptive biomarker levels are often reported by sex, but data pertaining to the effect of patient sex on the relationship between biomarkers and COVID-19 disease severity/outcomes are scarce. Such biomarkers may offer plausible explanations for the worse COVID-19 outcomes seen in men. There is the need for larger studies with sex-specific reporting and robust analyses to elucidate how sex modifies cellular and molecular pathways associated with SARS-CoV-2. This will improve interpretation of biomarkers and clinical management of COVID-19 patients by facilitating a personalized medical approach to risk stratification, prevention, and treatment.

 ■ Mayo Clin Proc. 2020;95(10):2189-2203

Reporting of disaggregated data by sex is uncommonly performed in the available literature, and current data relating to coronavirus disease 2019 (COVID-19) and attendant outcomes are no exception.[1] The Global Health 50/50 have collated international data from countries that provide sex-specific information and report a male-to-female case fatality ratio ranging from 1.6 to 2.8.[2] National data from China, Korea, and Europe report similar case fatality ratios and also a possible interaction with age.[3,4] Results from observational studies have been consistent, with males and older persons tending to be overrepresented among patients with severe disease,[5-7] intensive care unit admissions,[5,8-10] and death from the infection.[3,7,11-15] Studies stratified by sex have also identified male sex as a risk factor for worse outcomes and increased mortality.[16-19] Large, robust sex-stratified analyses, however, are limited due to the nature of studying an emerging disease.

The sex disparity of COVID-19–related morbidity and mortality is likely explained by a combination of biological sex differences (differences in chromosomes, reproductive organs, and related sex steroids) and gender-specific factors (differential behaviors and activities by social and cultural/traditional roles).[4] Men are more likely to engage in poor health behaviors such as smoking and alcohol consumption,[20,21] and have higher age-adjusted rates of pre-existing co-morbidities associated

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ARTICLE HIGHLIGHTS

- Most biomarkers associated with severe Covid-19 disease differ by sex when examined in experimental and epidemiological studies in the non-COVID-19 population.
- Sex specific genetic and hormonal modulation of the immune and renin angiotensin aldosterone system are complex, but important COVID-19 disease mechanisms which may provide insight into the observed sex disparity in case fatality rates.
- Future studies should address the relationship between biomarkers and COVID-19 disease severity including mortality, as current data are scarce.

with poor COVID-19 prognosis, including hypertension, cardiovascular disease (CVD), and chronic obstructive pulmonary disease (COPD).[7,9,13,18,22-24] Furthermore, a stratified analysis by sex showed that even after adjustment for age, the effect of co-morbidities on COVID-19 mortality was greater for men than women.[16]

Various biological pathways may contribute to the differing responses to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus by sex. The size and independence of the effect of sex on the association between biomarkers and COVID-19 health outcomes, however, rarely have been rarely reported or translated into preventive and clinical care settings. This review synthesizes the available evidence regarding the proposed cellular and molecular markers of COVID-19 severity by sex, including biomarkers of inflammation; coagulation; liver, renal, and cardiac function; and expressions of angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2). The available literature on such markers in the general population will also be discussed. The literature regarding biomarkers and COVID-19 available on PubMed, Embase, and the Chinese National Knowledge Infrastructure published until June 7, 2020, was systematically searched and reviewed. Our review synthesizes the current data and identifies

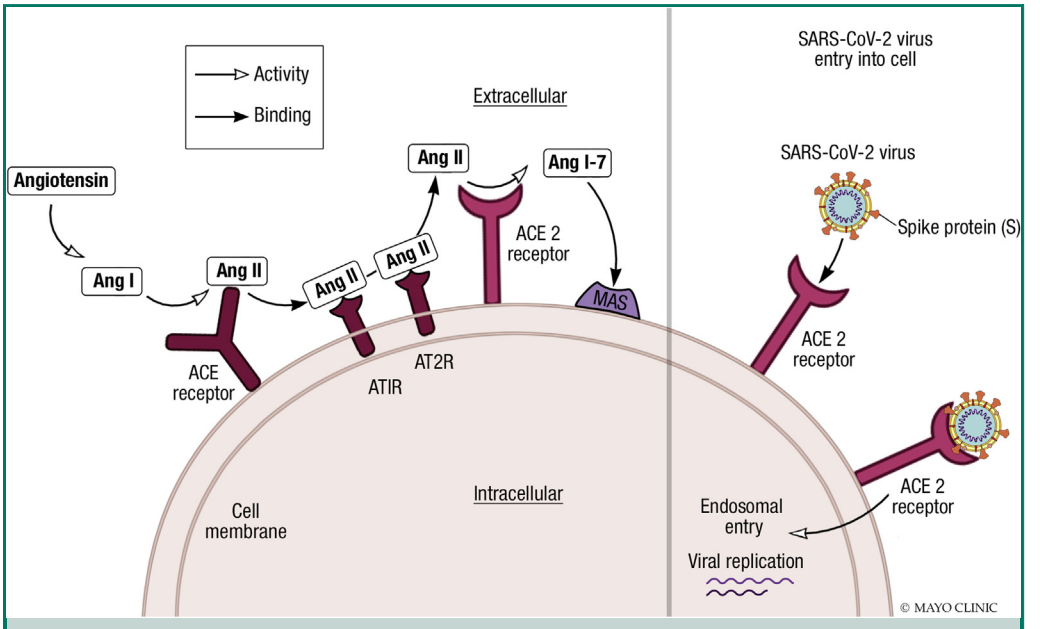

FIGURE. Cellular receptors of angiotensin II and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral entry. Angiotensin-converting enzyme 2 (ACE2) removes C-terminal amino acids from angiotensin II (Ang II), generating Ang 1-7 which activates MAS receptors. Ang 1-7 has a range of cardiovascular protective effects, thus attenuating the effect of Ang II. SARS-CoV-2 spike protein is primed by transmembrane serine protease 2 (not shown in figure) and interacts with the cell surface ACE2 receptor facilitating endosomal entry. AT1R = angiotensin type 1 receptor; AT2R = angiotensin type 2 receptor.

Mayo Clin Proc. ■ October 2020;95(10):2189-2203 ■ https://doi.org/10.1016/j.mayocp.2020.07.024
www.mayoclinicproceedings.org

the gaps in knowledge regarding the sex differences in COVID-19, which should be addressed in current and future studies to personalize evolving screening, preventive, and treatment strategies.

RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM AND SARS-COV-2 VIRAL CELL ENTRY

The pathogenesis of SARS-CoV2 disease involves tissues which express high levels of the ACE2 receptor. The infection typically starts in the oropharynx or nasopharynx, and then spreads to tissues that express ACE2: involvement of the upper airway and lungs occurs, the latter potentially leading to pneumonitis. Angiotensin-converting enzyme 2 is a key negative regulator of the renin–angiotensin–aldosterone system (RAAS) and counterbalances the actions of angiotensin-converting enzymes (ACEs) (Figure). The ACE converts angiotensin I (Ang I) to angiotensin II (Ang II), which binds to the angiotensin type 1 receptor (AT1R). This induces many deleterious effects, including vasoconstriction, fluid retention, enhanced cellular growth and migration, and oxidative stress promoting fibrosis and inflammation.[25] Angiotensin-converting enzyme and ACE2 share substantive sequence identity, but ACE2 shows substrate specificity and functions exclusively as a monocarboxypeptidase. It removes single C-terminal amino acids from Ang II, generating Ang 1-7 which binds and activates the G-protein–coupled Mas receptor. Angiotensin 1-7 attenuates the harmful effects of Ang II by eliciting a range of effects on the cardiovascular system, including vasodilatation; myocardial protection; and effects that are anti-arrhythmic, anti-hypertensive, anti-inflammatory, anti-thrombotic, and inotropic in nature. Angiotensin 1-7 also inhibits pathologic cardiac remodeling and insulin resistance.[26,27]

The SARS-CoV-2 spike protein interacts with the human cell surface ACE2 receptor, whereas TMPRSS2 primes the spike protein and may cleave the S1/S2 and S2' sites to assist attachment and membrane fusion.[28,29] Viral invasion increases activity of A disintegrin and metalloproteinase 17, which mediates the release of pro-inflammatory cytokines and ectodomain shedding of ACE2, reducing ACE2 cell surface expression.[27] The protective regulatory effect of the ACE2/Ang 1-7 axis against the RAAS is therefore lost.

ACE2 AND TMPRSS2: SEX DIFFERENCES IN EXPRESSION AND REGULATION

Increasing evidence supporting the roles of ACE2 and TMPRSS2 in viral entry and invasion of cells has led to numerous animal and human studies aiming to elucidate the relationship between their expressions/functions and risk for SARS-CoV-2 infection and COVID-19 severity. In addition, given previously known male/female differences in RAAS,[30] possible sex differences in ACE2 and TMPRSS2 have been postulated, but data are limited. It is likely that chromosomal/genetic differences, together with differential regulation of ACE2 and TMPRSS2 by sex hormones, which is life-cycle dependent, may be relevant considerations. Notably, the ACE2 gene lies on the X chromosome and escapes X-chromosome inactivation; however, sex-specific expression is inconsistent across multiple different tissues.[31] The receptor is predominantly expressed in the lung, heart, vascular endothelium, kidney, testis, and gastrointestinal tract and is also shed into circulating plasma.[27]

Estrogen through estrogen-receptor signaling on myocardium may decrease the ratio of ACE to ACE2 expression and upregulate Mas and angiotensin type 2 receptor (AT2R) expression levels,[32] which, unlike the effect of AT1R activation as described above, reduces inflammation and tissue fibrosis and promotes tissue repair.[33] However, a subsequent study showed no significant difference in ACE2 expression values in left ventricular tissue between males and females.[34] Preliminary results of an integrated bio-informatics analysis of single-cell RNA sequencing data indicate that the expression of androgen receptors positively correlates with ACE2 and that men may have increased pulmonary alveolar type II cells expressing ACE2 compared with women.[35] Other studies, in contrast, report no significant difference in lung tissue gene expression between males and females, or with differences in age.[34,36,37] Smoking

status, however, appears to correlate with ACE2 gene expression thus implicating differences in gender-specific behaviors. Current smokers as compared with never smokers have significantly upregulated ACE2 expression in the lung and oral epithelium.[37-39] COPD also has been independently associated with increased ACE2 expression by approximately 50% compared with those without COPD.[38] Given that smoking and COPD are more prevalent among males, higher expression of ACE2 due to these risk factors may, in part, explain the worse outcomes of COVID-19 in males. In summary, although studies have reported inconsistent sex differences related to ACE2 expression, it seems that, in general, ACE2 expression is increased in men and decreased in women. These effects may be modified/potentiated by gender-specific factors/behaviors, and should be investigated in future studies dedicated to sex differences.

Observed sex-related differences in the severity of COVID-19 may also be mediated via TMPRSS2 gene expression and activity. The expression of TMPRSS2 on non—sex-specific tissues does not appear to significantly differ between males and females.[34] However, the only known stimulus of TMPRSS2 gene transcription is androgens[40] and, interestingly, patients with COVID-19 who required hospital admission exhibit androgenic alopecia.[41] TMPRSS2 is a recognized protease associated with prostate cancer, and males with prostate cancer on androgen deprivation therapy may be at a significantly lower risk of SARS-CoV-2 infection compared with male patients who are not.[42] In addition, one haplotype that upregulates TMPRSS2 expression did so in response to androgens, whereas another is associated with increased risk of severe influenza, the latter also disproportionately affecting males.[43,44] The risk of severe infection mediated by androgen levels may, in part, explain why preadolescents are usually not severely affected by infection with SARS-CoV-2. However, studies directly comparing the expression of TMPRSS2 by sex and COVID-19 outcomes have not yet been conducted. Future investigation of ACE2 and TMPRSS2 expressions in various tissues and further stratification by sex with respect to disease severity is required.

IMMUNOLOGICAL AND INFLAMMATORY BIOMARKERS

Morbidity and mortality associated with COVID-19 is mediated through intense viral stimulated inflammation and increasing levels of inflammatory biomarkers and cytokines, commonly referred to as "cytokine storm." Together with reduced lymphocyte counts, cytokine storm is consistently associated with more severe COVID-19 disease. Among those exhibiting an excessive inflammatory profile, older and male patients are overrepresented.[7,45,46]

An early elevation in C-reactive protein (CRP) greater than 15 mg/L provides a marker of disease severity[46] and levels greater than 200 mg/L on admission are independently associated with five times the odds of death.[7] Males with severe COVID-19 reportedly have a higher CRP concentration compared with females, independent of age and co-morbidities.[19] Of the numerous interleukins (IL)—associated with COVID-19 severity, including IL-6, IL-2, IL-8, IL-10,[45-47] and compared with females, young and old males with COVID-19 exhibit significantly higher IL-2 and tumor necrosis factor alpha (TNF-alpha), respectively, independent of co-morbidities.[19] Moreover, data indicate that males with COVID-19 display greater upregulation of pro-inflammatory cytokines, including CCL14, CCL23, IL-7, IL-16, and IL-18, the latter possibly contributing to their higher susceptibility to developing cytokine storm and subsequent poorer COVID-19 outcomes.[35] Although IL-10, a cytokine with anti-inflammatory effects, has been shown to be higher among older males, a positive IL-10 feedback could be considered an attempt to decrease excessive inflammation and consequent tissue damage. Further, higher IL-10 expression diminishes the activity of antiviral T-cells.[48,49] Whether biological sex differences modify the associations among CRP and ILs and COVID-19 outcomes has yet to be examined.

Mayo Clin Proc. ■ October 2020;95(10):2189-2203 ■ https://doi.org/10.1016/j.mayocp.2020.07.024
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Adaptive Immune Response

Lymphocytes are among the first responders to viral agents, including SARS-CoV-2, and are associated with COVID-19 severity.[50] Although mild COVID-19 disease can be associated with either increased or decreased lymphocyte counts,[51] in severe disease, lymphocytes are consistently decreased. Although some COVID-19 studies have suggested that male sex is inversely associated with lymphocyte count,[17,19] a meta-analysis of the mean difference in admission lymphocyte counts between patients with and without severe COVID-19 outcomes showed that lymphopenia and disease severity were not modified by sex or co-morbidities.[52]

A single-center Wuhan study showed that in ill patients, concentrations of SARS-CoV-2 immunoglobulin G were significantly higher in females compared with males, and remained so until 4 weeks from hospital admission.[53] Sex-specific adaptive immune response is generally well recognized, with women mounting higher antibody production[54] and more efficacious vaccine responses.[54] Healthy females are known to have higher numbers of CD4+ T cells, greater CD4+: CD8+ ratios, and increased numbers of activated T cells, cytotoxic T cells, and B cells compared with males,[54-57] resulting in a prompt response to the presence of infectious agents. The role of sex steroids in the differential immune responses is supported by a study indicating testosterone exerts an immunosuppressant effect, whereas estrogen may be either immune enhancing[58] or immunosuppressive.[59]

Innate Immune System

Total white cell count was less consistently elevated among COVID-19 patients who required intensive care unit admission or died compared with patients who did not.[12,46,51,60] These studies did not investigate the effect of sex on this relationship, a question that merits attention as there are sex-specific differences in blood leukocyte composition within the general population. In the latter population, males have higher baseline numbers of total leukocytes, monocytes, neutrophils, eosinophils, and basophils compared with females. The total leukocyte and neutrophil counts increase progressively until the age of 55 years in males. Women have a bimodal distribution in total leukocyte counts, with the lowest counts occurring around menopause.[61,62] These known sex differences, together with the presence of underlying co-morbidities and concurrent infections, likely contribute to the inconsistent findings regarding white blood cell counts reported in current COVID-19 studies.

The neutrophil to lymphocyte ratio (NLR) is a well-known marker of inflammation and appears to reflect the severity of COVID-19, particularly among patients older than 50 years of age.[51,63] A single-center retrospective analysis observed that more males had an NLR above 11.75, which was associated with a lower survival rate.[64] The NLR exhibits distinct sexual dimorphism in the general population. Females 50 age years or younger have a higher NLR compared with males of the same age and compared with older females. The NLR is higher for males than females older than the age of 50 years.[61] The effects of sex and age on the prognostic value of NLR require further investigation.

Sex differences may have important implications in the efficacy of therapeutics that target particular viral signaling pathways. Notably, toll-like receptors (TLRs), which upregulate type 1 interferon (IFN), an important protective mechanism against viral infections,[65] may be up to 10-fold higher in females compared with males.[66-69] Furthermore, a recent study reported that after TLR7 stimulation, IFN levels were lower in men compared with women. Toll-like receptor 7—mediated IFN expression may be decreased in men due to the known negative effects of testosterone on IFN expression.[68] IFN therapy is under active investigation for COVID-19 patients, so additional research addressing sex differences in the IFN pathway may result in a targeted, sex-dependent therapeutic approach.

In addition to deriving benefit from the specific effects of estrogen, females may have stronger immune responses due to the intrinsic differences in the expression of genes on the sex chromosomes. Notably, several

genes which contain high numbers of immune-related alleles responsible for innate and adaptive immune responses to infection are located on the X chromosome.[70] Although X-chromosome inactivation is a mechanism of equalizing gene expression in females and males, some genes such as TLR7[71] may escape silencing, thereby conferring on females an immune advantage over males.[70]

SEX-SPECIFIC CONDITIONS AND COVID-19

Reproduction

One physiologic state that is associated with upregulation and increase in ACE2 is normal gestation, which raises the possibility that pregnant women may be at a greater risk for SARS-CoV-2 infection. We have recently reviewed the topic of pregnancy, its complications, and COVID-19.[72] Briefly, the upregulation of ACE2 and consequent conversion of Ang II to Ang 1-7 promote a general state of vasodilation with antithrombotic and anti-inflammatory activities in uncomplicated pregnancies. Preeclampsia is a pregnancy-specific, multisystem condition caused by abnormal placental vascular remodeling and systemic endothelial dysfunction which affects 3.3% of pregnancies,[73] and is characterized by decreased maternal plasma Ang 1-7 levels. SARS-CoV-2 directly binds and downregulates ACE2 expression; accordingly, ACE2 protein expression is expected to decrease during such infection. In pregnancy, this may potentiate these RAAS abnormalities because increased Ang II levels, relative to decreased Ang 1-7 levels, occur in preeclampsia (Figure).[72] Because of the overlapping mechanisms, certain clinical features and lab abnormalities, including thrombocytopenia[74] and liver function derangement,[75] may be seen in both preeclampsia and COVID-19, making the distinction between COVID-19 plus preeclampsia versus COVID-19 only complicated.[76] Consequently, pregnant women with COVID-19 must be evaluated critically, with particular consideration as to whether pre-eclampsia concomitantly exists or is incipient.

Sex Hormones, Menopause, and Hormone Replacement Therapy

Sex differences that are constant throughout the life cycle are likely chromosomal/genetic in origin, whereas those that occur with puberty and then fade with aging are suggestive of hormonal effects. Sex steroids, including testosterone, estrogen, and progesterone are potent regulators of immune and inflammatory responses due to the presence of sex-hormone responsive sequences in the respective genes. Estrogen during premenopause has anti-inflammatory effects, attended by lower levels of IL-6, IL-8, and TNF-alpha.[77] Conversely, the physiologic decline of estrogen levels during natural menopause results in increased levels of IL-6, IL-8, and TNF-alpha.[59] Estrogen depletion or oophorectomy in mice infected with SARS-CoV led to a worse prognosis compared with normal estrogen producing mice.[78] Clinical studies show that inflammation resolves more rapidly in women as compared with men, and these differences are thought to be due to hormonal effects on neutrophil apoptosis and bone marrow production.[79,80] Taken together, available studies provide strong evidence that estrogen exerts significant anti-inflammatory responses, thus suggesting a potential therapeutic role of hormone replacement therapy in older women. Similarly, low levels of testosterone in elderly men have been associated with upregulation of inflammatory markers and possible increased risk of lung damage, as well as respiratory muscle catabolism and increased need for assisted ventilation.[81] As advanced age remains one of the most important risks for poor COVID-19 outcomes, future research should address the role of hormone replacement therapy in elderly women and men who are diagnosed with COVID-19.

MARKERS OF CALCIUM HOMEOSTASIS AND COVID-19

Procalcitonin

Procalcitonin (PCT) is the precursor of calcitonin, a hormone that regulates calcium and phosphorus homeostasis by opposing the

action of parathyroid hormone. Procalcitonin levels are higher in severe cases of COVID-19.[7,82-84] Several meta-analyses showed that the risk of severe infection may be five-fold higher in patients with elevated levels of PCT.[83-85] Serum PCT levels are low in healthy persons and an elevated PCT level of greater than or equal to 0.5 ng/mL is typically considered a sign of bacterial but not viral infection.[86,87] Although no difference in PCT levels by sex occurs in healthy individuals,[88] one study of PCT levels in 14 patients with critical COVID-19 infection described that more males had a PCT level greater than or equal to 0.5 ng/mL compared with females.[17] Given this association with outcomes in COVID-19, ongoing studies should investigate the role of PCT as a sex-specific prognostic marker of disease severity.

Vitamin D

Apart from its role in calcium homeostasis through improving calcium reabsorption from the gut, vitamin D modulates inflammatory pathways associated with viral infections.[89] Meta-analyses indicate that vitamin D deficiency increases the risk of acute viral respiratory infection and community-acquired pneumonia, and that supplementation may prevent upper respiratory tract infections.[90] Vitamin D was found to decrease with age, and the strongest protective effect of supplementation was observed in those with the lowest 25-hydroxyvitamin D [25(OH)D] levels at baseline.[91,92] Whether sex modified the effect of supplementation on upper respiratory tract infection risk was not examined.[92,93] American men are uncommonly evaluated for this deficiency and often do not receive adequate supplementation, especially those who are older or obese.[94]

Ecological studies suggest a positive correlation between countries with low mean concentrations of 25(OH)D and higher COVID-19 infection and mortality rates.[95,96] A Swiss cohort study of 109 patients reported that 25(OH)D levels were significantly lower in patients with SARS-CoV-2 compared with those who were uninfected, although the association did not significantly differ when stratified by sex and age older than 70 years.[97] A larger analysis of 348,598 UK Biobank participants confirmed that despite a univariate association between 25(OH)D levels and the odds of COVID-19, following multivariable adjustment, the association was no longer significant. Modification by age or sex was not investigated.[98] It is plausible that lower vitamin D levels may contribute to worse disease observed in older men compared with younger or female individuals,[90,93] but there is insufficient epidemiologic evidence in support of this thesis. Given the relatively minimal risks of vitamin D supplementation, some experts have recommended vitamin D supplementation as a COVID-19 preventive strategy, especially in at risk elderly populations.[91]

ORGAN-SPECIFIC BIOMARKERS

Cardiac Biomarkers

Patients with COVID-19 may suffer direct cardiac damage or damage from associated systemic inflammation, hemodynamic instability, and multiple organ failure.[99-101] Cardiac biomarkers are routinely reported among hospital cohorts, and meta-analyses and subsequent studies have shown that mean levels of troponin,[7,101-105] N-terminal pro-B-type natriuretic peptide (NT-proBNP), and creatine kinase myocardial band[101,103,105] were all significantly higher in patients with more severe COVID-19. Furthermore, elevated troponin levels diagnostic of acute cardiac injury were associated with severe disease and at least a four-fold higher mortality.[102,103] These patients tended to be older, male, and have a history of CVD.[101,103] The most recent meta-analysis, however, reported that the standard mean difference of troponin and BNP levels between severe and less severe COVID-19 infections was modified by hypertension, but not by age, sex or other co-morbidities.[103] Subsequent studies of NT-proBNP, high sensitivity (hs)-troponin levels and COVID-19 outcomes also observed no association with sex.[17,106,107] Among healthy individuals, baseline levels of cardiac

biomarkers significantly differ by sex. For example, in females compared with age-matched males, hs-troponin and creatine kinase myocardial band levels are lower, whereas NT-proBNP levels are higher.[108,109] However, in an acute setting, sex-specific hs-troponin and NT-proBNP thresholds do not improve their predictive value of myocardial infarction or death.[109-111] Despite women having higher baseline NT-proBNP, in the setting of acute heart failure the absence of an NT-proBNP sex difference is likely because women more commonly have preserved ejection fraction and less of an increase in NT-proBNP levels compared with men who are more likely to develop low ejection fraction heart failure and greater elevations in NT-proBNP levels.[106,111] In addition, NT-proBNP levels are thought to be inversely related to androgen levels. Thus, the baseline sex difference is less pronounced as women and men age because estrogen levels decline in women and androgen levels decrease in men.[112] Similar to non-COVID patients, sex was not shown to modify the association between cardiac biomarker levels and patient outcomes among COVID-19 patients in the acute hospital setting.[103]

Liver Function

SARS-CoV-2 causes liver damage through varied mechanisms, from direct cellular toxicity to the effects of immune-related inflammation; concomitant drug toxicity may contribute to liver damage in patients with COVID-19.[75] Several studies reported increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of between 14% and 53% in COVID-19–affected patients. Significantly higher plasma levels associate with more severe infection and, in some studies, with mortality.[10,12,16,75,113-115] One study of 168 patients critically ill with COVID-19 reported significantly higher levels of ALT and AST in males compared with females.[17] Serum transaminase concentrations are generally lower in females compared with males,[116-119] in part due to differences in fat to muscle ratio, lipid metabolism, and hormonal effects on liver cells.[119] Premenopausal women are at lower risk of development of liver inflammation, nonalcoholic steatohepatitis, and the resulting increase in cardiovascular risk.[120] Furthermore, elevated ALT may be a predictor of coronary artery disease in males only.[121] With limited reporting on sex differences in liver markers, it is difficult to identify an effect of sex on the prognostic potential of transaminases in COVID-19 patients.

Renal Function

Renal injury occurs in COVID-19. A large prospective cohort study of 701 patients in Wuhan, China, noted that acute kidney injury (AKI) occurred in 5% of patients.[122] This is a lower percentage than is usually observed in other critical illnesses, and renal autopsies performed on COVID-19 patients with AKI showed evidence of renal histologic injury, with varying degrees of acute tubular necrosis.[123] Biomarkers of renal impairment, including an increase in creatinine, blood urea nitrogen, and presence of AKI have been reported in most studies.[16,113,122,124,125] Greater elevations in these renal biomarkers, along with proteinuria, and hematuria, occur in critically ill patients compared with patients with mild or moderate infection.[7,123] Furthermore, independent of age and sex, a higher baseline creatinine, underlying proteinuria, and hematuria were associated with a higher risk of mortality.[12,46,123,124] In patients with severe disease, creatinine and blood urea nitrogen levels were consistently higher in men compared with women,[17] and older males were more likely to have a higher baseline creatinine and develop AKI,[17,124] although studies have not investigated the effect of sex on renal biomarkers and COVID-19 severity.

Serum creatinine levels are affected by many factors including, age, sex, and muscle mass. The literature regarding susceptibility to AKI based on sex is controversial. A woman's hormonal environment, however, is thought to have a protective effect against the development of AKI,[126,127] and females have been previously shown to be at lower risk of AKI compared with males.[126] Similarly, smaller studies of renal transplantation patients have suggested that male sex may be a risk factor for AKI in

Mayo Clin Proc. ■ October 2020;95(10):2189-2203 ■ https://doi.org/10.1016/j.mayocp.2020.07.024
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TABLE. Coagulation Biomarkers, Sex, and Age Differences[a]

Coagulation biomarker	Sex with higher biomarker level[b]	Advancing age
Primary hemostasis		
Platelet count	Female[150]	Decrease[150]
Estrogen receptor associated platelet protein expression	Equal[151]	
VSM estrogen receptor beta: alpha ratio	Female[152,c]	
NO mediated vasodilation	Female[153-155]	
Platelet adherence + spreading response to vascular injury	Male[156]	
Platelet aggregation	Equal[156]	
Secondary hemostasis		
Factor VII	Female[157]	Increase[157]
Factor VIII	Female[157,158,d]	Increase[157,158]
Factor IX	Equal[157]	Increase[157]
vWF	Female[158,d]	Increase[158]
Fibrinogen	Female[157,d,e]	Increase[157]
PT	Equal[159]	
aPTT	Equal[159]	
Fibrinolysis		
Clot lysability	Equal[160]	
Plasminogen activator inhibitor-1 antigen	Male[161]	Increase[161,f]
Tissue plasminogen activator antigen	Male[161]	Increase[161,f]
Protein C/S levels	Varies by age[157]	Increase[157,g]
D-dimer	Female[162]	Increase[163,164]
General		
VTE	Male[165,h]	
VTE recurrence	Male[166]	
Ischemic stroke incidence	Male[167,i]	
Hemodynamically significant coronary stenosis at first MI in age <45 years	Male[168]	

[a]aPTT = activated partial thromboplastin time; MI = myocardial infarction; NO = nitric oxide; PT = prothrombin time; VSM = vascular smooth muscle; VTE = venous thromboembolism; vWF = von Willebrand factor.
[b]It is assumed that pre-menopausal females have significantly higher estrogen levels than males.
[c]Female levels decreased post-menopause.[169]
[d]Alterations seen in women on hormone replacement therapy/ pregnancy / menstrual cycle.
[e]Alterations seen with testosterone levels.[170]
[f]Levels are non-significantly higher in men than age-matched post-menopausal women.
[g]Female levels increased post-menopause more consistently than in men.[157]
[h]Excluding women on hormone replacement therapy, pregnant, or during the puerperium.
[i]Within the 46- to 64-year-old age group. Changes depending on race in the 65- to 74-year-old age group. No difference after 75 years.

COVID-19.[128-131] These data set the stage for future studies that should be adequately powered, and likely multicenter, to address how the interplay between sex and COVID-19 may affect the incidence of kidney dysfunction, both in native and transplanted kidneys.

COAGULATION BIOMARKERS

Thrombotic diatheses are commonly observed in persons with severe COVID-19.[132,133] COVID-19 patients with thrombotic complications generally follow a course of disease that is more aggressive. In one study, 71% of patients who died of COVID-19 fulfilled the International Society of Thrombosis and Hemostasis criteria for disseminated intravascular coagulopathy compared with just 0.6% of survivors.[134] Moreover, evidence consistently shows the negative prognostic value of individual coagulation parameters, including elevated D-

dimer[12,46,85,135,136] and reduced platelet counts,[45,46,74,135-138] both of which were significant after adjustment for multiple confounders.[139]

In studies of COVID-19 patients with coagulation dysfunction, the composition of the patient population more commonly includes male patients, and possibly reflects the more severe disease that occurs in males.[12,85,138] The underlying mechanism of coagulopathy in COVID-19 patients has yet to be elucidated, but it is hypothesized that a disproportionate inflammatory response results in endothelial cell dysfunction and a pro-thrombotic state.[140] Because of ACE2 receptor expression on endothelial cells, the COVID-19 virus may cause endotheliitis, which could result in not only arterial and venous inflammation, but also microcirculatory and lymphocytic endotheliitis; the consequences of such endotheliitis include widespread organ involvement, sudden vasoconstriction, abnormal angiogenesis, microthrombi formation, and ischemia.[140-142] Moreover, patients with severe COVID-19 develop a hypercoagulable state,[143] further demonstrated by increased levels of factor VIII and von Willebrand factor, marginally decreased anti-thrombin III activity,[144] and inactivation of the fibrinolytic system.[145] These derangements likely underlie venous thromboses; arterial thromboses that may present as ischemic stroke, mesenteric ischemia and acute limb ischemia; and the phenomenon of free-floating thrombi observed in COVID-19 infection—related thrombotic events.[133,146-149]

Studies of coagulation factors in the general population have consistently shown more favorable profiles for female subjects, and particularly for young women of premenopausal age; such profiles may confer lower risks for thrombotic events compared with men (Table). Future investigation into the associations of coagulation markers with respect to COVID-19 severity and sex differences would improve understanding of the disease pathology and inform treatment options.

CONCLUSION

The higher COVID-19 case fatality rate and increased severity of disease in males compared with females is likely due to a combination of behavioral/lifestyle risk factors, prevalence of co-morbidities, aging, and underlying biological sex differences. Several comorbidities, which disproportionally occur in men, likely contribute to worse COVID-19 outcomes, and concerns have been expressed whether ACE inhibitors or angiotensin receptor blockers may exert adverse effects in COVID-19. Experimental and epidemiologic evidence is conflicting as to whether the use of ACE inhibitors and angiotensin receptor blockers upregulate ACE2 expression and impacts susceptibility to infection and/or disease severity. Randomized clinical trials in progress may inform recommendations about the use of such therapy in COVID-19 patients and whether this will differ by sex.

Based on the available literature, we conclude that biological sex differences may affect the pathogenic mechanisms of COVID-19, the risk for infection, and the severity of the disease, its outcomes, and its biomarkers. Indeed, experimental and epidemiologic evidence suggests that most of the biomarkers that have been tested in the context of the risk of infection and the severity of COVID-19 differ by sex at baseline within healthy populations. However, the role of biological sex and risk for infection and disease severity is complex and available data are not uniformly consistent. A notable example is that of the immune response: although females generally have an overall stronger immune response, males are more likely to develop the cytokine storm associated with poor COVID-19 outcomes. Further investigation into immunomodulation by sex hormones, age, and X-linked gene expression may help explain the worse survival of men, and may identify sex-specific risk factors for SARS-CoV-2 infection and the course, outcomes, and prognosis for COVID-19.

Mayo Clin Proc. ■ October 2020;95(10):2189-2203 ■ https://doi.org/10.1016/j.mayocp.2020.07.024
www.mayoclinicproceedings.org

ACKNOWLEDGMENTS

The authors thank Joanna King, Creative Director of the Mayo Clinic Biomedical & Science Visualization Medical Illustration team, for her help in producing Figure 1. Drs Tu and Vermunt are first co-authors.

Potential Competing Interests: Dr Garovic has received grants from the National Institutes of Health (R01 HL136348). The remaining authors report no competing interests.

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