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The intersection of sex and gender in the treatment of influenza

Rosemary Morgan[1] and Sabra L Klein[2]

Males/men and females/women differ in the outcome of influenza A virus (IAV) infections, vaccination, and antiviral treatments. Both sex (i.e. biological factors) and gender (i.e. sociocultural factors) can impact exposure and severity of IAV infections as well as responses and outcomes of treatments for IAV. Greater consideration of the combined effects of sex and gender in epidemiological, clinical, and animal studies of influenza pathogenesis is needed.

Addresses
[1] Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[2] W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Corresponding author: Klein, Sabra L (sklein2@jhu.edu)

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Basic scientists, clinicians, and epidemiologists alike often use the terms 'sex' and 'gender' interchangeably in virology research, which is incorrect because these terms refer to different aspects of biology and behavior. The term 'sex' is a biological construct that defines males and females by the basic organization of chromosomes, reproductive organs, and circulating sex steroid hormone concentrations. Gender is defined as 'the socially constructed roles, behaviors, activities, attributes that a given society considers appropriate for men and women', and people with non-binary identities [1]. Gender is relational, shaping how men/boys, women/girls, and people with non-binary identities interact with each other and the world around them. Both sex and gender can impact the pathogenesis of infectious diseases caused by viruses by influencing the biology and behaviors of men and women differentially. Although we recognize that gender is non-binary, in this review we focus on men and women as we are interested in how biological sex (i.e. male or female) intersects with its corresponding gender (i.e. man or woman) to influence treatments for influenza, which remains an underexplored topic.

Published reports of differences between males/men and females/women in the diagnosis, presentation, and pathogenesis following infection with diverse viruses are rapidly increasing in number [2]. As a general rule, males/men are more susceptible to infection with diverse viruses than females/women, but the underlying causes for greater susceptibility in males/men are diverse and in many cases not known [3]. In addition to differences in the pathogenesis, prognosis, and outcome of infectious diseases, there are profound differences between the sexes in responses and outcomes of prophylactic and therapeutic treatments for infectious diseases, including those caused by viruses.

Influenza (family: *Orthomyxoviridae*) is a significant public health threat, with influenza A viruses (IAVs) causing seasonal epidemics, occasional outbreaks, and sporadic pandemics. Pulmonary disease following infection with IAV can be caused by excessive and aberrant inflammatory responses to the virus, which leads to immunopathology and tissue damage [4]. The severity of influenza disease is typically worse for young children, aged adults, individuals with compromised immune function, and pregnant women. Rarely do basic scientists, epidemiologists, or clinicians consider male–female differences in the pathogenesis of IAVs or in responses to treatments for IAVs. The goal of this review is to identify how biological and social differences between males/men and females/women can alter influenza pathogenesis and the efficacy of treatments and make recommendations for how these factors should be incorporated into public health efforts, to more effectively and efficiently treat IAV infections.

Male–female differences in human influenza pathogenesis

Seasonal epidemics

Most case reports of seasonal influenza do not analyze data for differences between males and females, or if they do, they often do not consider the interaction between sex and age. Examination of the limited available reported seasonal influenza cases that required hospitalization reveals that the severity of infection is typically greater in pre-pubertal males compared with age-matched females [5–7]. Data from Denmark suggest that differences between males and females in the risk of hospitalization from seasonal influenza virus shift at puberty. More recently, a surveillance study in Japan conducted during the 2016–17 season, which was an influenza season dominated by H3N2, reported that adult females were significantly more likely than adult males (median age = 45) to be hospitalized with confirmed influenza diagnosed with a rapid diagnostic test [8]. An analysis of age-associated IAV notifications in Australia from 2010–15 revealed that the female to male ratio was significantly reduced in children under the age of 15 and in adults 75 years and older; in contrast, among adults 20–65 years of age, notifications were significantly greater in females than males [9]. Available data suggest that males are more likely to have severe seasonal influenza illness before puberty, whereas females are more likely to have severe seasonal influenza illness after puberty and before menopause [10].

Pandemics

The 1918 H1N1 influenza pandemic was the most deadly influenza pandemic to date [11]. This influenza pandemic was disproportionately fatal in young adult males (i.e. 20–40 years of age) and was exacerbated by co-infection with tuberculosis, which is also considered to be a male-dominant disease [12,13]. Unlike the 1918 H1N1 pandemic, the 1957 H2N2 pandemic was the first pandemic that was lethal without a secondary bacterial infection. The 1957 H2N2 pandemic resulted in higher fatality rates among adult females than males under 50 years of age, despite the widespread use of vaccine therapy [14•,15]. Many of the fatal cases during the H2N2 pandemic had underlying cardiac or pulmonary conditions; thus, sex-biased co-morbid conditions may have contributed to the increased rates of severe disease and mortality among young adult females during the 1957 H2N2 pandemic [15].

During the 2009 H1N1 pandemic in the United States, females were more likely to develop severe disease than males (53.2% female versus 46.8% male hospitalizations) [16••]. Age at the time of infection, however, was strong predictor of the male–female differences in the incidence, severity of 2009 H1N1 pandemic infection, and mortality rates. Among adults, females were at a higher risk of hospitalization and death from 2009 H1N1 infection than males [17]. In Japan, for example, a female bias was observed in clinical disease following infection with 2009 H1N1 influenza among adults of reproductive ages, which contrasted with the male bias observed before puberty [18••]. In Canada, during the first wave of the pandemic, the majority of critically ill patients with confirmed or probable 2009 H1N1 influenza were young adult females [19]. The reason for the greater proportion of hospitalized adult females than males is not known, but many cases involved comorbid conditions, including chronic lung disease (e.g. asthma), which is typically more severe in adult females than males [20]. Gender norms, included reduced use of medical facilities among men may also be a factor. In countries, including Canada, Japan, and Austrialia, where investigators disaggregated and analyzed outcome data by sex, differences were observed. In other countries, including the United States, data were not analyzed for male–female differences and, therefore, not reported during the 2009 H1N1 pandemic.

Outbreaks

Avian H5N1 is a highly pathogenic influenza virus that affects the lower respiratory tract in humans and is primarily transmitted from diseased poultry to humans, with rare person-to-person transmission. Worldwide, the incidence and severity of H5N1 infection and mortality caused by H5N1 infection has been greater among young females (10–39 years of age) than males [21]. There is annual, as well as country, variation in the male-female differences suggesting that gender-related factors, including occupational exposure, play a significant role. Furthermore, during the 2013–14 H7N9 avian influenza outbreak, while aged males were more likely than any other sex and age-matched cohort to be hospitalized with severe disease, it was females of reproductive ages (i.e. 18–50 years of age) who were most likely to die from H7N9 infection [22]. Hypothesized mechanisms mediating age-associated male–female differences in IAV notifications and pathogenesis are explored below.

Sex differences

Immune responses to infection

Sex differences in influenza pathogenesis have been systematically evaluated using small animal models [23–27]. Using murine models, most, but not all [28], studies have shown that young adult females develop higher pulmonary inflammatory responses and experience a more severe outcome from IAV infection (i.e. with infection with H1N1, H3N1, H3N2, or H7N9 IAVs) than males, despite the sexes having comparable virus titers [23,24,29–31]. For example, pulmonary concentrations of proinflammatory cytokines (e.g. TNF-α, IFN-γ, IL-6, and IL-12) and chemokines (e.g. CCL2, CCL5, and CCL12) are greater during IAV infection in females than males [22,23]. Male mice also repair damaged pulmonary tissue faster than females [32•]. Repair of the damaged lung tissue following IAV infection is generally orchestrated by both immune cells (e.g. regulatory T cells and macrophages) and epithelial cells and involves the production of cytokines and growth factors [33,34]. In response to IAV infection, epithelial cells release growth factors, including amphiregulin (AREG), that can promote repair of damaged lung tissue [35]. Expression of AREG is greater in lung tissue as well as in respiratory epithelial cells derived from males as compared with females during IAV [32•]. Males also depend on AREG more than females for faster recovery from IAV, because when AREG is deleted from mice, the impact on pulmonary inflammation and function is significantly greater for male than for female mice [32•]. In our model, infection of young adult female mice with IAVs reduces ovarian function and concentrations of sex hormones [23,36] suggesting that inhibition of sex hormones, including estrogens and progesterone (P4) may contribute to severe outcomes from IAV in female mice.

Murine models of IAV pathogenesis demonstrate that estradiol (E2) treatment protects females against infection-induced morbidity and mortality [23,37,38]. Treatment of female mice with E2 protects against IAV disease by reducing the inflammatory responses that can cause tissue damage, including excessive production of IFNγ, TNFα, and CCL2, and by promoting higher antibody responses to influenza vaccination [23,37,38]. Some [38], but not all [23,25], studies suggest that treatment of females with E2 affects type I IFN responses and virus replication in the lungs. Estriol (E3) is another form of estrogen that significantly improves pulmonary function and reduced morbidity and clinical disease in females during IAV infection. Estriol reduces the transcriptional activity of genes associated with proinflammatory cytokines and chemokines during early IAV infection, which is associated with reduced recruitment of immune cells into the lungs [36].

In C57BL/6 mice infected with IAV, treatment with either progesterone (P4) or a synthetic progestin, leovonorgestrel (LNG), prevents severe outcome by decreasing pulmonary inflammation and promoting faster recovery during a primary infection [26,39]; in contrast, studies utilizing outbred CD-1 mice report minimal effects of P4 morbidity, mortality, and pulmonary inflammation following IAV infection [40]. P4-based treatments also promote pulmonary repair following clearance of IAVs by elevating levels of TGF-β, IL-6, and IL-22 and increasing the numbers of regulatory CD39+ Th17 cells in the lungs. Production of AREG is increased following P4 treatment, which promotes proliferation and repair of respiratory epithelial cells during IAV infection [26]. Treatment with either P4 of LNG also reduces IAV-specific antibody titers as well as IAV-specific memory CD8+ T cells numbers, which results in worse outcome following secondary IAV challenge in female mice [39]. While the anti-inflammatory effects of P4-based compounds protect against a primary virus infection, the reduction in memory T cell responses increase female susceptibility to secondary IAV challenge.

In male mice, lower concentrations of testosterone, either associated with aging (i.e. 16–18 months of age) or caused by surgical castration of young males, are associated with increased IAV-associated pulmonary inflammation and morbidity, with no effect on the control of virus replication [32•,27]. Importantly, administration of exogenous testosterone to either aged male or castrated young male mice significantly improves the outcome of IAV, independent of changes in pulmonary viral load [27]. Taken together, these data illustrate a role for sex steroids in IAV pathogenesis.

Efficacy of antiviral drugs

Following infection, neuraminidase inhibitors can be administered to alleviate symptoms of disease and virus shedding [41]. Oseltamivir (Tamiflu) is administered orally, absorbed in the gastrointestinal tract, and converted to the active metabolite, oseltamivir carboxylate, by an esterase in the liver [41]. Zanamivir (Relenza) is an inhaled powder delivered as the active compound directly into the respiratory tract [41]. In patients with confirmed IAV infection and treated with oseltamivir, alleviation of symptoms of disease is faster and the reduction of nasal virus load is greater among males than females [42]. Data also suggest that females clear oseltamivir more rapidly than males, at least in newborns [43]. Oseltamivir has to be converted into oseltamivir carboxylate to have antiviral effects. Oseltamivir activation is catalyzed by carboxylesterase 1 (CES1) in the liver, with females having greater amounts of CES1 and greater CES1-mediated oseltamivir hydrolysis than males [44]. In contrast, in IAV-infected patients treated with zanamivir, no sex differences in either alleviation of symptoms or virus load are observed, suggesting that sex differences in drug absorption or metabolism may contribute to the dimorphic outcome of treatment with oseltamivir, but not zanamivir [42].

Immune responses and efficacy of vaccines

Influenza vaccines are recommended for all individuals aged six months and older and are administered annually. Sex differences in response to influenza vaccines in both adult and aged individuals have been reported [45]. Data from human trials have shown that when young adults, ages 18–49 years, are administered either a full or half dose of the seasonal trivalent inactivated influenza vaccine (TIV), females generate hemagglutination inhibition (HAI) antibody titers that are twice as high as those of males [46]. Similarly, adult females 20–89 years of age (not partitioned by age or reproductive status [i.e. premenopause versus post-menopause) generate higher neutralizing antibody titers to the H3N2 and influenza B antigens following seasonal TIV than males, and males who have the highest circulating testosterone concentrations tend to have the lowest neutralizing antibody titers [47], but this effect has been challenged [48]. A more recent report of seasonal influenza vaccine effectiveness in Canada reported that the overall efficacy of TIV was significantly higher for females (49%; age not specified) than males (38%) [49].

In response to the pandemic monovalent 2009 H1N1 vaccine administered to older individuals (ages 61–86 years), aged females generate higher HAI antibody titers than males, which results in a two to three times higher seroconversion rate for females as compared to males [50]. It also has been demonstrated, at least in one study, that among community-dwelling older individuals in Taiwan who received the seasonal influenza vaccine, higher HAI titers were associated with lower hospitalization rates and mortality in females as compared to males [51]. These data suggest that influenza vaccine efficacy may be greater for females as compared to males. Overall, there are no clinical studies that have adequately partitioned and analyzed data for age-related sex differences in the context of reproductive status.

Animal models provide further evidence for sex differences in the immunological responses to and protection provided from influenza vaccines. In response to live influenza virus infection, adult female mice have higher influenza-specific antibody responses and are better protected from secondary heterosubtypic challenge than male mice [29]. Following vaccination with either whole inactivated IAV, TIV, or the quadrivalent inactivated vaccine (QIV), adult female mice generate greater quantity and quality of influenza-specific antibody responses than males [52,53,54••]. Antibody derived from vaccinated females is also better at protecting both naïve males and females than antibody from males, and this protection is associated with increased antibody specificity and avidity to the H1N1 virus [54••]. The toll-like receptor-7 (*Tlr7*) gene is encoded on the X chromosome, is also expressed in B cells, and plays a role in isotype switching [55]. The expression of *Tlr7* is greater in B cells from vaccinated females than males and is associated with reduced DNA methylation in the *Tlr7* promoter region, higher neutralizing antibody, class switch recombination, and antibody avidity in females [54••]. Deletion of *Tlr7* reduced sex differences in vaccine-induced antibody responses and protection following challenge and had a greater impact on responses in females than males. Taken together, these data illustrate that greater TLR7 activation in B cells and antibody production in females improves the efficacy of vaccination against influenza.

Gender differences

Gender and its intersection with sex and other social stratifiers (i.e. age, race, education, etc.) influences patterns of exposure to pathogens, vulnerability to illness, and outcome of illness, resulting in differences in incidence, duration, severity, and fatality rates [16••]. Understanding these interactions and considering how this leads to differences between and among men/boys and women/girls can increase the effectiveness and efficacy of influenza prevention and control programs.

Although it is difficult to disentangle sex and gender (as well as other social stratifiers), it is important to consider the ways in which each impact vulnerability, exposure, and treatment. Gender norms, roles, and relations, for example, can lead to differences in men/boys and women/girls: vulnerability and exposure to illness [56], acceptance of vaccination or vaccination hesitancy [57–59], prevalence of vaccination [45], response to adverse reactions to vaccines [57], and treatment to disease [60]. Gender differences in vulnerability to disease, acceptance of influenza vaccines, and treatment to influenza are discussed below.

Vulnerability to disease

Men/boys and women/girls have different roles and occupations that increase the likelihood of coming into contact and being infected with IAVs [16••]. Occupational roles can differentially influence the vulnerability (i.e. likelihood of becoming infected) to IAVs that are transmitted through animals, such as poultry or swine. Professions that influence the likelihood of exposure to IAVs include health workers and people who work with children – which are professions predominately employed by women [16••,56,61]. When family members fall ill, the caregiving roles within the home are most likely to be performed by women, putting them at greater risk of acquiring IAVs [16••]. Caregiving roles and responsibilities may delay health-care seeking in women, where as other gender norms may delay health-care seeking in men.

Acceptance of influenza vaccines

A limited body of evidence suggests that the acceptance of influenza vaccines differs between men and women. For example, while vaccine hesitancy is often reported as being higher among women than men [57,62], and receipt of an influenza vaccine has been found in some instances to be higher among men than women [63–65], there is little explanation of the cause for these differences. Although sex can lead to differences in biological response to vaccines, it does not explain why more women than men are reluctant to be vaccinated, or why in some instances more men than women receive a flu vaccination. Greater acceptance and receipt of influenza vaccines is reported among both older and younger adults. One hypothesis for reluctance to receive the influenza vaccine is that pregnant women are more likely to have concerns about vaccine safety than the general population; studies suggest, however, that pregnancy cannot be the only explanation of these gender differences [57]. A gender analysis of vaccination acceptance is needed to help explain these differences.

When considering vaccination acceptance and its role in the control and prevention of influenza, it is also important to consider how sex and gender intersect with other social stratifiers, such as race, socio-economic status, education, and disability. Evidence shows that in the United States, for example, race plays a role in influenza vaccine attitudes and uptake [66••,67], with African Americans having greater vaccine hesitancy and lower uptake of vaccination. Factors that contribute to lower rates of immunizations among different races include the following: personal beliefs, trust in health providers, limited access to health care, and anticipated negative side effects [60]. There is very little research, however, which explores how gender and race intersect to affect an individual's attitudes toward and uptake of influenza vaccines. Such research may show that vaccine acceptance and uptake among African American women is different than that of African American men or their white counterparts.

Antiviral treatment of influenza

In the United States, while data have shown that the rate of prescribing antivirals to those infected with influenza is similar between men and women, the rate of inappropriate prescription of antivirals has been found to be higher among women than men [16••]. Again, these differences cannot be explained by biological sex alone and more research is needed to explore how gender norms, roles, and relations lead to disparities in treatment to influenza between men/boy and women/girls.

Racial disparities in treatment to influenza have also been found. A study in the United States, for example, found that treatment rates among those diagnosed with influenza were three times higher for White patients compared to African American patients [60]. If the previous data had been disaggregated by sex and race concurrently, the rate of prescribing antivirals to those infected with influenza may have been different across different groups of men and women. This is also an area where more research is needed to understand how gender intersects with race to create differential marginalization in influenza treatment.

Conclusions

The interaction of sex and gender must be considered in studies of IAV pathogenesis, influenza vaccines, and antiviral treatments. There are gaps in our understanding of the precise mechanisms mediating sex-biased immune responses and how this affects the outcome of IAV infection. Future research should define the mechanistic pathways mediating how hormones, genes, and even microbiota differently affect immunity to IAV and vaccines in males compared with females.

Very little attention has been paid to the role of gender in the incidence, prevalence, and severity of IAV. Sex and gender do not exist independently from one another and it can be difficult to disentangle the role of each in the treatment of influenza [16••]. Sex and gender also intersect with other social stratifiers, including age, race, socioeconomic status, and disability, and similar difficulties exist in disentangling the role of gender from the role of other social stratifiers, particularly in relation to understanding marginalization and vulnerability in relation to diseases, including influenza.

We recommend that clinicians, epidemiologists, and basic biomedical scientists design experiments that include both males/men and females/women, develop *a priori* hypotheses that males/men and females/women will differ in their responses to and the outcome of IAV infection, vaccination, and antiviral treatments across the life course, and statistically analyze outcome data by sex and other social stratifiers. In the United States, the National Institutes of Health (NIH) implemented a policy in 2016 that requires investigators seeking federal funds for preclinical research to consider how biological sex impacts research findings. A recent analysis of NIH study section members and their understanding and opinion of the NIH sex-reporting policy revealed growing acceptance and appreciation of the value of this policy over time [68].

In order to explore the intersection of sex and gender, we recommend that gender should also be mainstreamed into experiments where appropriate (i.e. through the inclusion of gender-related questions and indicators), and that follow-up quantitative and qualitative studies which explore the role of gender norms, roles, and relations in responses to and outcomes of IAV infection, vaccination and antiviral treatments are conducted. Such research will help to explain *why* differences exist between and among males/men and females/women, providing a more robust and accurate understanding of sex and gender differences in the treatment of influenza.

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