THE INTERSECTION OF SEX, GENDER, AND AGING ON INFLUENZA AND COVID-19 VACCINE OUTCOMES

by
Janna R. Shapiro

A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland
May 2022

ABSTRACT

The influenza and COVID-19 vaccines are essential public health tools for older adults, who are vulnerable to severe disease following infection. For both diseases, although sex and gender are known to influence epidemiologic outcomes, their role in the context of vaccination is largely unexplored. The three aims of this dissertation each address how sex or gender differences can be leveraged to successfully vaccinate older adults against viral respiratory diseases. First, in data collected from a longitudinal cohort of older adults (≥ 75 years of age) who repeatedly received the high-dose trivalent inactivated influenza vaccine, pre-vaccination titers predicted vaccine responses. Turning to pre-vaccination titers as an outcome of importance, an interaction between age and sex emerged. Titers to two influenza strains decreased with age in males but not in females, suggesting that older females mount more durable responses to vaccination than older males. Second, using the same cohort of older adults, we measured humoral responses induced by three doses of a SARS-CoV-2 mRNA vaccine. Age and frailty were associated with reduced antibody responses in males, but not females, suggesting that older males may be vulnerable to breakthrough infections following two doses. The third vaccine dose restored functional antibody responses, eliminated disparities caused by sex, age, and frailty, and boosted responses to variants of concern, highlighting the importance of third dose coverage in older adults, especially males. Finally, turning to the role of gender in vaccine behavior, we performed in-depth interviews to understand how older adults made the decision to receive the COVID-19 vaccine, and how intersections between gender and race shaped this process. While some participants eagerly

accepted vaccination, others had significant hesitations due perceived risks associated with the vaccine product and infrastructure, which varied by gender and race. Most ultimately accepted vaccination due to fear of COVID-19, with additional motivators depending on lived experiences of gender and race. Taken together, these data support a central role for sex and gender in vaccine outcomes for older adults and more tailored approaches to vaccinology. By harnessing the heterogeneity in vaccine outcomes, we can better serve this vulnerable population.

Thesis Readers

Advisor: Sabra L. Klein	International Health - BSPH
Co-advisor: Rosemary Morgan	International Health - BSPH
Sean X. Leng	Geriatric Medicine & Gerontology - SOM
Scott L. Zeger	Biostatistics - BSPH

Alternates

Kawsar Talaat	International Health - BSPH
Michelle Kaufman	Health, Behavior, and Society - BSPH

ACKNOWLEDGEMENTS

The research presented in this dissertation, and my personal and professional development over the past four years, would not have been possible without the support of many wonderful mentors, colleagues, friends, and family members.

First and foremost, I would like to thank my co-supervisors, Drs. Sabra Klein and Rosemary Morgan, for the many opportunities you have trusted me with, everything you have taught me, and for being such wonderful role models. You have both taught me how to use research as advocacy, and that lesson will leave an indelible mark on my career. Sabra – thank you for being a fierce leader and teaching me to think strategically about my research and my career. Rosemary – thank you for being patient as I muddled through new research techniques and for always refocusing my thoughts by asking 'so what?'.

Thank you to Dr. Sean Leng for trusting me with your precious data and samples, for welcoming me into your team, and for your guidance. To the clinical staff, Engle Abrams, Denise Kelly, and Eileen Sheridan-Malone, thank you for being wonderful colleagues.

I would also like to thank the members of the Klein lab, past and present, for your contributions to the work presented in this dissertation, and for your support and friendship. Special thanks to Han-Sol Park, Christopher Caputo, and John Lee for many long ELISA days, to Santosh Dhakal for many helpful conversations, and to Patrick Shea for making everything run smoothly. To Helen Kuo, thank you for being patient with me in my early days of Stata, and for your friendship. Thank you to Katherine Merport for all your contributions to this work.

I want to thank the members of my thesis advisory and oral exam committees for helpful feedback as I developed my research. Special thanks to Dr. Scott Zeger and Lois Privor-Dumm, whose expertise and perspective were pivotal to this work. Thank you to the Department of International Health, especially Cristina Salazar and Kawsar Talaat, and my adopted family in MMI for helping me to navigate the many requirements of a PhD program. I am extremely grateful for the opportunities I was afforded at the School of Public Health, notably the PAVE program and the Gordis Teaching Fellowship, for adding diversity and richness to my training. Thank you to Dr. Brian Ward for making all of this seem possible, and to everyone at McGill who gave me the foundation I needed to tackle my PhD.

~

To my friends in Baltimore: It took me a long time to feel settled here, and your friendship has made me feel at home. Thank you for all the girls' nights and all the adventures.

To my partner Mario, who has been by my side every single step of the way: You have grounded me, kept me on track, and been an endless source of encouragement, perspective, and laughter. Special shout-out to FaceTime for sustaining a long-distance relationship across an international border during a global pandemic.

Finally, none of this would have been possible without the support of my family. My grandparents, aunts, uncles, cousins, brother, and sister-in-law have listened patiently to my slightly too-long answers to their questions about vaccines during the pandemic, kept me entertained in weekly Zoom calls, and only made fun of my 'made-up' science words occasionally. Thank you to my Mom, Adele, for being there for every single twist and turn along

the way, and to my Dad, Michael, who is my sounding board for all big ideas. Together, my parents created a home filled with love and laughter, laying a foundation that allowed me to keep reaching for bigger and better things.

DEDICATIONS

To my friend, Jessica Yudcovitch, who passed away suddenly five years ago. I have often thought of all the good you would have put out into the world, both through your career and as a respected member of the community. This has been a constant source of inspiration for me, and I will always strive to honor your legacy.

To my grandfather, David Marshall, who, after completing his bachelor's degree in Chemistry at McGill, earned a PhD in Chemistry from MIT in the early 1950's, publishing several papers along the way. I am proud to walk in your footsteps.

TABLE OF CONTENTS

LIST OF TABLES

MAIN TABLES

SUPPLEMENTAL TABLES

LIST OF FIGURES

MAIN FIGURES

SUPPLEMENTAL FIGURES

LIST OF ABBREVIATIONS

ACE2	Angiotensin converting enzyme 2
ACIP	Advisory Committee on Immunization Practices
AIC	Akaike's Information Criterion
ANOVA	Analysis of variance
AS03	Adjuvant system 03
AUC	Area under the curve
BCA	Bicinchoninic acid
BEI	Biodefense and emerging infections
BMI	Body mass index
CD	Cluster of differentiation
CDC	Centers for Disease Control and Prevention
CEIRS	Centers of Excellence in Influenza Research and Surveillance
CI	Confidence interval
CLIP	Chronic low-grade inflammatory phenotype
CM	Complete media
CMV	Cytomegalovirus
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immune absorbent spot
GM-CSF	Granulocyte macrophage-colony stimulating factor
GMT	Geometric mean titer
HA	Hemagglutinin
HAI	Hemagglutination inhibition
HC	Healthy control
HCoV	Human coronavirus
HCW	Healthcare worker
HD	High dose
HKU1	Human coronavirus HKU1
HPV	Human papillomavirus
HRP	Horseradish peroxidase
IAV	Influenza A virus
IFN	Interferon
IIV3	Trivalent inactivated influenza vaccine
IL	Interleukin

IM	Intra-muscular
IQR	Inter-quartile range
IRB	Institutional review board
LTCFR	Long-term care facility resident
MERS	Middle East respiratory syndrome
mRNA	Messenger ribonucleic acid
nAb	Neutralizing antibodies
NL63	Human coronavirus NL63
NT50	50% neutralization titer
OC43	Human coronavirus OC43
OD	Optical density
OPD	o-phenylenediamine dihydrochloride
PBMC	peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline - tween
PD1	Post dose 1
PD2	Post dose 2
PD3	Post dose 3
RBD	Receptor binding domain
RT	Room temperature
SADII	Sex and age differences in immunity to influenza
SARS	Severe acute respiratory syndrome
SCORE	Specialized centers of research excellence
SCR	Seroconversion rate
SFU	Spot forming units
SI	Stimulation index
SPR	Seroprotection rate
VE	vaccine effectiveness
VOC	Variant of concern
VPD	Vaccine-preventable disease
WHO	World Health Organization

CHAPTER 1

ROADMAP FOR SEX-RESPONSIVE INFLUENZA AND COVID-19 VACCINE RESEARCH IN OLDER ADULTS

Janna R. Shapiro, Rosemary Morgan, Sean X. Leng, and Sabra L. Klein

Frontiers in Aging, 2022

1.1 Abstract

Sex differences in the immune system are dynamic throughout the lifespan and contribute to heterogeneity in the risk of infectious diseases and the response to vaccination in older adults. The importance of the intersection between sex and age in immunity to viral respiratory diseases is clearly demonstrated by the increased prevalence and severity of influenza and COVID-19 in older males compared to older females. Despite sex and age biases in the epidemiology and clinical manifestations of disease, these host factors are often ignored in vaccine research. Here, we review sex differences in the immunogenicity, effectiveness, and safety of the influenza and COVID-19 vaccines in older adults and the impact of sex-specific effects of age-related factors, including chronological age, frailty, and the presence of comorbidities. While a female bias in immunity to influenza vaccines has been consistently reported, understanding of sex differences in the response to COVID-19 vaccines in older adults is incomplete due to small sample sizes and failure to disaggregate clinical trial data by both sex and age. For both vaccines, a major gap in the literature is apparent, whereby very few studies investigate sex-specific effects of aging, frailty, or multimorbidity. By providing a roadmap for sex-responsive vaccine research, beyond influenza and COVID-19, we can leverage the heterogeneity in immunity among older adults to provide better protection against vaccine-preventable diseases.

1.1 Introduction

Throughout the lifespan, sex and age are fundamental modifiers of immunity to infectious diseases and the response to vaccination. Females tend to mount stronger immune responses than males (1, 2), and immunosenescence leads to impaired immune function and a heightened inflammatory state in older adults (3). There is an important intersection between these host factors, whereby the impact of aging on the immune system differs in males and females (4, 5). The implications of the interaction between sex and age are clearly demonstrated by the epidemiology and clinical manifestations of respiratory viral diseases, such as influenza and COVID-19 (6, 7).

Influenza and COVID-19 represent the largest proportion of the vaccine-preventable diseases that occur in older adults and are thus the focus of this review (8, 9). Despite high coverage with seasonal influenza vaccines in the United States, there are an estimated 4 million incident cases per year in older adults, accounting for 90% of the deaths associated with influenza (8, 10). Globally, it has consistently been reported that at older ages, males are at greater risk of infection (11), hospitalization (12-14), and mortality (12, 15). Similarly, the disproportionate burden of COVID-19 in older adults was recognized early in the pandemic (16, 17), with male sex being a significant predictor of severe outcomes at older ages (18-22).

Vaccines prevent the morbidity and mortality associated with influenza and COVID-19 in older adults. Despite the clear sex and age biases in epidemiology, the impact of these host factors on vaccine responses is often ignored or controlled for in analyses, instead of thoroughly investigated. Here, we review sex differences in the immunogenicity, effectiveness,

and safety of influenza and COVID-19 vaccines in older adults, and the available evidence on how sex modifies the impact of age-related factors on vaccine outcomes (**Table 1**). After identifying major gaps in the literature, we provide a framework for sex-responsive vaccine research to leverage the heterogeneity of older populations to provide optimal protection against vaccine-preventable diseases, beyond influenza and COVID-19.

1.2 Sex differences in the response to influenza vaccination in older adults

Sex differences in the immune response to influenza vaccination in older adults have been reported for multiple types of inactivated influenza vaccines (IIV). For the standard-dose IIV, older females have greater influenza-specific memory B cells, post-vaccination antibody titers, fold-rises in titers, rates of seroconversion, and rates seropositivity (23-27). For the high-dose IIV, which contains four times the amount of antigen as standard dose vaccines and is targeted to older adults, females have greater post-vaccination titers and rates of seroconversion than males (25-27). In addition, the 2009 pandemic H1N1 (pH1N1) vaccine generates stronger responses in females in the oldest age groups (28). The consistency of the female-bias in immunogenicity across various vaccine formulations implores continued consideration of sex as a variable of importance in influenza vaccine research.

Sex differences in vaccine effectiveness (VE) in older adults have also been observed, but the evidence is less robust (29, 30). Looking across seven influenza seasons, VE was significantly greater among females than males, and this difference was more pronounced in older adults (31). A recent systematic review, however, concluded that there is insufficient evidence of a sex difference in effectiveness in older age groups (30). The authors note that many studies are

either not designed to assess sex differences or do not present data that is sufficiently disaggregated by age and sex. More evidence is needed to understand how the sex differences in the immunogenicity of influenza vaccines translate to effectiveness.

In terms of vaccine safety, older females consistently report more adverse events (AE) following influenza immunization than males. This has been studied for both the standard-dose (24, 32, 33) and high-dose (34, 35) IIV, and has been confirmed in several systematic reviews (30, 36, 37). In one study that disaggregated data by both sex an age, sex differences were greater at older than younger adult ages (38). Differences in rates of AE may reflect a gender difference in reporting or a biological sex difference in reactogenicity (4).

1.3 Sex differences in the response to COVID-19 vaccines in older adults

In contrast to the well-documented sex differences in response to influenza vaccination, minimal sex- and age-disaggregated data are currently available to interrogate sex differences in COVID-19 vaccine outcomes in older adults. Published studies including older adults focus primarily on the mRNA vaccines (BNT162b2 and mRNA-1273), and often rely on relatively small sample sizes. In multivariable analysis, female long-term care facility residents (LTCFR) had significantly greater IgG titers and functional antibodies than males after the first mRNA vaccine dose, but not after the second dose (39, 40). Similarly, in fully vaccinated older adults, there are no significant sex differences in antibody titers (41-43). Among LTCFR who recovered from SARS-CoV-2 infection, however, there is a trend of higher antibody levels in females than males (44). While sex differences in immune responses are currently not apparent among older adults who received mRNA vaccines, data are missing for other vaccine platforms, and more research

is needed to understand how sex differences may be modified by prior infection or affect immunity against variants of concern (e.g., Omicron).

The COVID-19 vaccine clinical trials revealed remarkably high efficacy against the ancestral virus at all ages but did not provide estimates disaggregated by sex within each age group (45-48). Sex differences observed in COVID-19 outcomes in unvaccinated older adults are not observed in fully vaccinated populations, such that VE with respect to hospitalization and mortality is the same in males and females (49). Similarly, sex does not impact the risk of breakthrough infection in LTCFR (50) or in the general older adult population (51, 52). Like immunogenicity, sex differences in COVID-19 VE are currently not apparent in older adults, but failure to disaggregate data by both sex and age may be obscuring an important effect.

Few studies have provided both sex- and age-disaggregated safety data for the SARS-CoV-2 vaccines. Among older adults, local, systemic, and medically attended AE are more common in females than in males (53, 54). Both among females and overall, older individuals report fewer AE than younger individuals (53, 54), but the proportion of events classified as serious increases with age (55). Sex differences have been reported for several serious AE, including a female bias in anaphylaxis (56-58) and thrombosis with thrombocytopenia syndrome (59), and a male-bias in myocarditis (60), but these events predominantly occur in younger age groups. It is currently unclear what age- and sex-dependent protective factors may explain the absence of these events following immunization in older populations.

1.4 The intersection of sex with age-related factors

In addition to sex differences in vaccine outcomes in older adults, sex can also modify the effect of chronological age, frailty, or the presence of comorbidities on immunogenicity or VE. Intersectional analyses investigating differences among males and females caused by age-related factors are crucial to a robust understanding of the heterogeneity of vaccine responses.

1.4.1 The intersection of sex and aging

Age-associated changes in immunity (e.g., heightened pro-inflammatory state and deficits in both cellular and humoral immunity) (3, 61, 62) are coupled with changes in the hormonal milieu in both males and females, which can cause sex-specific effects of aging (29). For example, decreasing concentrations of estrogens with menopause are associated with reduced B and T cell numbers and lower concentrations of IL-6 in females (63-65), while decreases in testosterone in males are inversely correlated with levels of soluble IL-6 receptor (66). Furthermore, profiling of peripheral blood mononuclear cells across the lifespan revealed that the decline in naïve T cell activity and increase in monocyte function observed with age occur to a greater extent in males than females, and are accompanied by a male-specific decline in B cell transcriptional activity (67). This analysis also found that abrupt age-associated epigenetic changes occur earlier in males than females, and that while older females have higher adaptive immune cell activity, older males have higher inflammatory and monocyte activity (67). These findings have been replicated in multiple other studies (68-72), and together suggest that the effects of aging on the immune system are dampened and occur at a slower rate in females than males.

Sex-specific effects of aging have been reported in the humoral immune response to influenza vaccination. In the case of repeated vaccination with the high-dose IIV, pre-vaccination titers to A/H3N2 and influenza B viruses decrease with age in males but not in females, suggesting that older females enter each influenza season with greater immunity than their male counterparts (73). In contrast, in response to the pH1N1 vaccine, age-associated declines in immunogenicity are not observed in males, but are observed in females, where they are associated with declining concentrations of estradiol (74). Although the results of these studies may appear conflicting, it is important to note that the high-dose IIV study included only older adults (aged ≥75 years), while the pH1N1 study compared younger (aged 18-45 years) and older (aged ≥65 years) cohorts. Furthermore, pandemic viruses and vaccines provide a unique opportunity to evaluate responses to a novel viral antigen, whereas during seasonal influenza vaccination, the influence of prior exposure to influenza on vaccine immunogenicity may be sex differential (73). Thus, the discordance in the two studies may be explained by the pandemic versus seasonal nature of the vaccines investigated.

For COVID-19 vaccines, multiple studies that either control for or ignore sex have reported that vaccine-induced antibody responses decrease with age in older adults (39, 43, 44, 75-77). There is conflicting evidence on the effect of age on VE, with some studies reporting a negative effect (78-80), and others reporting no effect (81-83). For antibody responses (84, 85) and VE (86-88), several studies have identified both male sex and old age as risk factors, but have not investigated sex-specific effects of aging, with the result that it is currently unknown whether the effects of aging on COVID-19 vaccine outcomes differ in males and females.

1.4.2 The intersection of sex and frailty

Frailty is defined as reduced physiological function leading to increased vulnerability and is associated with profound immune dysregulation that can impact vaccine responses in a sex-specific manner (89-91). Importantly, the prevalence of frailty is higher, but mortality is lower, in older females than older males, suggesting fundamental sex differences in pathophysiology (92, 93). For example, frailty is associated with increased frequency of pro-inflammatory late memory B cells only in males (94), and baseline concentrations of CRP and fibrinogen are associated with increased incidence of frailty only in females (95). The relationship between frailty and vaccine responses is debated in the literature, and few studies have considered how sex may modify this relationship.

For influenza, frailty is not associated with pre- or post-vaccination HAI titers in either males or females, nor is a sex difference in the impact of frailty observed (73, 96). In addition, no association between frailty and antibody responses is observed either when controlling for sex in statistical analysis (25, 97) or when ignoring sex altogether (98-100). In contrast, it has also been reported that frailty has both a negative (101) and a positive effect on antibody responses (26), and that frailty may impact measures of vaccine-induced cell-mediated immunity (25). Evidence of the effect of frailty on influenza VE is also conflicting, with one study reporting that VE decreases significantly with frailty (102), and another reporting that VE estimates are not confounded by frailty (103).

The impact of frailty on COVID-19 vaccine outcomes has only been investigated without consideration of sex. Frailty does not impact vaccine-induced antibody responses against

BNT162b2 when controlling for sex and other covariates (42) or when ignoring sex (104, 105). Frailty does, however, increase the risk of post-vaccination infection when controlling for sex and age (50, 51). In analyses that control for sex, living in a long-term care facility, a proxy for frailty, was associated with increased risk of severe COVID-19 outcomes post-vaccination (86) and individuals with breakthrough infections were more likely to be LTCFR than unvaccinated infected individuals (52). Finally, VE is lower and wanes faster in both frail individuals and males, but sex-specific effects of frailty were not investigated (87). Together, the data support a role for frailty in impairing COVID-19 VE beyond the impact of chronological age, but whether this effect is different in males or females remains unknown.

For both influenza and COVID-19, the frailty literature is complicated by different methods used to measure frailty, small sample sizes, and lack of consideration of biological sex. More research is needed to address the discordance and gaps in the literature.

1.4.3 The intersection of sex and comorbidity

There is a high prevalence of comorbid conditions in older adults, which can have immunomodulatory effects that impact infectious disease epidemiology and vaccine responses (106). For example, the prevalence of health conditions that increase the risk of influenza-related complications (e.g.: chronic pulmonary or cardiovascular disease, metabolic disorders, etc.) rises drastically with age and is significantly higher in older males than older females (107). Similarly, a greater percentage of older males than females are at high-risk of requiring hospitalization if infected with COVID-19 due to the presence of an underlying condition (e.g.: cardiovascular disease, diabetes, cancer, etc.) (108).

Despite the clear age-by-sex bias in the prevalence of comorbidities that may impact influenza and COVID-19 vaccine responses, sex-specific effects of chronic conditions have not been studied. For influenza vaccines, studies that either control for or do not consider sex report that influenza vaccine immunogenicity (27) and relative VE (98, 109) do not differ by the presence of high-risk conditions. For COVID-19 vaccines, analyses that control for sex reveal that multimorbidity is not associated with immunogenicity in older adults (42, 43), but is associated with VE (52, 86, 87, 110, 111). These data reveal a gap in the literature, whereby the role of sex in modifying the effect of multimorbidity on vaccine responses remains poorly understood.

1.5 Discussion

Both sex and age-related factors have important consequences on vaccine responses in older adults, but the intersection of sex with age, frailty, and comorbidity remain incompletely elucidated (**Table 1**). This literature gap suggests that a roadmap is needed for sex-responsive vaccinology research in older adults (**Figure 1**). Sex-responsive research requires careful thought at the study planning, data collection, analysis, and dissemination phases.

First, study planning must begin with a review of the literature to identify gaps and generate hypotheses related to sex differences and sex-specific effects. Following hypothesis generation, sample size calculations are required to adequately power studies for sub-group analyses and interrogation of sex as an effect-modifier (112). In the literature reviewed above, small sample sizes suggest that many studies are not appropriately powered, and are thus prone to type II errors, whereby the null hypothesis of no sex difference is erroneously

accepted (113). Larger sample sizes, with adequate numbers of males and females, are thus necessary for correct statistical inference. Instead of interpreting larger sample sizes as a burden, sex should be viewed as an important modifier of vaccine-induced immunity and outcomes that could improve study design and interpretation (114)

Second, recruitment of diverse participants and inclusion of populations that are typically under-represented in research (e.g.: populations of color, frail individuals, gender minorities) is essential. Once recruited, explicit strategies are needed for participant retention. For example, home visits that do not require participants to travel to study sites are an effective method to retain participants with reduced mobility. Data collection should also utilize validated measures of frailty, and multi-morbidity, along with sex-specific questions (e.g.: use of hormone replacement therapy) to thoroughly understand underlying differences among and between male and female participants.

Third, sex must be considered as a variable of importance, rather than a confounder to be controlled for, during data analysis and dissemination of results (112). This begins by disaggregating data by sex for relevant sub-groups (i.e., age, frailty status). In formal analysis, use of interaction terms between sex and other variables allows for interrogation of how trends differ between males and females. Finally, dissemination of results should underscore whether findings are true for both males and females and highlight the clinical and public health implications of any sex-specific findings.

In conclusion, we identified sex differences in influenza and COVID-19 vaccine outcomes in older adults but uncovered a significant gap in the literature in terms of the sex-specific

effects of age-related factors. While the present review focused on influenza and COVID-19 vaccines, the conclusions and research roadmap extend to other vaccines administered to older adults, such as the herpes zoster and pneumococcal vaccines, as well as other public health interventions. Implementation of the roadmap requires engagement at all levels, including funders, regulatory agencies, vaccine manufacturers, and academic institutions. Ultimately, it is through sex-responsive research that that we can leverage the heterogeneity of older populations to provide optimal protection against vaccine-preventable diseases.

1.6 Table

Table 1.1 Summary of sex differences and sex-specific effects of age-related factors on influenza and COVID-19 vaccine outcomes in older adults.

Influenza vaccines	COVID-19 vaccines
Sex differences in older adults	
• Immunogenicity of inactivated influenza vaccines is greater in females than in males. • Evidence of greater VE in females, but insufficient sex- and age-disaggregated data to support a definitive conclusion.	• No sex differences are observed in the immunogenicity of mRNA vaccines. • Preliminary evidence that females mount greater antibody responses than males in the context of prior infection. • No evidence of sex differences in VE
Sex-specific effects of aging	
• Pre-vaccination titers to the high-dose inactivated influenza vaccine decrease with age in males, but not in females. • Antibody titers to the 2009 pandemic H1N1 vaccine decrease with age in females, but not in males.	• Both old age and male sex are risk factors for reduced immunogenicity and VE, but sex-specific effects of aging have not been studied.
Sex-specific effects of frailty	
• The impact of frailty on vaccine responses and VE is debated, but no sex-specific effects have been observed.	• Both frailty and male sex are associated with reduced VE, but sex-specific effects of frailty have not been studied.
Sex-specific effects of comorbidity	
• Not determined	• Not determined

Abbreviations: VE: vaccine effectiveness

1.7 Figure

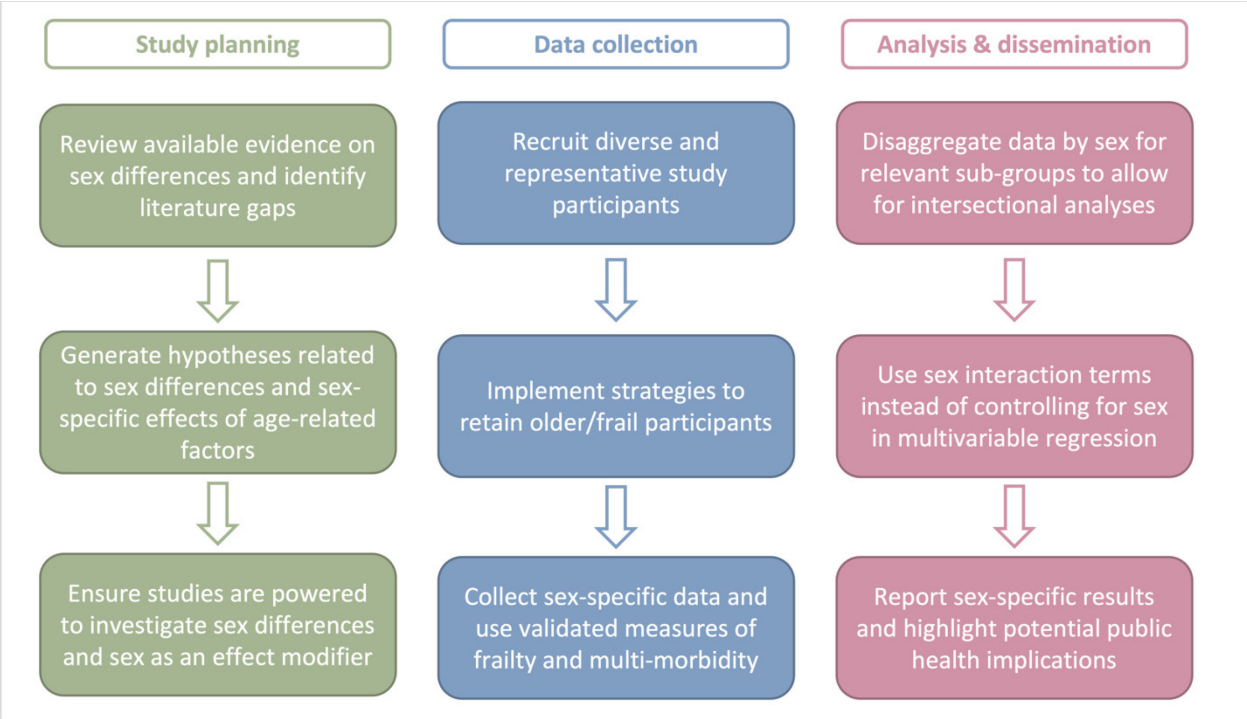


Figure 1.1 Roadmap for sex-responsive vaccinology research in older adults.
Sex-responsive vaccine research in older adults requires careful thought and at the study planning, data collection, analysis, and dissemination phases. Action items for each phase are provided.

1.8 Dissertation rationale & aims

As highlighted in the literature review above, sex is rarely considered as a variable of importance in vaccinology research, particularly in older adult populations. In parallel to the role of biological sex in the immunological response to vaccination, gender has important, but largely unexplored, consequences in vaccine hesitancy and decision-making. In the context of global population aging, where by 2050, the number of people above 65 years of age is expected to double and the number of people above 80 years of age is projected to triple (115), successful vaccination programs for older adults are increasingly important to global health. The three aims of this dissertation each highlight how sex or gender contribute to heterogeneity in vaccine outcomes in older adults, and how this heterogeneity can be leveraged to better protect this vulnerable population from viral respiratory diseases.

1.8.1 Aim 1

The first aim was to estimate the impact of repeat vaccination on the antibody response to the high-dose trivalent inactivated seasonal influenza vaccine in older adults, and its dependence on the intersection of age, sex, frailty, BMI, and pre-existing immunity. This aim consisted of quantitative secondary data analysis using clinical and hemagglutination inhibition titer data collected during the 2014-15 to 2019-20 influenza seasons and is discussed in Chapter 2.

1.8.2 Aim 2

The second aim was to investigate sex differences and sex-specific effects of aging in the magnitude, durability, and breadth of the humoral immune response induced by three doses of

a SARS-CoV-2 mRNA vaccine. This aim consisted of laboratory and quantitative analysis of clinical data and samples collected from older adults prior to and at several timepoints following SARS-CoV-2 vaccination and is discussed in Chapter 3.

1.8.3 Aim 3

The third aim was to understand how the intersection of gender and race shaped the vaccine decision-making processes for older adults during the COVID-19 pandemic, with the goal of understanding how vaccine programs and public health messaging could better meet the diverse needs of this population. This aim consisted of primary data collection in the form of in-depth interviews and qualitative analysis and is discussed in Chapter 4.

CHAPTER 2

SEX-SPECIFIC EFFECTS OF AGING ON HUMORAL IMMUNE RESPONSES TO REPEATED INFLUENZA VACCINATION IN OLDER ADULTS

Janna R. Shapiro, Huifen Li, Rosemary Morgan, Yiyin Chen, Helen Kuo, Xiaoxuan Ning, Patrick Shea, Cunjin Wu, Katherine Merport, Rayna Saldanha, Suifeng Liu, Engle Abrams, Yan Chen, Denise C. Kelly, Eileen Sheridan-Malone, Lan Wang, Scott L. Zeger, Sabra L. Klein, and Sean X. Leng

npj vaccines, 2021

2.1 Abstract

Older adults (≥65 years of age) bear a significant burden of severe disease and mortality associated with influenza, despite relatively high annual vaccination coverage and substantial pre-existing immunity to influenza. To test the hypothesis that host factors, including age and sex, play a role in determining the effect of repeated vaccination and levels of pre-existing humoral immunity to influenza, we evaluated pre- and post-vaccination strain-specific hemagglutination inhibition (HAI) titers in adults over 75 years of age who received a high-dose influenza vaccine in at least four out of six influenza seasons (NCT02200276). Pre-vaccination titers, rather than host factors and repeated vaccination, were significantly associated with post-vaccination HAI titer outcomes, and displayed an age-by-sex interaction. Pre-vaccination titers to H1N1 remained constant with age. Titers to H3N2 and influenza B viruses decreased substantially with age in males, whereas titers in females remained constant with age. Our findings highlight the importance of pre-existing immunity in this highly vaccinated older adult population and suggest that older males are particularly vulnerable to reduced pre-existing humoral immunity to influenza.

2.2 Introduction

Seasonal influenza is an important public health burden in older adults (people ≥65 years of age), particularly the oldest and frail subset (116-118). In the United States (U.S.), there are an estimated 4 million incident cases per year in older adults, accounting for 90% of deaths associated with influenza (8, 10). The U.S. Centers for Disease Control and Prevention (CDC) recommends annual influenza vaccination for prevention of influenza infection and complications in people 6 months and older (119). The high-dose inactivated influenza vaccine (HD-IIV) is available to older adults and has demonstrated superior efficacy over the standard-dose vaccine in older age groups (119, 120). Seasonal influenza vaccination coverage is relatively high in older adults, with >60% of older Americans being vaccinated annually, compared to <40% vaccination coverage in the 18-49 age group (121).

Age-related immunosenescence, defined by a decline in cellular and humoral immune function combined with a chronic low-grade inflammatory phenotype (CLIP), or inflammaging (3, 90, 122), is believed to be the primary reason for the reduced effectiveness of influenza vaccines observed in older adults (123-125). Repeated annual vaccination may also have a negative effect on vaccine-induced humoral immune responses as well as vaccine effectiveness (VE). For example, a recent observational test-negative study using ten years of vaccination history found that in older adults, VE decreases with increasing numbers of previous vaccinations but that vaccination continues to offer some level of protection (126). Another study over eight seasons in the general adult population found that VE to H3N2, but not influenza B virus, is reduced among individuals with frequent vaccination history compared to those without prior vaccination (127). Age-specific effects have also been observed in this

context, with a reduction in immunogenicity observed with repeat vaccination in teenagers but not adults (128). In addition, a meta-analysis found heterogeneous effects of repeated vaccination overall and that when negative effects are observed, they are most pronounced for H3N2 (129). In contrast, a recent systematic review and meta-analysis concluded that the available evidence does not support a reduction in VE with consecutive repeat vaccination, but that certainty in the evidence is low (130). Case control studies in both Australia and Spain found beneficial effects of repeated annual vaccination on VE in older adults (131, 132), and an observational population-based study in Sweden found no differences in VE between those who had been vaccinated in the current season only and those who had been vaccinated in both the current and previous seasons (133). Based on the conflicting evidence, multi-season clinical studies to address the effects of aging and repeated vaccination have been recommended (129).

Mechanistically, pre-existing immunity generated to various influenza virus exposures over time can have an important impact on the outcome of vaccination. According to the immune imprinting theory, the memory response established by an individual's first influenza exposure has a lifelong effect on subsequent immune responses to infection or vaccination (134). Broad pre-existing immunity is thought to have negative consequences, as pre-existing antibodies can suppress the response to novel influenza virus strains by reducing the amount of available antigen or epitope masking (135, 136). A theoretical benefit of HD-IIV is that pre-existing antibodies cannot sequester the increased amount of antigen delivered, and, thus, more antigen is available to activate memory B cells and elicit a protective response (137-139).

To our knowledge, however, the impact of pre-existing immunity in the context of the HD-IIV has not been adequately characterized.

In addition to age, other host factors including sex, frailty, and body mass index (BMI) can impact vaccine responses in older adults. Females have been found to mount greater antibody responses to HD-IIV than males (27). The immunological differences between males and females in immune responses are largely attributed to sex hormones and sex chromosomes (74, 140, 141). Inflammaging likely also contributes significantly to age- and sex-related differences in immune responses and vulnerability to infections, as discussed in two recent reviews in the context of SARS-CoV-2 infection and ongoing pandemic (140, 142). The relationship between frailty and influenza vaccine responses is debated in the literature, with one study reporting frailty having a negative effect (101), others reporting no effect (25, 96, 97, 99, 100), and others still reporting a positive effect (26). Finally, in older adults, obesity, as measured by BMI, is significantly associated with decreased hemagglutination inhibition (HAI) titers and percentage of switched memory B cells (143). Whether host related factors, including sex and age, explain variation in pre-existing immunity following repeated vaccination has not been reported. We hypothesize that the variation across studies in estimates of the effects of pre-existing immunity and repeated annual vaccination is partly caused by failure to adequately account for heterogeneity and interactions among host factors that likely differ across studies. To address this knowledge gap, we used a longitudinal cohort of older adults over 75 years of age who had received high-dose, trivalent inactivated influenza vaccine (HD-IIV3) in at least four out of six influenza seasons to estimate the impact of repeated vaccination on the

antibody response to HD-IIV3 and its dependence on the intersection of age, sex, frailty, BMI, and pre-existing immunity.

2.3 Results

2.3.1 Study Participants and annual influenza immunization with HD-IIV3

Over the six influenza seasons from 2014-2015 to 2019-2020, 90 individuals participated in at least four study seasons and 433 doses of HD-IIV3 were administered. The strains included in each vaccine and the study protocol are described in **Figure 1**. **Table 1** shows demographic and clinical characteristics of the study participants. There were slightly more females (55.6%), and yearly study enrollment increased over time. There were missing data in our study (i.e. individuals who did not participate in all six influenza seasons), but this did not substantially depart from the missing at random assumption, so multi-level models were used to account for this missingness. Baseline characteristics, measured during the first year of participation, were similar between males and females. The median age at study enrollment was 80, and >50% of participants were classified as pre-frail as per the Fried Frailty Phenotype (144). Trends in the change of frailty status since baseline were calculated as the difference between the first and last years of participation and differed by sex. A greater proportion of males improved in frailty status, whereas more females either did not change or progressed in frailty status. A greater proportion of males also experienced changes in BMI over the course of the study, while BMI did not change for females.

2.3.2 Pre- and post-vaccination strain-specific HAI titers are high among repeatedly vaccinated older adults

Pre- and post-vaccination strain-specific HAI titer outcomes to the three HD-IIV3 vaccine strains are summarized and disaggregated by sex in **Table 2**. Pre- and post-vaccination outcomes stratified by influenza season are detailed in **Supplementary tables 1-6**. As expected in this highly vaccinated elderly population, titers and seroprotection rates (defined as an HAI titer ≥40 (145-147)) were high. This was particularly true for influenza B, where 98% of the participants were seroprotected prior to immunization. Post-vaccination, >94% of participants achieved seroprotection for H1N1 and H3N2, while 100% of participants were seroprotected against influenza B. Because of the lack of variability in post-vaccination seroprotection, this outcome was omitted from further analysis. Fold-rise in titers and rates of seroconversion, as defined by ≥4-fold titer increase (148), were relatively low but were highest for H3N2. There were no sex differences in any post-vaccination outcomes. Together, these data indicate that pre-existing and post-vaccination strain-specific HAI titers remained high among older adults who were repeatedly vaccinated with HD-IIV3.

2.3.3 Age, sex, BMI, frailty status and repeated vaccination are not associated with post-vaccination outcomes

We then assessed the relationships between pre-defined host factors (i.e., age, sex, BMI, and frailty status) and post-vaccination strain-specific HAI titer outcomes. When controlling for pre-vaccination titers and influenza season, neither age, frailty, nor BMI individually had statistically significant associations with post-vaccine titers (**Supplementary figure 1A-C**), the fold-rise in titers (**Figure 2A-C**), or the odds of seroconversion (**Supplementary figure 2A-C**) for either H1N1, H3N2, or influenza B. Inclusion of interaction terms in the models

allowed for analysis of sex-specific contributions of age, frailty, and BMI, as well as sex differences in the effects of these host factors. None of the host factors had a statistically significant association with post-vaccination titers (**Supplementary figure 1A-C**), the fold-rise (**Figure 2A-C**), or the odds of seroconversion (**Supplementary figure 2A-C**) for either males or females.

The relationship between the number of years of study participation (measured as a time-varying predictor) and post-vaccination HAI titers outcomes was investigated to evaluate potential negative effects of repeated annual vaccination on the humoral immune response to future vaccines. Vaccination status prior to study enrollment could not be verified, but leveraging the longitudinal design of our study, we sought to quantify whether post-vaccination outcomes declined with additional annual vaccination, and whether this effect differed by sex. There was a non-significant negative trend between the number of years participated and the fold rise in titers (**Figure 2D-G**). These trends were also observed when analyses were repeated using post-vaccination titers (**Supplementary figure 1D-G**) and rates of seroconversion (**Supplementary figure 2D-G**) as outcomes of interest.

2.3.4 Pre-vaccination HAI titers strongly predict post-vaccination outcomes

Overall, host factors and increasing number of annual vaccinations did not significantly predict any of the post-vaccination antibody titer parameters. Pre-vaccination titers, however, were strong predictors of the fold-rise for H1N1 (slope = -0.15; 95% CI: -0.18; -0.13) (**Figure 2H**), H3N2 (slope = -0.12; 95% CI: -0.15; -0.10) (**Figure 2I**), and influenza B (slope = -0.13; 95% CI: -0.15; -0.11) (**Figure 2J**) ($p<0.0001$ for testing the null hypotheses that the slope equals zero for

each vaccine strains), such that greater pre-vaccination titers were associated with a smaller fold rise. Analyses were repeated using post-vaccination titers (**Supplementary Figure 1H-K)** and odds of seroconversion (**Supplementary Figure 2H-K)** as outcomes of interest, and similarly strong associations were observed with the pre-vaccination titers. The strength of these associations suggests that post-vaccination outcomes are primarily determined by pre-existing humoral immunity. Thus, in highly vaccinated populations, such as the older adult participants in this study, pre-vaccination titers are not just confounders to be controlled for in the analysis of post-vaccination humoral immunity but are an outcome of public health importance that illustrate the durability of immunity to influenza from one season to the next. Given the importance of pre-vaccination titers, we focused subsequent analyses on exploring the relationships between host factors and pre-vaccination HAI titers in the context of advanced age and repeated annual vaccination.

2.3.5 Sex modifies the relationship between age and pre-vaccination HAI titers

Next, we assessed the relationships between age, frailty, BMI, and pre-vaccination titers (**Figure 3A-C**). Neither frailty nor BMI were statistically significantly associated with pre-vaccination HAI titers for all participants or in sex-disaggregated subgroups. Further, there were no statistically significant sex differences in the effects of frailty or BMI on pre-vaccination HAI titers against either H1N1, H3N2, or influenza B. A statistically significant sex by age interaction, however, was observed for H3N2 (**Figure 3B**) and for influenza B (**Figure 3C**), in which HAI titers declined with age among male but not female participants.

To further interrogate the sex-specific effects of aging, expanded models controlling for frailty and BMI, in addition to influenza season, were constructed. Coefficients from the base (i.e., controlling for influenza season only) and expanded models are shown in **Table 3**, and results from expanded models are plotted in **Figure 3D-F**. For H1N1, HAI titers tended to increase with age for both males and females, but the increase was not statistically significant (males: 0.49 units per decade, p = 0.152; females: 0.35 units per decade, p = 0.267), nor was the sex difference in slope (p = 0.676) (**Figure 3D**). For H3N2, while titers again tended to increase with age in females (0.62 units per decade, p = 0.121), they tended to decrease with age in males (-0.75 units per decade, p = 0.097), leading to a significant sex difference in age slopes (sex by age interaction = 0.137; p = 0.010) (**Figure 3E**). For influenza B, titers again tended to increase with age in females (0.33 units per decade, p = 0.275) but decreased by 0.78 units per decade in males (p = 0.023) (**Figure 3F**). Like H3N2, the sex difference in the effect of age was significant for influenza B (p = 0.005).

Both the base and expanded models were then amended to include cubic splines to obtain more granular estimates of the effects of age on HAI titers for males and females. Coefficients for base and expanded non-linear models are shown in **Table 3**, and results from expanded models are plotted in **Figure 3G-I**. The non-linear model for H1N1 did not differ from the linear model, and no significant effects of age within each sex or difference between the sexes were observed (**Figure 3G**). Although the trends in the linear models were similar for H3N2 and influenza B, using age as a non-linear predictor revealed that different age categories were driving the overall effects. For H3N2, the increase in pre-vaccination HAI titers with age in females was driven by individuals in the 80-85 age category (increase of 0.67 units; p = 0.037),

and the decrease in males was driven primarily by people in the 75-80 age category (decrease of 1.78 units; p = 0.012) (**Figure 3H**). Thus, the sex difference was greatest at the younger end of the cohort (p = 0.036 at 75 years of age). Conversely, increasing titers to influenza B with age in females were driven by individuals in the 75-80 age category (increase of 1.2 units; p = 0.004), whereas for males there was a sharp decline in HAI titers that occurred in participants 90-95 years of age (decrease of 1.65 units; p < 0.0001) (**Figure 3I**). Here, the sex difference in pre-vaccination titers was only significant at the oldest end of the cohort (p = 0.003 at 95 years of age). Taken together, these data illustrate sex-specific effects of aging on pre-vaccination antibody titers to H3N2 and influenza B, but not H1N1.

To account for the fact that there were several consecutive influenza seasons where the strain included in the vaccine remained constant for either H1N1, H3N2 or influenza B, the above analyses were repeated controlling for viral vaccine stain rather than influenza season. Re-analysis of pre-vaccination antibody titers using viral vaccine strain rather than influenza season in the models did not change any of the associations between age and sex described above, and the trend of declining pre-vaccination titers in males to H3N2 and influenza B remained (**Supplementary Table 7**). Further, to illustrate the consequences of ignoring sex as a biological variable, as is commonly encountered in biomedical research (112, 149), analyses were repeated controlling for sex rather than allowing age effects to vary by sex. For H3N2 and influenza B, where the effect of aging was found to be significantly different in males as compared to females, the estimates derived by controlling for sex (black lines in **Figure 3E, F, H, I**) were not representative of either males or females. In the linear models, for example, controlling for sex led to the incorrect inference that titers remain constant with age, while the

interaction models demonstrate that this is false for both males and females. The goodness-of-fit of models controlling for sex and using an age-by-sex interaction term were compared using Akaike's Information Criterion (AIC) in **Table 4**, where lower values indicate better relative goodness-of-fit. For antibody titers to H3N2 and influenza B, despite the penalty for increasing model complexity, fit was improved by including an age-by-sex interaction term that allowed the effect of age to differ by sex. Thus, incorporating sex differences into vaccinology research can lead to more robust analysis.

2.4 Discussion

In this multi-season, longitudinal study of older adults over 75 years of age, pre-vaccination titers, rather than host factors or repeated vaccination, strongly predicted all post-vaccination antibody titer outcomes. While it has previously been reported that pre-existing immunity predicts the outcome of vaccination across various age groups (126, 128), the role of sex and age in explaining variability in pre-existing immunity has not been characterized. We report that pre-existing humoral immunity, which reflects the durability of humoral immunity against influenza from previous seasons, displayed an age-by-sex interaction. We found that HAI titers to all three vaccine strains stayed constant in females with age but that HAI titers to H3N2 and influenza B decreased with age in males, leading to significant sex differences in the effect of age for these two vaccine viruses. Thus, sex is a fundamental predictor of the effect of age on pre-existing immunity in this vulnerable population. It has previously been reported that at older ages, there is a male-bias in influenza B infection and hospitalization (11, 13). Our results therefore provide a potential mechanism for this sex difference and highlight the need to develop better vaccines or vaccination strategies against influenza for older males.

Because older adults are disproportionally burdened by severe disease and mortality from seasonal influenza, significant effort has been devoted to improving annual vaccination coverage for this vulnerable population. Older adults, particularly those who are over 75 years of age, can thus have decades of repeated annual influenza vaccination. Cumulatively, repeated annual vaccination can lead to high pre-vaccination titers, which we observed in this study and previously reported in younger adult healthcare workers, where mandatory vaccination policies result in exceptionally high rates of immunization (150). Particularly important in older adults, where formation of *de novo* responses is impaired by immunosenescence (151), the breadth of pre-existing humoral immunity and the positive predictive value for post-vaccination titers can thus be harnessed to elicit protection (138). The clinical and scientific implications of this notion are far-reaching and long-term, as the Advisory Committee on Immunization Practices (ACIP) of the CDC has recommended annual influenza vaccination for anyone aged 6 months and older since 2010 (152).

For many vaccines, antibody titers wane over time (153-156). Influenza vaccines are unique in this respect due to the recommendation for yearly immunization and exceptional antigenic diversity, which alter the dynamics of waning immunity. The constant pre-vaccination HAI titers with age in females seen in our study suggest that females benefit from a booster effect from each successive annual vaccination that appears to prevent antibody waning. This influenza-specific effect has been reported elsewhere, where samples collected from individuals over a 20-year period revealed longitudinal increases in neutralizing titers to influenza (157). However, our data suggest that this effect is absent in males for H3N2 and influenza B. The reasons for this sex difference are unknown, but may be attributable to the

compounding effects of females developing stronger responses to influenza infection and vaccination throughout adulthood (4), leading to a more robust repertoire of memory B cells that recognize conserved epitopes on drifted virus strains. It is speculated that in older adults, consistently inferior responses among males may manifest as a lack of memory B cells that can be boosted by drifted viruses to counteract waning of antibody over time, thus resulting in decreasing pre-vaccination titers with age.

Notably the sex difference was absent for responses to H1N1 vaccine antigens. A possible explanation for this lies in the differing evolutionary rates of the three vaccine viruses. H1N1 viruses experience slower evolution than H3N2 viruses (158) and the influenza B/Victoria lineage (159). In addition, the global co-circulation of B/Yamagata and B/Victoria lineages leads to increased exposure to divergent antigens (160). Accordingly, over the six influenza vaccine seasons included in our study, and in the past decade, vaccine antigens were significantly more variable for H3N2 and influenza B than for H1N1 (161). It is thus possible that repeated exposure to the same H1N1 strain sufficiently boosted male steady-state immunity to mask sex differences in the immune response. Conversely, for H3N2 and influenza B, sequential exposure to drifted viruses required robust and broad responses to allow for boosting of steady-state immunity, which may have only been present in females. Another possible explanation is immunological imprinting in youth, as it has a lifelong impact on subsequent immune responses to influenza infection and vaccination (134, 162). Individuals in our cohort, born from 1916-1941, may have been exposed to H1N1 in their youth, while the 1918 pandemic virus continued to circulate, but were likely exposed to H3N2 and influenza B later in life (163). It is therefore

possible that strong immune imprinting to H1N1 virus strains masked the sex differences otherwise observed for H3N2 and influenza B.

Our study had several strengths and limitations. First, this was an observational study that was not specifically designed to interrogate sex differences in the immune response to influenza vaccination. To overcome small yearly sample sizes, six influenza seasons were pooled together, and statistical methods were used to control for annual variation in vaccine virus strains and repeated measurements on participants. The resulting multi-season nature of this work improves generalizability to future influenza seasons. Secondly, the humoral immune response to vaccination was measured by strain-specific HAI titers, which are the standard in the field, but lack the functional quality of microneutralization assays (164). Relying solely on serological samples also prohibited mechanistic investigation at the cellular level. In-depth studies of cellular and transcriptional mechanisms underlying the sex differences observed in this cohort are on-going. Third, the lack of racial diversity in our cohort must be noted, as it prohibited us from investigating race as a host factor of interest, which should be considered in future studies. Although the study lacked racial diversity, the cohort was diverse in terms of age at vaccination, allowing us to study effects in the 'oldest' old subset. In addition, we were unable to ascertain vaccination history for the participants prior to enrollment in the study. Previous research suggests that influenza vaccination coverage is similar among older men and women (165), such that it is unlikely that gender differences in vaccination history confounded the results observed. Finally, a major strength of this study is the intersectional approach to analysis, which allowed for interrogation of effects both between and within groups (i.e.,

between and among males and females), leading to a richer and more nuanced interpretation (166).

In conclusion, we demonstrated that in highly vaccinated older adults, pre-vaccination HAI titers, rather than age, sex, BMI, frailty, or repeated vaccination, predict post-vaccination parameters of humoral immunity. These pre-vaccination titers change with age in a sex-specific manner, such that older males are particularly vulnerable to lower levels of pre-existing humoral immunity. These findings provide a basis for future studies to investigate the predictive value of host factors and vaccination history in protection from influenza, which could ultimately be a valuable tool in a clinical setting. Further research should focus on elucidating the mechanisms underlying this sex difference, as well as novel vaccination strategies to harness the breadth of pre-existing immunity in older adults to provide better protection against influenza for this vulnerable population.

2.5 Methods

2.5.1 Study population and protocol

During the 2014-2015 to 2019-2020 influenza seasons, we enrolled community-dwelling older adults above 75 years of age who had not yet received a seasonal influenza vaccine. Individuals who had a history of allergic reaction to influenza vaccines or to eggs, were currently taking oral steroids, or had worsening or new-onset of immune-modulating conditions (e.g., rheumatoid arthritis, hematologic malignancies, etc) were excluded. Study participants came to the Clinical Research Unit at Johns Hopkins Institute of Clinical and Translational Research on the Johns Hopkins Bayview Medical Center campus, or study visits were conducted

at participants' home as needed. A detailed medical history was obtained, vital signs were measured and frailty was assessed as per the Fried Frailty Phenotype (144). After a pre-vaccination blood draw, participants received HD-IIV3 (Fluzone®High-Dose, Sanofi Pasteur, PA, USA). A second blood sample was collected between 21 and 28 days after vaccine administration (**Figure 1**). To focus on the context of repeated annual vaccination, only individuals who participated in a minimum of 4 influenza seasons were included in this analysis.

2.5.2 Ethics

Written, informed consent was obtained from all participants. The study protocol was approved by the Johns Hopkins School of Medicine Institutional Review Board. The study is registered on clinicaltrials.gov (NCT02200276).

2.5.3 Hemagglutination inhibition assays

Validated HAI assays were performed by Sanofi Pasteur and used to quantify antibody titers against the three influenza virus strains (H1N1, H3N2, and B) included in each season's vaccine (167). Briefly, serum was incubated with type III neuraminidase to eliminate non-specific inhibitors and then with turkey red blood cells to adsorb non-specific agglutinins. Two-fold serial dilutions of sera, beginning at a 1:10 dilution, were then performed in duplicate, and sera were incubated with influenza virus (4 hemagglutination units/25µl). Turkey red blood cells were then added, and the titer defined as the highest dilution in which hemagglutination of turkey red blood cells was inhibited.

2.5.4 Definitions and categorization of predictor variables

Sex was used as a dichotomous variable based on self-report. Age was calculated based on the date of vaccination and used as a continuous variable. The frailty assessment was based on the presence or absence of five measurable characteristics: slowed motor performance (by walking speed), poor endurance and energy (by self-report of exhaustion), weakness (by grip strength), shrinking (by unintentional weight loss), and low physical activity (by self-report) (101, 144). Participants with three or more out of these five characteristics were defined as frail, those with one or two as prefrail, and those with none as non-frail. BMI was calculated based on measured height and weight and used as a continuous variable. Influenza season (i.e., 2014-2015 to 2019-2020) was used as categorical variable so as not to imply a linear relationship from year-to-year. The influenza season was included as a dummy variable in analyses to account for possible confounding due to variation in the vaccine composition over time. In sensitivity analyses, viral strain was used as an alternative approach to control for confounding due to antigenic drift. Number of years of study participation was defined as the number of vaccines administered to an individual as part of the study at the time that the outcome was measured each year. Number of years of study participation was used as a time-varying continuous predictor ranging from 1 to 6 (i.e., set to one the first year an individual participated, two the second year an individual participated, etc…).

2.5.5 Outcome variables

Geometric mean titers were calculated both pre- and post-vaccination. For regression analysis, titers were transformed to a $\log_2$ scale to achieve an approximately normal distribution. The fold-rise in titer was calculated as post-vaccination titers divided by pre-

vaccination titers, and $\log_{10}$ transformed to achieve a normal distribution. Seroconversion was defined as achieving a fold-rise ≥4 and used as a binary outcome. Seroprotection was defined as a titer ≥ 40 and used as a binary outcome.

2.5.6 Statistical analysis

To account for repeated measures on participants, multi-level mixed effects models with random intercepts on the individual were used. Although there was missing data in our study, we do not anticipate substantial departure from the missing at random assumption. The mixed-effects method was selected because it is considered to be robust in addressing 'non-informative' missing data (168). Following standard risk factor analysis procedure, the contributions of host factors of interest were first assessed individually. Based on the *a priori* hypotheses of this analysis, fixed effects of the base models for post-vaccination outcomes adjusted for influenza season, pre-vaccination titers and included interaction terms to allow the effect of the host factor to differ for males and females. Fixed effects of the base models for pre-vaccination titers adjusted for influenza season and included interaction terms to allow effects to differ by sex. Where significant sex differences were found, further analysis controlled for additional covariates, and used cubic B-splines to investigate non-linear relationships (169). The relative goodness-of-fit of various models were compared using Akaike's Information Criterion. For graphs, predictions were capped at 95 years of age due to low sample size and large uncertainty in estimates above 95 years. Coefficients were considered statistically significant if 95% confidence intervals did not span the null value of zero (i.e., $p<0.05$). Analysis was performed in Stata 15 (StataCorp).

2.6 Acknowledgements

The authors thank the participants, as well as the clinical staff at the Healthy Aging Studies Unit and the Johns Hopkins University Institute for Clinical and Translational Research. The authors would also like to than Dr. Andrew Pekosz for helpful discussion and feedback. Sanofi Pasteur provided HD-IIV3 and performed HAI testing but remained blinded to the results and had no further scientific input. This work was supported in part by National Institute of Health (NIH)/National Institute of Allergy and Infectious Diseases R01 AI108907 to S.X.L., NIH/National Institute on Aging Specialized Center of Research Excellence U54 AG062333 awarded to S.L.K., and funding from Irma and Paul Milstein Program for Senior Health, Milstein Medical Asian American Partnership (MMAAP) Foundation of USA to S.X.L. J.R.S was supported by a training award from the Fonds de recherche du Québec – Santé (File #287609). C.J.W, X.X.N, and S.F.L. were supported by Irma and Paul Milstein Program for Senior Health fellowship awards from MMAAP Foundation of USA (www.mmaapf.org).

2.7 Tables

Table 2.1 Summary of study population characteristics.

	All	Male	Female
Person-seasons - n (%)	433	192 (44.3)	241 (55.7)
Individuals - n (%)	90	40 (44.4)	50 (55.6)
Yearly participation - n (%)			
2014	45 (50.0)	19 (47.5)	26 (52.0)
2015	68 (75.6)	28 (70.0)	40 (80.0)
2016	68 (75.6)	31 (77.5)	37 (74.0)
2017	87 (96.7)	38 (95.0)	49 (98.0)
2018	88 (97.8)	40 (100.0)	48 (96.0)
2019	77 (85.6)	36 (90.0)	41 (82.0)
Number of seasons participated			
4	40 (44.4)	18 (45.0)	22 (44.0)
5	27 (30.0)	12 (30.0)	15 (30.0)
6	23 (25.6)	10 (25.0)	13 (26.0)
Birth year - Median (p25-p75)	1934 (1930 - 1938)	1934 (1929 - 1938)	1934 (1930 - 1938)
Range	1916 - 1941	1922 - 1941	1916 - 1940
Baseline characteristics[1]			
Age - Median (p25-p75)	80 (77 - 83)	80 (77 - 84)	80 (77 - 83)
Frailty - n (%)			
Non-frail	37 (41.1)	17 (42.5)	20 (40.0)
Pre-frail	48 (53.3)	21 (52.5)	27 (54.0)
Frail	5 (5.6)	2 (5.0)	3 (6.0)
BMI - Median (p25-p75)	26.8 (24.5 - 30.4)	27.0 (25.3 - 29.6)	26.5 (23.2 - 30.5)
Change from baseline[2]			
Frailty - n (%)			
Improved	11 (12.2)	8 (20.0)	3 (6.0)
No change	44 (48.9)	18 (45.0)	26 (52.0)
Worsened	35 (38.9)	14 (35.0)	21 (42.0)
BMI			
Decreased	39 (43.3)	19 (47.5)	20 (40.0)
No change (+/- 1)	38 (42.2)	14 (35.0)	24 (48.0)
Increased	13 (14.4)	7 (17.5)	6 (12.0)

[1] Value for first year participated

[2] Difference between first and last year participated

Table 2.2 Pre- and post-vaccination hemagglutination antibody inhibition (HAI) titer outcomes.

	All	Males	Females	Sex difference[1]
Person-seasons - n (%)	433	192 (44.3)	241 (55.7)	
H1N1				
Pre-vaccination - GMT (95% CI)	74.3 (66.8 - 82.8)	81.7 (70.7 - 94.4)	69.0 (59.0 - 80.6)	0.4451
Post-vaccination - GMT (95% CI)	192.3 (174.6 - 211.9)	185.9 (161.5 - 213.9)	197.7 (173.0 - 225.9)	0.8922
Pre-vaccination SPR - n (%)	325 (75.1)	149 (77.6)	176 (73.0)	0.4116
Post-vaccination SPR - n (%)	415 (95.8)	184 (95.8)	231 (95.9)	0.7837
Fold-rise (log10) - mean (95% CI)	0.413 (0.376 - 0.450)	0.357 (0.308 - 0.406)	0.457 (0.405 - 0.510)	0.1711
Seroconversion rate - n (%)	134 (30.9)	47 (24.5)	87 (36.1)	0.1932
H3N2				
Pre-vaccination - GMT (95% CI)	89.3 (77.0 - 103.5)	91.8 (73.0 - 115.4)	87.3 (71.9 - 106.0)	0.8246
Post-vaccination - GMT (95% CI)	363.1 (316.3 - 416.8)	365.0 (292.3 - 455.9)	361.5 (303.5 - 430.6)	0.8793
Pre-vaccination SPR - n (%)	314 (72.5)	141 (73.4)	173 (71.8)	0.8855
Post-vaccination SPR - n (%)	408 (94.2)	179 (93.2)	229 (95.0)	0.4556
Fold-rise (log10) - mean (95% CI)	0.609 (0.559 - 0.660)	0.600 (0.527 - 0.672)	0.617 (0.548 - 0.687)	0.8554
Seroconversion rate - n (%)	207 (47.8)	84 (43.8)	123 (51.0)	0.1807
B				
Pre-vaccination - GMT (95% CI)	262.8 (235.3 - 293.4)	236.3 (204.8 - 272.6)	286.0 (243.1 - 336.4)	0.2847
Post-vaccination - GMT (95% CI)	571.1 (520.2 - 627.1)	508.5 (445.8 - 580.1)	626.5 (549.7 - 714.0)	0.1735
Pre-vaccination SPR - n (%)	424 (97.9)	191 (99.5)	233 (96.7)	0.2887
Post-vaccination SPR - n (%)	433 (100.0)	192 (100.0)	241 (100.0)	
Fold-rise (log10) - mean (95% CI)	0.337 (0.304 - 0.370)	0.333 (0.288 - 0.378)	0.341 (0.294 - 0.387)	0.9482
Seroconversion rate - n (%)	105 (24.2)	47 (24.5)	58 (24.1)	0.8386

[1] Sex difference p-values derived from multi-level linear (GMT) or logistic regressions (SPR and SCR). Fixed effects included a term for sex and controlled for study year. Random effects included a random intercept on the individual.

Abbreviations & definitions: CI: confidence interval; Fold-rise: post-vaccination titer divided by pre-vaccination titer, transformed on the $\log_{10}$ scale; GMT: geometric mean titer; SPR: seroprotection rate, the proportion of individuals who achieved a titer ≥40; Seroconversion rate: the proportion of individuals who achieved a fold-rise in titer ≥ 4.

Table 2.3 Sex-specific effects of age on pre-vaccination hemagglutination antibody inhibition (HAI) titers

	Base Models[1]						Expanded Models[2]					
	Male age effects		Female age effects		Sex difference[3]		Male age effects		Female age effects		Sex difference[3]	
	Change	p-value	Change	p-value	Difference	p-value	Change	p-value	Change	p-value	Difference	p-value
H1N1												
Linear	0.047	0.164	0.034	0.286	-0.014	0.674	0.049	0.152	0.035	0.267	-0.014	0.676
Non-linear[4]												
75-80	0.379	0.300	0.146	0.650	-0.023	0.963	0.383	0.301	0.134	0.685	-0.010	0.984
80-85	0.133	0.564	0.233	0.251	-0.256	0.465	0.131	0.574	0.243	0.238	-0.259	0.462
85-90	0.205	0.397	0.109	0.589	-0.156	0.657	0.220	0.377	0.113	0.593	-0.147	0.678
90-95	0.421	0.188	0.066	0.830	-0.253	0.547	0.430	0.186	0.071	0.817	-0.254	0.553
95					-0.609	0.31					-0.612	0.313
H3N2												
Linear	-0.067	0.133	0.065	0.103	**0.131**	**0.014**	-0.075	0.097	0.062	0.121	**0.137**	**0.010**
Non-linear[4]												
75-80	**-1.725**	**0.015**	-0.156	0.801	**-1.898**	**0.029**	**-1.776**	**0.012**	-0.297	0.637	**-1.833**	**0.036**
80-85	-0.139	0.711	**0.653**	**0.039**	-0.328	0.469	-0.102	0.786	**0.670**	**0.037**	-0.355	0.435
85-90	0.023	0.953	0.114	0.716	0.463	0.326	-0.048	0.904	0.149	0.651	0.417	0.379
90-95	-0.496	0.39	-0.415	0.430	0.553	0.351	-0.591	0.307	-0.421	0.431	0.614	0.308
95					0.634	0.503					0.784	0.412
B												
Linear	**-0.098**	**0.005**	0.009	0.762	**0.108**	**0.007**	**-0.078**	**0.023**	0.033	0.275	**0.111**	**0.005**
Non-linear[4]												
75-80	-0.315	0.516	**0.965**	**0.023**	-1.051	0.087	-0.189	0.693	**1.203**	**0.004**	-1.048	0.080
80-85	-0.106	0.695	0.161	0.486	0.229	0.511	0.038	0.886	0.219	0.330	0.344	0.298
85-90	-0.291	0.306	**-0.534**	**0.019**	0.496	0.165	-0.181	0.515	-0.111	0.633	0.525	0.123
90-95	**-1.799**	**0.000**	-0.434	0.248	0.253	0.572	**-1.649**	**0.000**	-0.233	0.526	0.595	0.168
95					**1.618**	**0.020**					**2.011**	**0.003**

[1] Base models controlled for study year.

[2] Expanded models controlled for study year, frailty, and BMI.

[3] For linear models, the sex-difference is the age-sex interaction term. For non-linear models, the sex difference is at the beginning of each five-year interval.

[4] Non-linear models include cubic B-splines with knots at 5-year age intervals from 75-95 years.

Statistically significant values are bolded.

Table 2.4 Goodness-of-fit comparison of pre-vaccination age models

	Base models[1]		Expanded models[2]	
Age-sex interaction	**-**	**+**	**-**	**+**
H1N1				
Linear age	1138.86	1140.69	**1131.05**	1132.87
Non-linear age	1144.47	1151.51	1136.73	1143.72
H3N2				
Linear age	1693.38	1689.57	1666.94	**1662.83**
Non-linear age	1694.58	1694.83	1667.28	1667.95
B				
Linear age	1398.31	1393.17	1355.04	1349.28
Non-linear age	1388.09	1378.85	1344.94	**1337.26**

[1] Base models controlled for study year.

[2] Expanded models controlled for study year, frailty, and BMI.

The lowest AIC, corresponding to the best-fit model, is bolded for each virus.

Supplementary Table 2.1 Pre- and post-vaccination HAI titer outcomes, 2014-2015 season

2014-2015 season	All	Males	Females	Sex difference[1]
Vaccinations - n	45	19	26	
H1N1				
Pre-vaccination - GMT (95% CI)	83.3 (57.5 - 120.7)	93.5 (60.7 - 143.8)	76.6 (42.7 - 137.3)	0.5987
Post-vaccination - GMT (95% CI)	301.5 (223.3 - 407.0)	322.2 (213.3 - 486.7)	287.2 (183.7 - 448.9)	0.7074
Pre-vaccination SPR - n (%)	33 (73.3)	16 (84.2)	17 (65.4)	0.1676
Post-vaccination SPR - n (%)	44 (97.8)	19 (100.0)	25 (96.2)	.
Fold-rise (log10) - mean (95% CI)	0.559 (0.416 - 0.702)	0.537 (0.382 - 0.693)	0.574 (0.344 - 0.804)	0.8024
Seroconversion rate - n (%)	23 (51.1)	10 (52.6)	13 (50.0)	0.8615
H3N2				
Pre-vaccination - GMT (95% CI)	159.0 (106.2 - 238.0)	178.0 (87.0 - 364.3)	146.4 (88.4 - 242.7)	0.6354
Post-vaccination - GMT (95% CI)	485.2 (320.1 - 735.4)	672.1 (326.4 - 1383.6)	382.4 (228.7 - 639.2)	0.1801
Pre-vaccination SPR - n (%)	40 (88.9)	17 (89.5)	23 (88.5)	0.9150
Post-vaccination SPR - n (%)	43 (95.6)	18 (94.7)	25 (96.2)	0.8205
Fold-rise (log10) - mean (95% CI)	0.484 (0.363 - 0.606)	0.577 (0.334 - 0.820)	0.417 (0.292 - 0.541)	0.1937
Seroconversion rate - n (%)	15 (33.3)	8 (42.1)	7 (26.9)	0.2890
B				
Pre-vaccination - GMT (95% CI)	458.4 (315.5 - 666.1)	454.0 (298.9 - 689.5)	461.7 (254.4 - 838.3)	0.9644
Post-vaccination - GMT (95% CI)	1153.9 (868.5 - 1533.0)	1296.0 (909.7 - 1846.2)	1060.0 (685.1 - 1640.0)	0.4875
Pre-vaccination SPR - n (%)	44 (97.8)	19 (100.0)	25 (96.2)	
Post-vaccination SPR - n (%)	45 (100.0)	19 (100.0)	26 (100.0)	
Fold-rise (log10) - mean (95% CI)	0.401 (0.302 - 0.500)	0.456 (0.347 - 0.564)	0.361 (0.204 - 0.518)	0.3464
Seroconversion rate - n (%)	18 (40.0)	10 (52.6)	8 (30.8)	0.1432

[1] Sex difference p-values derived from simple linear (GMT) or logistic regressions (SPR and SCR).
Abbreviations & definitions: GMT: geometric mean titer; SPR: seroprotection rate, the proportion of individuals who achieved a titer ≥1:40; Fold-rise: post-vaccination titer divided by pre-vaccination titer, transformed on the log10 scale. Seroconversion rate: the proportion of individuals who achieved a fold-rise in titer ≥ 4.

Supplementary Table 2.2 Pre- and post-vaccination HAI titer outcomes, 2015-2016 season

2015-2016 season	All	Males	Females	Sex difference[1]
Vaccinations - n	68	28	40	
H1N1				
Pre-vaccination - GMT (95% CI)	68.1 (51.4 - 90.1)	80.2 (54.6 - 117.7)	60.7 (40.6 - 90.9)	0.3338
Post-vaccination - GMT (95% CI)	199.5 (157.0 - 253.5)	175.3 (125.3 - 245.1)	218.4 (155.1 - 307.5)	0.3714
Pre-vaccination SPR - n (%)	49 (72.1)	20 (71.4)	29 (72.5)	0.9228
Post-vaccination SPR - n (%)	65 (95.6)	27 (96.4)	38 (95.0)	0.7787
Fold-rise (log10) - mean (95% CI)	0.467 (0.373 - 0.560)	0.340 (0.243 - 0.436)	0.556 (0.415 - 0.696)	**0.0217**
Seroconversion rate - n (%)	24 (35.3)	7 (25.0)	17 (42.5)	0.1411
H3N2				
Pre-vaccination - GMT (95% CI)	65.8 (48.4 - 89.4)	77.1 (45.2 - 131.4)	58.8 (40.2 - 86.2)	0.3909
Post-vaccination - GMT (95% CI)	673.7 (486.8 - 932.4)	619.4 (363.1 - 1056.8)	714.6 (466.8 - 1093.9)	0.6691
Pre-vaccination SPR - n (%)	47 (69.1)	20 (71.4)	27 (67.5)	0.7302
Post-vaccination SPR - n (%)	68 (100.0)	28 (100.0)	40 (100.0)	0.7302
Fold-rise (log10) - mean (95% CI)	1.011 (0.866 - 1.155)	0.905 (0.693 - 1.117)	1.084 (0.884 - 1.284)	0.2245
Seroconversion rate - n (%)	53 (77.9)	21 (75.0)	32 (80.0)	0.6252
B				
Pre-vaccination - GMT (95% CI)	127.8 (96.6 - 169.0)	158.7 (110.6 - 227.8)	109.8 (72.9 - 165.3)	0.1974
Post-vaccination - GMT (95% CI)	382.6 (302.4 - 484.1)	422.9 (304.8 - 586.7)	356.7 (254.3 - 500.2)	0.4813
Pre-vaccination SPR - n (%)	63 (92.6)	28 (100.0)	35 (87.5)	
Post-vaccination SPR - n (%)	68 (100.0)	28 (100.0)	40 (100.0)	
Fold-rise (log10) - mean (95% CI)	0.476 (0.383 - 0.570)	0.426 (0.319 - 0.532)	0.512 (0.368 - 0.655)	0.3696
Seroconversion rate - n (%)	25 (36.8)	9 (32.1)	16 (40.0)	0.5091

[1] Sex difference p-values derived from simple linear (GMT) or logistic regressions (SPR and SCR).
Abbreviations & definitions: GMT: geometric mean titer; SPR: seroprotection rate, the proportion of individuals who achieved a titer ≥1:40; Fold-rise: post-vaccination titer divided by pre-vaccination titer, transformed on the log10 scale. Seroconversion rate: the proportion of individuals who achieved a fold-rise in titer ≥ 4.

Supplementary Table 2.3 Pre- and post-vaccination HAI titer outcomes, 2016-2017 season

2016-2017 season	All	Males	Females	Sex difference[1]
Vaccinations - n	68	31	37	
H1N1				
Pre-vaccination - GMT (95% CI)	90.0 (69.0 - 117.4)	92.4 (62.6 - 136.3)	88.0 (60.2 - 128.9)	0.8588
Post-vaccination - GMT (95% CI)	174.1 (135.4 - 224.0)	157.7 (106.2 - 234.2)	189.2 (134.8 - 265.4)	0.4770
Pre-vaccination SPR - n (%)	59 (86.8)	28 (90.3)	31 (83.8)	0.4326
Post-vaccination SPR - n (%)	64 (94.1)	29 (93.5)	35 (94.6)	0.8553
Fold-rise (log10) - mean (95% CI)	0.287 (0.205 - 0.369)	0.232 (0.113 - 0.351)	0.332 (0.216 - 0.448)	0.2284
Seroconversion rate - n (%)	13 (19.1)	5 (16.1)	8 (21.6)	0.5674
H3N2				
Pre-vaccination - GMT (95% CI)	119.6 (80.7 - 177.2)	134.1 (74.0 - 243.1)	108.7 (62.8 - 188.0)	0.5990
Post-vaccination - GMT (95% CI)	413.4 (292.8 - 583.5)	477.0 (268.3 - 847.7)	366.7 (237.6 - 565.8)	0.4522
Pre-vaccination SPR - n (%)	54 (79.4)	25 (80.6)	29 (78.4)	0.8180
Post-vaccination SPR - n (%)	65 (95.6)	30 (96.8)	35 (94.6)	0.6663
Fold-rise (log10) - mean (95% CI)	0.539 (0.421 - 0.656)	0.551 (0.366 - 0.736)	0.528 (0.369 - 0.688)	0.8484
Seroconversion rate - n (%)	31 (45.6)	13 (41.9)	18 (48.6)	0.5802
B				
Pre-vaccination - GMT (95% CI)	241.1 (183.5 - 316.7)	213.3 (153.6 - 296.0)	267.1 (173.5 - 411.3)	0.4161
Post-vaccination - GMT (95% CI)	601.8 (467.7 - 774.4)	542.5 (388.2 - 758.1)	656.5 (448.3 - 961.4)	0.4560
Pre-vaccination SPR - n (%)	67 (98.5)	31 (100.0)	36 (97.3)	
Post-vaccination SPR - n (%)	68 (100.0)	31 (100.0)	37 (100.0)	
Fold-rise (log10) - mean (95% CI)	0.397 (0.300 - 0.495)	0.405 (0.269 - 0.542)	0.391 (0.247 - 0.534)	0.8801
Seroconversion rate - n (%)	21 (30.9)	7 (22.6)	14 (37.8)	0.1788

[1] Sex difference p-values derived from simple linear (GMT) or logistic regressions (SPR and SCR).
Abbreviations & definitions: GMT: geometric mean titer; SPR: seroprotection rate, the proportion of individuals who achieved a titer ≥1:40; Fold-rise: post-vaccination titer divided by pre-vaccination titer, transformed on the log10 scale. Seroconversion rate: the proportion of individuals who achieved a fold-rise in titer ≥ 4.

Supplementary Table 2.4 Pre- and post-vaccination HAI titer outcomes, 2017-2018 season

2017-2018 season	All	Males	Females	Sex difference[1]
Vaccinations - n	87	38	49	
H1N1				
Pre-vaccination - GMT (95% CI)	70.6 (54.8 - 90.9)	72.2 (49.7 - 104.7)	69.4 (48.6 - 99.1)	0.8793
Post-vaccination - GMT (95% CI)	207.4 (164.2 - 261.9)	203.2 (139.9 - 295.3)	210.7 (154.8 - 286.7)	0.8802
Pre-vaccination SPR - n (%)	63 (72.4)	27 (71.1)	36 (73.5)	0.8025
Post-vaccination SPR - n (%)	83 (95.4)	36 (94.7)	47 (95.9)	0.7946
Fold-rise (log10) - mean (95% CI)	0.468 (0.383 - 0.553)	0.450 (0.320 - 0.579)	0.482 (0.365 - 0.600)	0.7073
Seroconversion rate - n (%)	31 (35.6)	11 (28.9)	20 (40.8)	0.2534
H3N2				
Pre-vaccination - GMT (95% CI)	141.4 (100.3 - 199.2)	145.5 (82.8 - 255.4)	138.3 (88.8 - 215.4)	0.8858
Post-vaccination - GMT (95% CI)	335.9 (246.8 - 457.2)	319.2 (201.1 - 506.9)	349.4 (227.7 - 536.1)	0.7748
Pre-vaccination SPR - n (%)	69 (79.3)	30 (78.9)	39 (79.6)	0.9413
Post-vaccination SPR - n (%)	82 (94.3)	37 (97.4)	45 (91.8)	0.2963
Fold-rise (log10) - mean (95% CI)	0.376 (0.300 - 0.452)	0.341 (0.236 - 0.447)	0.402 (0.292 - 0.513)	0.4329
Seroconversion rate - n (%)	23 (26.4)	8 (21.1)	15 (30.6)	0.3182
B				
Pre-vaccination - GMT (95% CI)	264.8 (212.0 - 330.8)	223.3 (160.6 - 310.5)	302.2 (222.5 - 410.6)	0.1817
Post-vaccination - GMT (95% CI)	468.8 (387.3 - 567.4)	385.0 (290.5 - 510.2)	546.2 (421.3 - 708.1)	0.0706
Pre-vaccination SPR - n (%)	87 (100.0)	38 (100.0)	49 (100.0)	
Post-vaccination SPR - n (%)	87 (100.0)	38 (100.0)	49 (100.0)	
Fold-rise (log10) - mean (95% CI)	0.248 (0.177 - 0.319)	0.236 (0.110 - 0.363)	0.257 (0.173 - 0.341)	0.7783
Seroconversion rate - n (%)	12 (13.8)	5 (13.2)	7 (14.3)	0.88

[1] Sex difference p-values derived from simple linear (GMT) or logistic regressions (SPR and SCR).
Abbreviations & definitions: GMT: geometric mean titer; SPR: seroprotection rate, the proportion of individuals who achieved a titer ≥1:40; Fold-rise: post-vaccination titer divided by pre-vaccination titer, transformed on the log10 scale. Seroconversion rate: the proportion of individuals who achieved a fold-rise in titer ≥ 4.

Supplementary Table 2.5 Pre- and post-vaccination HAI titer outcomes, 2018-2019 season

2018-2019 season	All	Males	Females	Sex difference[1]
Vaccinations - n	88	40	48	
H1N1				
Pre-vaccination - GMT (95% CI)	97.6 (77.5 - 122.9)	110.3 (80.9 - 150.5)	88.2 (62.7 - 124.0)	0.3377
Post-vaccination - GMT (95% CI)	233.4 (192.2 - 283.3)	239.9 (182.1 - 316.2)	228.1 (172.3 - 301.9)	0.7978
Pre-vaccination SPR - n (%)	72 (81.8)	32 (80.0)	40 (83.3)	0.6868
Post-vaccination SPR - n (%)	87 (98.9)	40 (100.0)	47 (97.9)	.
Fold-rise (log10) - mean (95% CI)	0.379 (0.302 - 0.455)	0.337 (0.221 - 0.454)	0.413 (0.309 - 0.517)	0.3313
Seroconversion rate - n (%)	23 (26.1)	7 (17.5)	16 (33.3)	0.0970
H3N2				
Pre-vaccination - GMT (95% CI)	119.4 (86.0 - 165.8)	121.8 (75.1 - 197.7)	117.5 (74.0 - 186.5)	0.9136
Post-vaccination - GMT (95% CI)	319.3 (237.0 - 430.2)	322.0 (194.4 - 533.4)	317.1 (219.7 - 457.5)	0.9594
Pre-vaccination SPR - n (%)	70 (79.5)	34 (85.0)	36 (75.0)	0.2512
Post-vaccination SPR - n (%)	83 (94.3)	36 (90.0)	47 (97.9)	0.1470
Fold-rise (log10) - mean (95% CI)	0.427 (0.333 - 0.521)	0.422 (0.303 - 0.542)	0.431 (0.287 - 0.575)	0.9247
Seroconversion rate - n (%)	33 (37.5)	10 (25.0)	23 (47.9)	**0.0292**
B				
Pre-vaccination - GMT (95% CI)	271.0 (210.7 - 348.4)	208.2 (146.4 - 295.9)	337.6 (236.7 - 481.4)	0.0566
Post-vaccination - GMT (95% CI)	576.9 (468.0 - 711.1)	448.9 (328.2 - 613.9)	711.0 (538.9 - 938.0)	**0.0287**
Pre-vaccination SPR - n (%)	86 (97.7)	39 (97.5)	47 (97.9)	0.8962
Post-vaccination SPR - n (%)	88 (100.0)	40 (100.0)	48 (100.0)	
Fold-rise (log10) - mean (95% CI)	0.328 (0.262 - 0.394)	0.334 (0.240 - 0.428)	0.324 (0.228 - 0.419)	0.8795
Seroconversion rate - n (%)	21 (23.9)	10 (25.0)	11 (22.9)	0.8195

[1] Sex difference p-values derived from simple linear (GMT) or logistic regressions (SPR and SCR).
Abbreviations & definitions: GMT: geometric mean titer; SPR: seroprotection rate, the proportion of individuals who achieved a titer ≥1:40; Fold-rise: post-vaccination titer divided by pre-vaccination titer, transformed on the log10 scale. Seroconversion rate: the proportion of individuals who achieved a fold-rise in titer ≥ 4.

Supplementary Table 2.6 Pre- and post-vaccination HAI titer outcomes, 2019-2020 season

2019-2020 season	All	Males	Females	Sex difference[1]
Vaccinations - n	77	36	41	
H1N1				
Pre-vaccination - GMT (95% CI)	49.3 (39.2 - 62.0)	56.8 (42.1 - 76.6)	43.6 (30.8 - 61.8)	0.2556
Post-vaccination - GMT (95% CI)	115.2 (94.0 - 141.0)	114.8 (87.2 - 151.3)	115.5 (85.1 - 156.6)	0.9792
Pre-vaccination SPR - n (%)	49 (63.6)	26 (72.2)	23 (56.1)	0.1449
Post-vaccination SPR - n (%)	72 (93.5)	33 (91.7)	39 (95.1)	0.5438
Fold-rise (log10) - mean (95% CI)	0.368 (0.287 - 0.450)	0.306 (0.197 - 0.415)	0.423 (0.302 - 0.544)	0.1547
Seroconversion rate - n (%)	20 (26.0)	7 (19.4)	13 (31.7)	0.2245
H3N2				
Pre-vaccination - GMT (95% CI)	27.5 (21.1 - 35.8)	24.0 (16.9 - 34.1)	30.9 (20.7 - 46.1)	0.3433
Post-vaccination - GMT (95% CI)	200.2 (142.8 - 280.7)	184.4 (105.7 - 321.7)	215.2 (140.3 - 330.0)	0.6524
Pre-vaccination SPR - n (%)	34 (44.2)	15 (41.7)	19 (46.3)	0.6803
Post-vaccination SPR - n (%)	67 (87.0)	30 (83.3)	37 (90.2)	0.3731
Fold-rise (log10) - mean (95% CI)	0.863 (0.740 - 0.986)	0.886 (0.697 - 1.074)	0.843 (0.673 - 1.012)	0.7301
Seroconversion rate - n (%)	52 (67.5)	24 (66.7)	28 (68.3)	0.8792
B				
Pre-vaccination - GMT (95% CI)	370.5 (297.1 - 462.1)	304.6 (221.0 - 419.9)	440.1 (323.7 - 598.4)	0.0981
Post-vaccination - GMT (95% CI)	636.4 (523.4 - 773.9)	522.2 (395.1 - 690.1)	757.2 (576.1 - 995.1)	0.0584
Pre-vaccination SPR - n (%)	77 (100.0)	36 (100.0)	41 (100.0)	
Post-vaccination SPR - n (%)	77 (100.0)	36 (100.0)	41 (100.0)	
Fold-rise (log10) - mean (95% CI)	0.235 (0.178 - 0.292)	0.234 (0.156 - 0.312)	0.236 (0.150 - 0.321)	0.9792
Seroconversion rate - n (%)	8 (10.4)	6 (16.7)	2 (4.9)	0.11

[1] Sex difference p-values derived from simple linear (GMT) or logistic regressions (SPR and SCR).
Abbreviations & definitions: GMT: geometric mean titer; SPR: seroprotection rate, the proportion of individuals who achieved a titer ≥1:40; Fold-rise: post-vaccination titer divided by pre-vaccination titer, transformed on the log10 scale. Seroconversion rate: the proportion of individuals who achieved a fold-rise in titer ≥ 4.

2.8 Figures

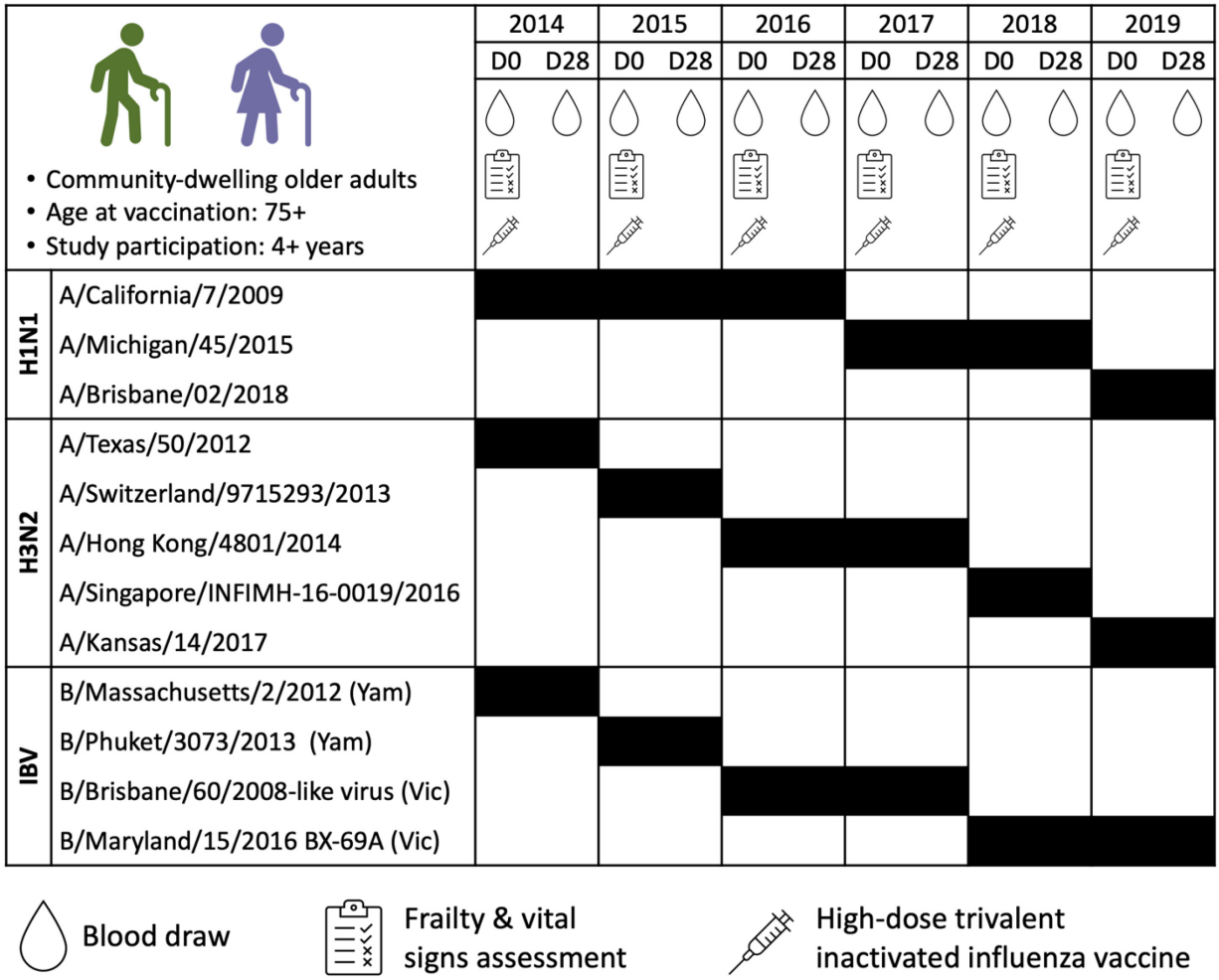


Figure 2.1 Study design
Study procedures and the three strains included in each seasonal HD-IIV3 are shown. Serum from blood draws was used to evaluate pre- and post-vaccination strain-specific hemagglutination antibody inhibition (HAI) titers, and frailty was assessed using the Frailty Phenotype. Images were created with BioRender.com.

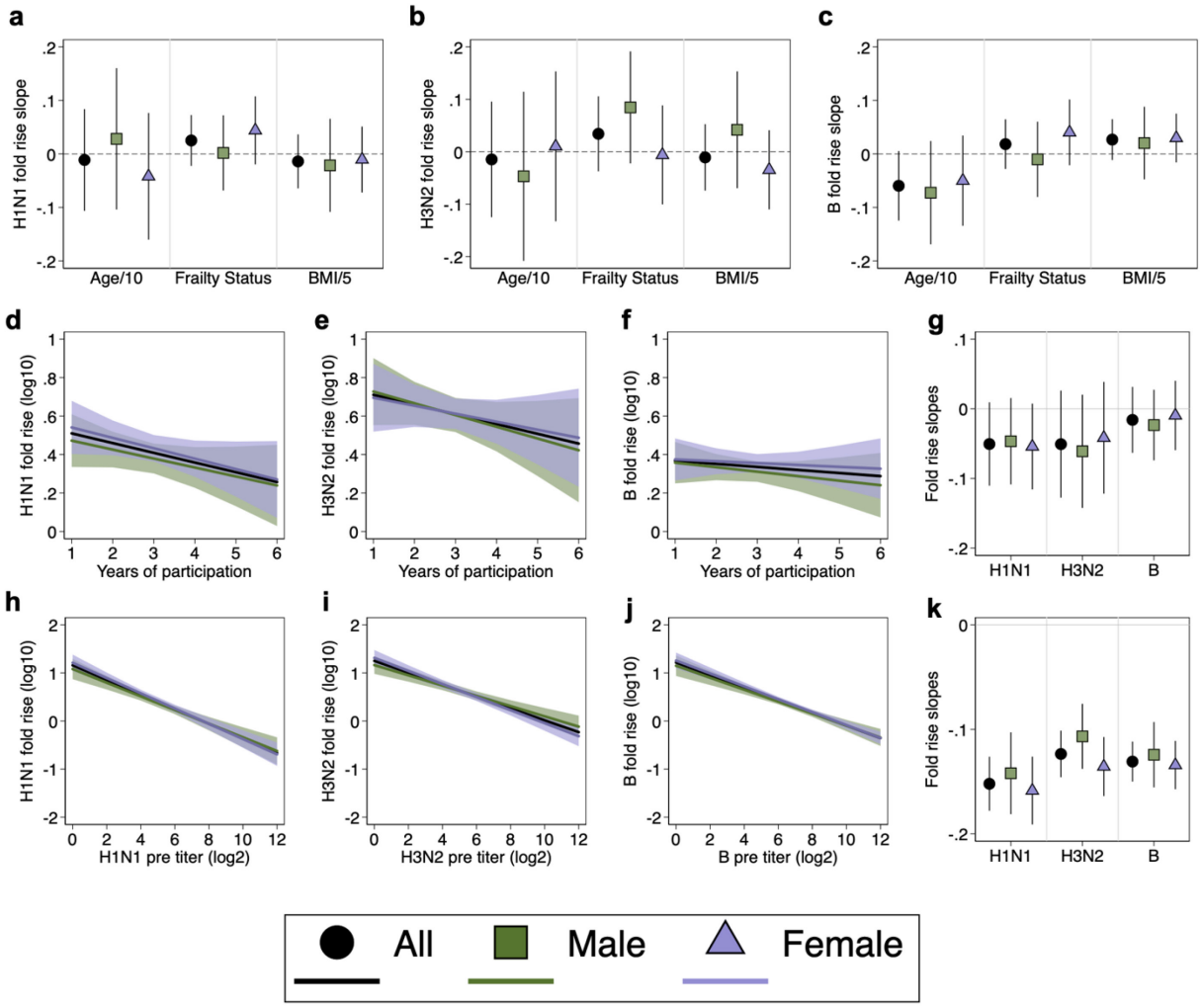


Figure 2.2 Impact of host factors, repeated vaccination, and pre-vaccination titers on the fold rise in HAI titers

The relationship of age (in decades, Age/10), frailty status, and BMI (five-unit intervals, BMI/5) with log_{10}-transformed fold-rise in titers (post-titer/pre-titer) are shown as slopes for H1N1 (**a**), H3N2 (**b**) and influenza B (**c**). The relationship between increasing years of vaccination and the log_{10}-transformed fold-rise in titers are shown for H1N1, H3N2, and influenza B (**d-f**), with the slopes summarized (**g**). The relationships between pre-vaccination HAI titers and the log_{10}-transformed fold-rise in titers are shown for each vaccine antigen (**h-j**), with the slopes summarized (**k**). Estimates and 95% confidence intervals were derived from multi-level mixed effects models with random intercepts on the individual participant. Models controlled for influenza season and pre-vaccination HAI titers (**a-g**), and either controlled for sex (whole population estimates) or used interaction terms between sex and the host factor of interest to derive sex-specific estimates.

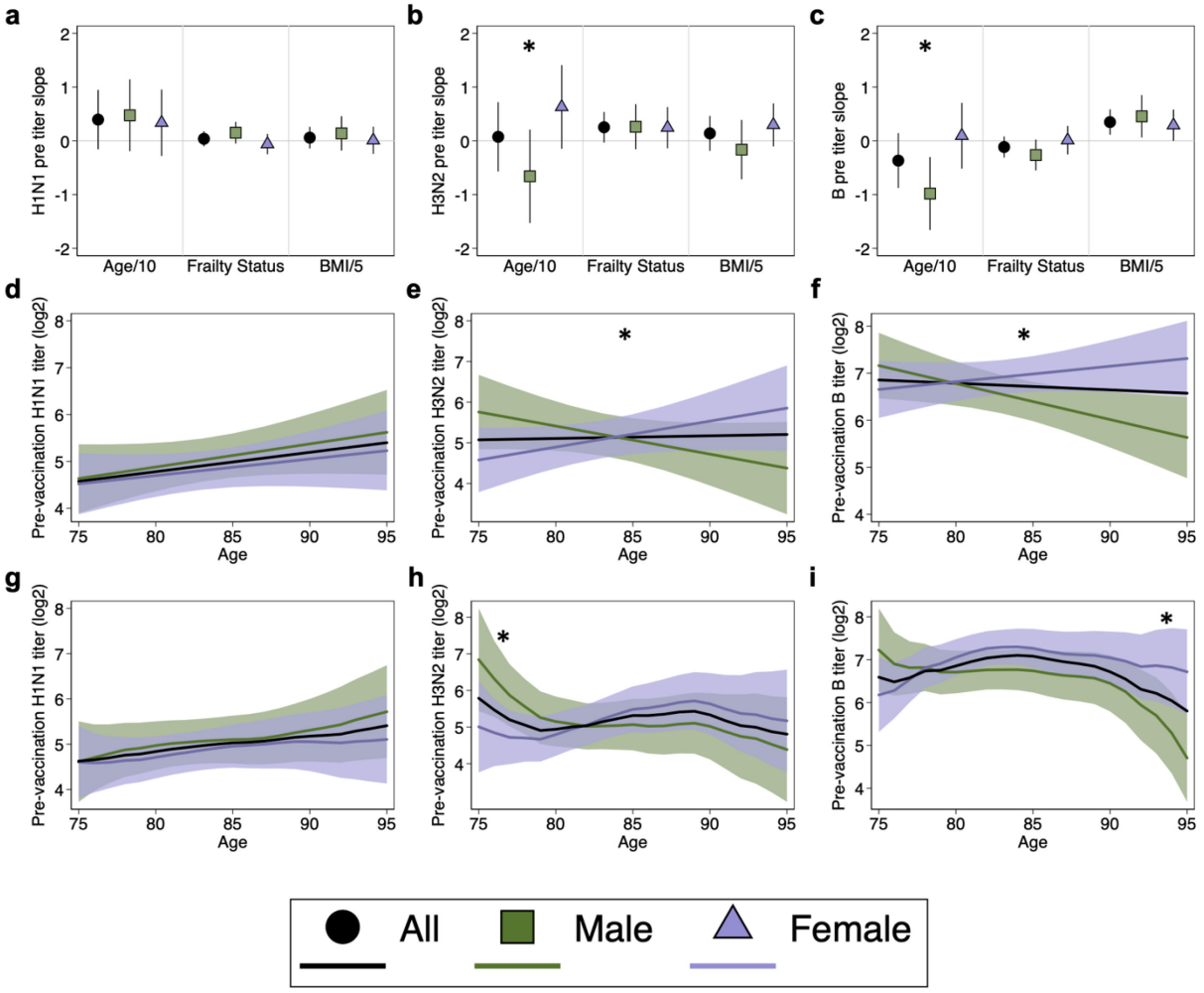
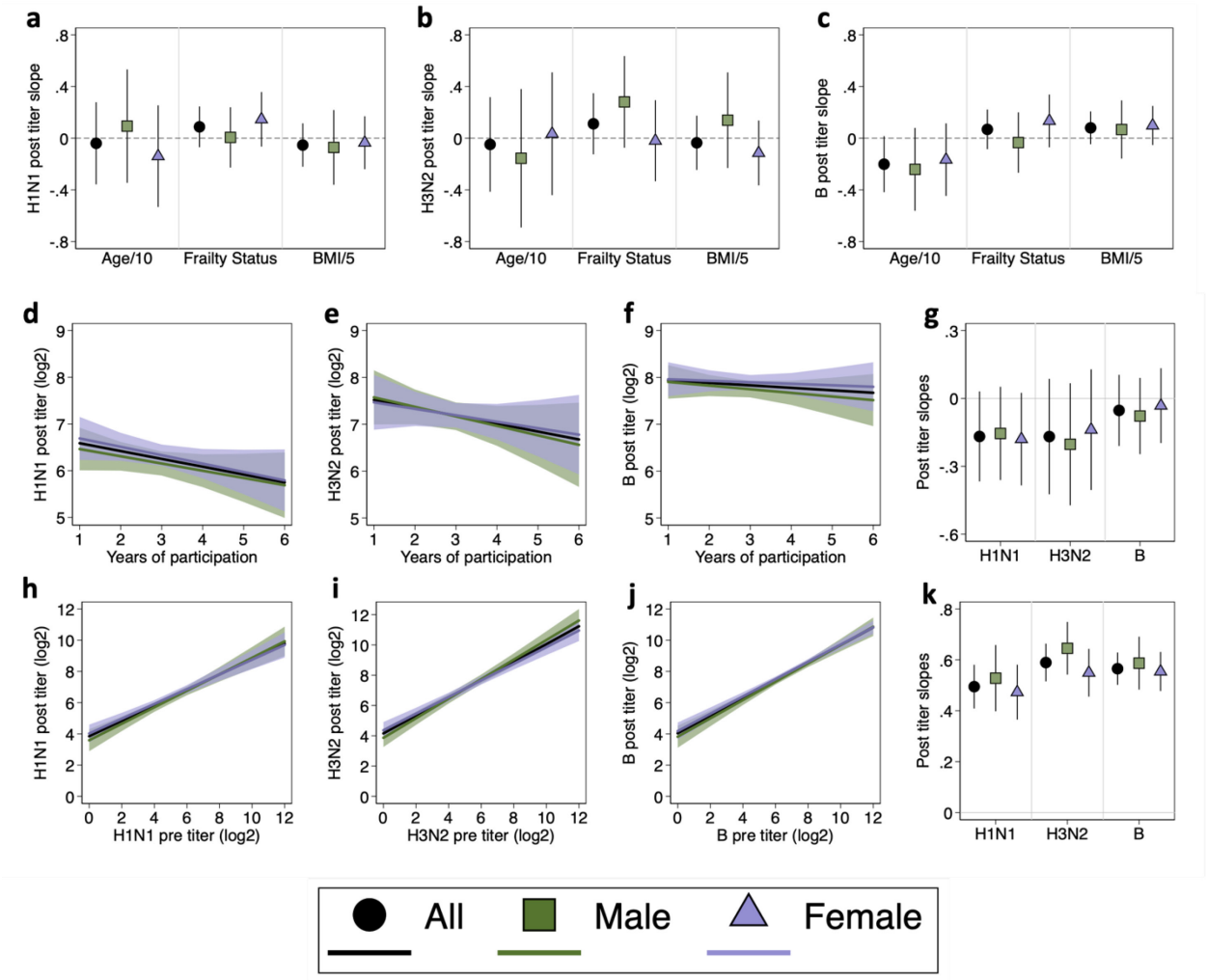


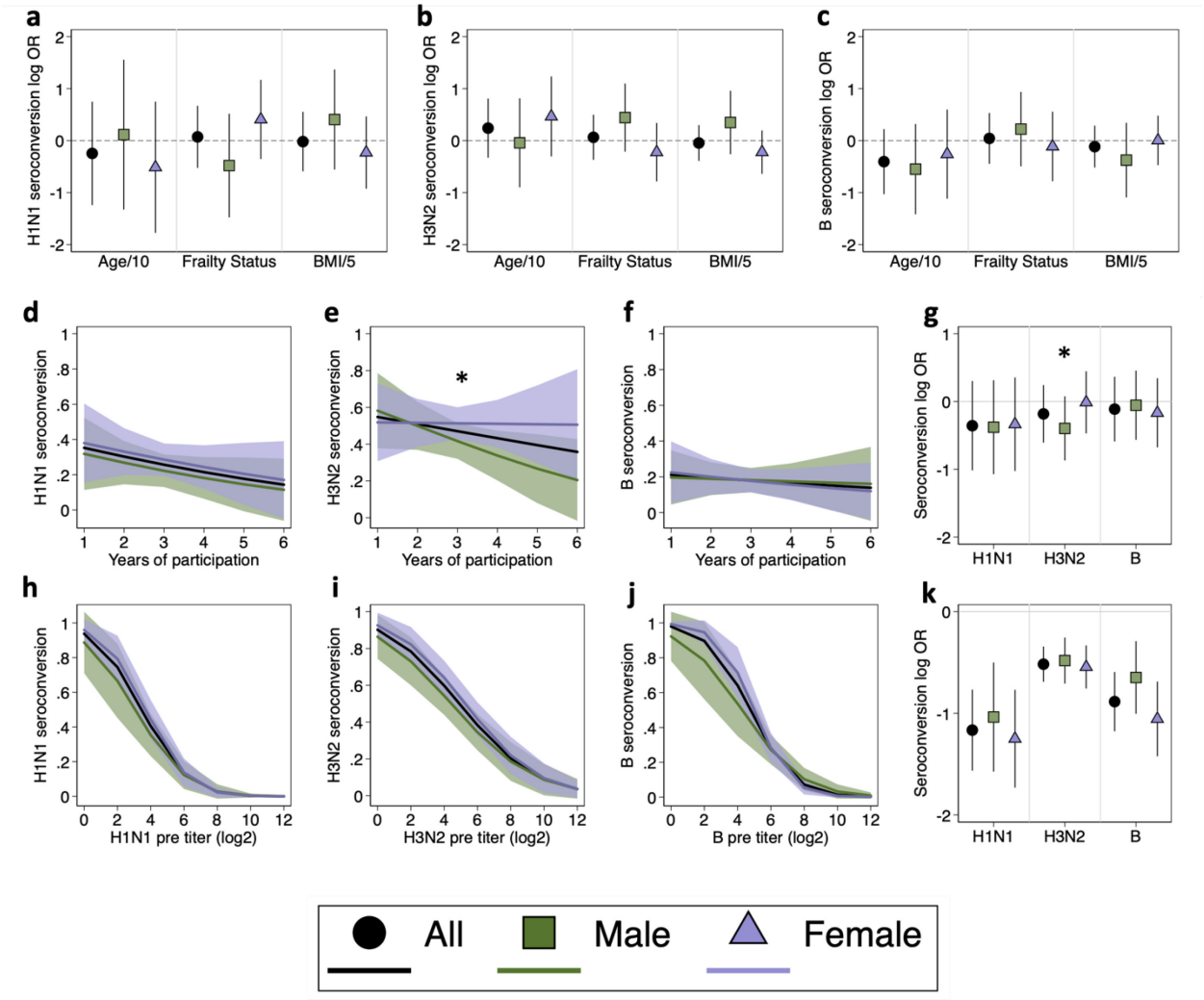
Figure 2.3 Relationship of age, frailty status, and BMI to pre-vaccination hemagglutination antibody inhibition (HAI) titers

Estimates for the relationship of age in decades (Age/10), frailty status, and BMI (five-unit intervals, BMI/5) to pre-vaccination HAI titers were derived from multilevel mixed-effects models controlling for study year for H1N1 (**a**), H3N2 (**b**), and influenza B (**c**). Expanded age models controlling for frailty and BMI are shown for responses to H1N1 (**d**), H3N2 (**e**), and influenza B (**f**). Expanded models for responses to H1N1 (**g**), H3N2 (**h**), and influenza B (**I**) were then amended to include cubic B-splines for age with knots at 5-year intervals. Models for the whole study population adjusted for sex, while sex-specific estimates included an interaction term allowing effects to differ by sex and are shown with 95% confidence intervals. Asterisks indicate significant sex differences.



Supplementary Figure 2.1 Impact of host factors, repeat vaccination and pre-vaccination titers on post-vaccination titers

The relationship of age (in decades, Age/10), frailty status, and BMI (five-unit intervals, BMI/5) with log_2-transformed post-vaccination HAI titers are shown as slopes for H1N1 (**a**), H3N2 (**b**) and influenza B (**c**). The relationship between increasing years of vaccination and the log_2-post-vaccination HAI titers are shown for H1N1, H3N2, and influenza B (**d-f**), with the slopes summarized (**g**). The relationships between pre-vaccination HAI titers and the log_2-transformed post-vaccination HAI titers are shown for each vaccine antigen (**h-j**), with the slopes summarized (**k**). Estimates and 95% confidence intervals were derived from multi-level mixed effects models with random intercepts on the individual participant. Models controlled for influenza season and pre-vaccination HAI titers (**a-g**), and either controlled for sex (whole population estimates) or used interaction terms between sex and the host factor of interest to derive sex-specific estimates.



Supplementary Figure 2.2 Impact of host factors, repeat vaccination and pre-vaccination titers on the odds of seroconversion

The relationship of age (in decades, Age/10), frailty status, and BMI (five-unit intervals, BMI/5) with log odds of seroconversion are shown H1N1 (**a**), H3N2 (**b**) and influenza B (**c**). The relationship between increasing years of vaccination and the probability of seroconverting are shown for H1N1, H3N2, and influenza B (**d-f**), with the log odds summarized (**g**). The relationships between pre-vaccination HAI titers and the probability of seroconversion are shown for each vaccine antigen (**h-j**), with the log odds summarized (**k**). Estimates and 95% confidence intervals were derived from multi-level mixed effects models with random intercepts on the individual participant. Models controlled for influenza season and pre-vaccination HAI titers (**a-g**), and either controlled for sex (whole population estimates) or used interaction terms between sex and the host factor of interest to derive sex-specific estimates.

CHAPTER 3

ASSOCIATION OF FRAILTY, AGE, AND BIOLOGICAL SEX WITH SARS-COV-2 mRNA VACCINE-INDUCED IMMUNITY IN OLDER ADULTS

Janna R. Shapiro, Ioannis Sitaras, Han-Sol Park, Tihitina Y. Aytenfisu, Christopher Caputo, Maggie Li, John Lee, Trevor S. Johnston, Huifen Li, Camille Wouters, Pricila Hauk, Henning Jacobsen, Yukang Li, Engle Abrams, Steve Yoon, Andrew J. Kocot, Tianrui Yang, Yushu Huang, Steven M. Cramer, Michael J. Betenbaugh, Amanda K. Debes, Rosemary Morgan, Aaron M. Milstone, Andrew H. Karaba, Andrew Pekosz, Sean X. Leng, and Sabra L. Klein

Provisionally accepted at *Clinical Infectious Diseases*

3.1 Abstract

Background: Male sex and old age are risk factors for severe COVID-19, but the intersection of sex and aging on antibody responses to SARS-CoV-2 vaccines has not been characterized.

Methods: Plasma samples were collected from older adults (75-98 years) before and after three doses of SARS-CoV-2 mRNA vaccination, and from younger adults (18-74 years) post-dose two, for comparison. Antibody binding to SARS-CoV-2 antigens (spike protein [S], S-receptor binding domain [S-RBD], and nucleocapsid [N]), functional activity against S, and live-virus neutralization were measured against the vaccine virus and the Alpha, Delta, and Omicron variants of concern (VOC).

Results: Vaccination induced greater antibody titers in older females than males, with both age and frailty associated with reduced antibody responses in males, but not females. Responses declined significantly in the six months following the second dose. The third dose restored functional antibody responses and eliminated disparities caused by sex, age, and frailty in older adults. Responses to the VOCs, particularly the Omicron variant, were significantly reduced relative to the vaccine virus, with older males having lower titers to the VOCs than females. Older adults had lower responses to the vaccine and VOC viruses than younger adults, with disparities being greater in males than females.

Conclusion: Older and frail males may be more vulnerable to breakthrough infections due to low antibody responses before receipt of a third vaccine dose. Promoting third dose coverage in older adults, especially males, is crucial to protecting this vulnerable population.

3.2 Introduction

The disproportionate burden of COVID-19 in older adults was recognized early in the pandemic (16, 17, 142). Phase III trials for the two mRNA vaccines (mRNA-1273 and BNT162b2) revealed high efficacy in older adults (45, 170), for whom immunosenescence is thought to impair vaccine-induced immune responses (3). The clinical trials, however, failed to represent the oldest and frailest subset of the population. Accordingly, wide-spread use of the vaccines in long-term care facility residents revealed that old age is a risk factor for poor antibody responses (39, 43, 171).

Male sex is also a significant predictor of severe COVID-19 outcomes at older ages (18-20, 22, 172). There is extensive evidence that the effects of aging on the immune system differ between the sexes, including that immunosenescence occurs at a slower rate in females than males (29, 67). The implications of biological sex are evident in the response to repeated seasonal influenza vaccination in older adults, where pre-vaccination titers decrease with age in males but not in females, suggesting that older females enter each influenza season with greater immunity than their male counterparts (73). In the context of SARS-CoV-2 vaccines, however, studies have failed to provide sex-disaggregated data within each age group (173, 174), and little is known about how biological sex may modify the effects of age, and age-related factors like frailty, on vaccine immunogenicity and the durability of protection. Here, we investigate sex differences and sex-specific effects of aging in the humoral immune response to the vaccine virus and variants of concern (VOC) induced by three doses of a SARS-CoV-2 mRNA vaccine in a cohort of adults above 75 years of age. We illustrate that the age- and frailty-associated declines in antibody responses occur to a greater extent in males than females.

3.3 Methods

3.3.1 Cohorts

Older adults (75-98 years) were recruited from the Johns Hopkins Longitudinal Influenza Immunization Study of Aging over 75 years of age (JH LIISA 75+) cohort (73) (**Table 1**). Individuals who had worsening or new-onset of immune-modulating conditions (e.g., rheumatoid arthritis, hematologic malignancies, or other cancers) or a previous diagnosis of COVID-19 were excluded. Participants came to the Johns Hopkins Bayview Medical Center, or study visits were conducted at participants' homes, as needed. At pre-vaccination visits (Pre), frailty status was assessed using the Fried Frailty Phenotype (144) and a baseline blood draw was obtained. Subsequent receipt of two (primary vaccination series) or three doses of a SARS-CoV-2 mRNA vaccine, either mRNA-1273 or BNT162b2, was confirmed via vaccination cards, and blood samples were collected 14-30 days (i.e., average ≤ 1 month [M]) post dose 1 (<1M_PD1)), 14-30 days post dose 2 (<1M_PD2), 90 (± 15) days post dose 2 (3M_PD2), 180 (± 15) days post dose 2 (6M_PD2), and 14-60 days post dose 3 (1M_PD3).

Younger adult healthcare workers from the Johns Hopkins Health System were also sampled as a comparison group. Recruitment of these younger adults has been reported elsewhere (175). To be eligible for the present study, participants needed to be younger than 75 years, not have a history of COVID-19, and have two samples collected at least 90 days apart, with the first one collected at least 14 days after receiving the second dose of a SARS-CoV-2 mRNA vaccine. Due to low plasma volumes, these samples were not tested for ACE2-inhibition or virus neutralization, and antibody titration against antigens from VOCs could not be performed for some participants. Exact sample sizes are included in figure legends. For both

cohorts, written, informed consent was obtained from all participants, and the study protocols were approved by the Johns Hopkins School of Medicine Institutional Review Board.

3.3.2 Laboratory methods

Detailed ELISA, ACE2 inhibition, and virus neutralization methods can be found in the **Supplemental materials**. Briefly, plasmids expressing recombinant nucleocapsid (N), Spike (S), or S receptor-binding domain (S-RBD) of the vaccine strain and the Alpha, Delta, and Omicron variants of SARS-CoV-2 were engineered at Johns Hopkins as described previously (176) or obtained through the NCI Serological Sciences Network for COVID-19 (177) (**Supplemental Table 1**). Recombinant proteins were used to coat plates for indirect ELISA measuring plasma IgG against N, S, or S-RBD. Results were expressed as the $\log_{10}$-transformed area under the curve (AUC) generated from ten three-fold serial plasma dilutions, as previously described (176). The ability of plasma antibodies to inhibit ACE2 binding to S was measured using Meso Scale Diagnostics (MSD) V-PLEX SARS-CoV-2 ACE2 kits according to the manufacturer's protocol at a dilution of 1:100 (178). Data were expressed as the $\log_{10}$-transformed concentration (μg/ml) of ACE2-inhibiting antibodies (ACE2iAb), which are equivalent to anti-S monoclonal antibodies. For comparison between the vaccine virus and VOCs, data were expressed as percent ACE2 inhibition. Live-virus microneutralization assays were performed as previously described, using two-fold dilutions of plasma incubated with infectious virus and then VeroE6-TMPRSS2 cells to measure cytopathic effect (179). Results were expressed as the $\log_{10}$-transformed neutralizing antibody (nAb) AUC. Because pre-vaccination IgG and ACE2iAb responses were low or non-detectable, live virus neutralization was only performed on post-vaccination samples. IgG binding to seasonal and epidemic β-coronavirus S proteins was

measured using the multiplex chemiluminescent MSD V-PLEX COVID-19 Coronavirus Panel 3 (IgG) Kit according to the manufacturer's protocol at a dilution of 1:5000.

3.3.3 Statistical methods

Longitudinal data in the older adult cohort were analyzed using mixed-effects models with random intercepts on the individual to account for repeated measures, and interaction terms between study timepoint (categorical) and sex (self-report), age (categorized based on terciles) and frailty status. Linear regression models including interaction terms between sex and age or frailty were used to investigate sex-specific effects at individual timepoints. To compare the older and younger cohorts, the number of days post-dose 2 was used as a continuous predictor and cubic splines were included to study non-linear relationships (169). Cubic spline knots were placed at 30-, 100-, and 160-days post-vaccination, points chosen to approximately divide the data into quartiles. Mixed-effects models included an interaction term between time and cohort and were repeated separately for males and females. Differences between cohorts were tested at three sentinel points (14-, 90- and 180- days post dose 2). All p-values <0.05 were considered statistically significant. Analyses were performed using Stata 15 (StataCorp).

3.3.4 Supplemental Methods

3.3.4.1 SARS-CoV-2 ELISAs

The ELISA protocol was adapted from a protocol published by the Florian Krammer laboratory (180). Antigens were either engineered at Johns Hopkins as previously described (176) or were obtained through the NCI Serological Sciences Network for COVID-19 (177) (**Supplemental Table 1**). S and S-RBD antigens were diluted to 2ug/ml in PBS. Nucleocapsid

antigen was diluted to 1ug/ml in PBS. Ninety-six-well plates (Immulon 4HBX, Thermo Fisher Scientific) were coated overnight at 4°C with 50µL of the antigen solution. Plates were then washed. All washing steps consisted of 3 washes with 300µL PBS plus 0.1% Tween-20 (PBST) (Thermo Fisher Scientific). Plates were blocked with 200µL PBST with 3% nonfat milk (milk powder, American Bio) for 1 hour at room temperature (RT). Plasma samples were heat inactivated at 56°C for 1 hour and then diluted to 1:20 in PBST + 1% nonfat milk before performing ten 3-fold serial dilutions. Pre-pandemic plasma samples were diluted to 1:100 and used as negative controls. A monoclonal antibody against the SARS–CoV-2 S protein (1:5000; catalog 40150-D001, Sino Biological) was used as a positive control for vaccine, Alpha and Delta S and S-RBD ELISAs. Convalescent plasma diluted to 1:100 was used as a positive control for N and Omicron ELISAs. 100µL of samples and controls were added to ELISA plates in duplicate and incubated for 2 hours at RT. Plates were then washed and 50µL of Fc-specific total IgG HRP (1:5000 dilution in PBST plus 1% nonfat milk, catalog A18823, Invitrogen, Thermo Fisher Scientific) was added and incubated at RT for 1 hour. Plates were washed and 100µL of SIGMAFAST OPD (*o*-phenylenediamine dihydrochloride) solution (MilliporeSigma) was added to each well and incubated in the dark at RT for 10 minutes. To stop the reaction, 50µL of 3M HCl (Thermo Fisher Scientific) was added to each well. The OD of each plate was read at 490 nm (OD_{490}) on a SpectraMax i3 ELISA Plate Reader (BioTek Instruments). The cutoff value for each plate was calculated by adding the average of negative control OD values to three times the standard deviation of the negative control OD values. For each sample, the highest dilution above the cut-off value was considered the endpoint titer. The cut-off value was then subtracted from all sample OD values, and negative values set to zero. Background-subtracted

OD values were plotted against the dilution factor to calculate the area under the curve (AUC). For each S and S-RBD antigen, the limit of detection was set to half of the lowest measured AUC for samples with a detectable titer (i.e., titer ≥ 20). For samples with an undetectable titer (i.e., titer < 20), the AUC was arbitrarily set to half of the limit of detection. For nucleocapsid assays, the threshold for seropositivity was set to a titer of 1:180 based on pre-pandemic samples.

3.3.4.2 ACE2 inhibition Assays

The MSD ACE2 inhibition assay measures the ability of participant plasma to inhibit ACE2 binding to spike protein. Plasma was thawed and ACE2 blocking was measured using the ACE2 MSD V-PLEX SARS-CoV-2 ACE2 kits according to the manufacturer's protocol at a dilution of 1:100. Plates come pre-coated with spike proteins corresponding to variants of interest. They were washed and incubated with plasma for one hour, human ACE2 protein conjugated with a SULFO-TAG (light-emitting label) added for another hour, washed, read buffer added, and read with a MESO QuickPlex SQ 120 instrument. An 8-point calibration curve was included on each plate such that results could be expressed as ACE2-inhibiting activity corresponding to 1 µg/ml of monoclonal antibody to the vaccine strain of SARS-CoV-2 S protein. Values below the manufacturer-specified limit of detection of 0.448 µg/ml were arbitrarily set to half of that value. For the VOC, results were expressed as percent inhibition ([1 – average sample electrochemiluminescence/average electrochemiluminescence signal of blank well] × 100), due to the lack of calibrator for the Omicron variant. based on the equation provided by the manufacturer. The limit of detection was defined as 20% based on correlation with live virus-neutralizing antibody (178).

3.3.4.3 Viruses and cells

Vero-E6-TMPRSS2 cells (181) were cultured in complete media (CM) consisting of DMEM containing 10% FBS (Gibco, Thermo Fisher Scientific), 1 mM glutamine (Invitrogen, Thermo Fisher Scientific), 1 mM sodium pyruvate (Invitrogen, Thermo Fisher Scientific), 100 U/mL penicillin (Invitrogen, Thermo Fisher Scientific), and 100 µg/mL streptomycin (Invitrogen, Thermo Fisher Scientific). Cells were incubated in a 5% CO2 humidified incubator at 37°C.

The SARS–CoV-2/USA-WA1/2020 virus was obtained from BEI Resources. The Alpha (hCoV19/USA/MD-HP01101/2021, EPI_ISL_825013), Delta (SARS-CoV-2/USA/MD-HP05647/2021, EPI_ISL_2331496)(182) and Omicron BA.1 (SARS-CoV-2/USA/MD-HP20874/2021, EPI_ISL_7160424)(183) variant of SARS-CoV-2 were isolated on Vero-TMPRSS2 cells (184). The consensus sequence of the virus isolate did not differ from the sequence derived from the clinical specimen. The infectious virus titer was determined on Vero-TMPRSS2 cells using a 50% tissue culture infectious dose (TCID50) assay as previously described for SARS-CoV-2 (185, 186). Serial 10-fold dilutions of the virus stock were made in infection media (IM) (identical to CM except the FBS was reduced to 2.5%), and then 100 µL of each dilution was added to the cells in a 96-well plate in sextuplicate. The cells were incubated at 37°C for 4 days, visualized by staining with naphthol blue-black, and scored visually for cytopathic effect. A Reed and Muench calculation was used to determine the TCID50 per mL (187).

3.3.4.4 Microneutralization assays

Plasma nAbs were determined as previously described for SARS-CoV (188) and SARS-CoV-2 (179). Two-fold dilutions of plasma (starting at a 1:20 dilution and ending in 1:2560) were made in infection media (IM). Infectious virus was added to the plasma dilutions at a final

concentration of 1×10^4 $TCID_{50}$/mL (100 $TCID_{50}$ per 100 µL). The samples were incubated for 1 hour at room temperature, and then 100 µL of each dilution was added to 96-well plates of VeroE6-TMPRSS2 cells in hexaplicate. Cells were incubated for 6 hours at 37°C, 5% CO_2. The inocula were removed, fresh IM was added, and cells were incubated at 37°C, 5% CO_2 until cytopathic effect was evident in all of the control cells infected with virus containing no serum or plasma. The time ranged from 2-4 days depending on the specific virus used. The cells were fixed by the addition of 100 µL of 4% formaldehyde per well, incubated for at least 4 hours at room temperature, and then stained with Napthol Blue Black (MilliporeSigma). The nAb titer was calculated as the highest serum dilution that eliminated the cytopathic effect in 50% of the wells (NT50) and the area under the curve (AUC) was calculated using Graphpad Prism, with the lower limit of detection set to 1.7.

3.4 Results

3.4.1 Study population demographics

Eighty-six older adults were recruited from the Baltimore area, with three participants excluded from analysis due to high SARS-CoV-2 N antibody titers (i.e., titer >180), suggesting prior COVID-19 infection (**Supplemental figure 1**). One additional participant was excluded from analysis due to evidence of severe immunosuppression, such that their responses could not be accurately captured in population-level models. Characteristics of the 82 participants included in analysis are detailed in **Table 1**. The population had more females (59%) than males, and a median age of 84 years. Most participants were classified as pre-frail (64%) and a greater percentage of males than females were frail. All participants received two doses of a SARS-CoV-

2 mRNA vaccine, with the majority (70%) receiving BNT162b2. Sixty participants (73%) received a third vaccine dose at least six months after the second dose.

Demographic information for the younger adult cohort is provided in **Table 2**. Of 84 eligible participants from the affiliated study (175), three were excluded due to high anti-N titers (**Supplemental figure 1**). In the younger population included in analysis, there were more females than males (60% vs 40%), most participants were between 30 and 49 years of age, and a majority of samples were collected 21-43 days and 125-150 days after receipt of the second vaccine dose.

3.4.2 Older females mount greater responses to vaccination than older males

Among older adults, IgG binding to S and S-RBD of the vaccine strain increased significantly in response to the first two vaccine doses and then decreased significantly in the 6 months following immunization ($p<0.001$ for all comparisons; **Figure 1A-B & E**). Geometric mean titers (GMT) decreased 11- and 12-fold, for S and S-RBD, respectively, from <1M_PD2 to 6M_PD2 (**Supplemental Table 2**). Females mounted greater IgG responses to S and S-RBD relative to their baseline than males at all pre dose 3 timepoints ($p<0.02$ for all comparisons, **Figure 1A-B & E**). Older females also had greater titers of IgG against S and S-RBD at each visit, and this difference was significant for anti-S IgG at <1M_PD1 ($p=0.020$) and at 3M_PD2 ($p=0.026$). Although differences appear attenuated on the log scale, GMT ratios reveal a consistent sex difference of 1.2 – 3-fold higher titers in females than males (**Figure 1F**). After receipt of a third vaccine dose, IgG titers increased significantly in both males and females ($p<0.001$), leading to GMT that were 2- and 4-fold greater than the post-dose 2 peak for S and

S-RBD, respectively, and to reductions in the female-to-male GMT ratios (**Figure 1F; Supplemental Table 2**).

The functional ability of antibodies to inhibit S from binding to ACE2 followed similar kinetics as IgG in response to the primary immunization series, but then decreased more rapidly in the 6-months following immunization, resulting in a 28-fold decrease in GMT from <1M_PD2 to 6M_PD2 (**Figure 1C & Supplemental Table 2**). By 6M_PD2, 79% of males and 77% of females had undetectable ACE2iAb. Sex differences were apparent at all timepoints and were significant at 3M_PD2 (p=0.046), with females mounting stronger responses than males (**Figure 1F**). Post dose 3, all but one participant had detectable ACE2iAb, and the geometric mean was 7-fold higher than the post-dose 2 peak (**Supplemental Table 2**). Neutralizing capacity declined 6-fold in the 3 months following the second dose, and titers were then restored to 3-times the post dose 2 peak by the third dose (**Figure 1D**). As with the other outcomes, nAb titers were consistently 1.3-1.9-fold higher for females than for males, reaching statistical significance at 3M_PD2 (p=0.001) and 6M_PD2 (p=0.028; **Figure 1D-F**)

Despite differences in kinetics over time between the binding and functional assays, the four readouts of humoral immunity correlated well with each other (R > 0.67; **Supplemental figure 2**). As expected, correlations became weaker at the lower range of the ACE2-inhibition and virus neutralization assays. Taken together, these data suggest that older females mount stronger response to SARS-CoV-2 vaccination than males, and that a third vaccine dose is necessary to boost functional antibody responses in both males and females.

3.4.3 The effects of age and frailty are greater in males than in females

We next assessed the overall and sex-specific effects of age on the humoral response to vaccination. Among all older participants, age was significantly associated with reduced anti-S IgG, anti-S-RBD IgG, ACE2iAb, and nAb in the six months following the primary vaccination series (**Figure 2A-D**). This effect was largely driven by the oldest tercile of the population (≥88 years). At 3M_PD2, nAb GMT were 1.8-fold higher in the youngest tercile (75-82 years) compared to the oldest tercile and the percent of participants with undetectable ACE2iAb by 6M_PD2 was 67% and 85% in the youngest and oldest terciles, respectively. In sex-disaggregated analyses focusing on the 3M_PD2 timepoint (i.e., a time point when all study participants were represented), age significantly impaired responses in males, but not females, leading to statistically significant sex differences in the effect of age for anti-S IgG (p=0.025), ACE2iAb (p = 0.001), and nAb (p=0.037; **Figure 2E-H**). The trend of greater age effects in males than females was consistent at other timepoints following the primary immunization series (**Supplemental figure 3A-H**), and by 6M_PD2, 100% of males in the oldest age group, compared to 77% of females, had undetectable ACE2iAB. After receipt of a third dose, the effect of age was no longer significant in the overall population or within either sex, suggesting that a third vaccine dose eliminated sex and age disparities in vaccine-induced immunity (**Figure 2A-D & Supplemental figure 3I-L**).

Frailty had an important overall effect, with frail participants mounting significantly weaker responses to vaccination than robust and pre-frail participants (**Figure 2I-L**). By 6M_PD2, 90% of frail participants had undetectable ACE2iAb, compared to 75% of pre-frail and robust participants. For the nAb, responses in frail participants were 1.8-, 2.3- and 1.9-fold

lower at <1M_PD2, 3M_PD2, and 6M_PD2, respectively. Like with age, the effect of frailty at 3M_PD2 was significant in males, but not females for all readouts (**Figure 2M-P**). No significant sex differences in the effect of frailty were observed, however, and trends were less consistent over time (**Supplemental figure 3M-X**). The effect of frailty was also attenuated by the third dose but remained significant for ACE2iAb (p=0.005; **Figure 2K**). From these data, we conclude that the effects of age and frailty in older adults are largely driven by males, not females.

3.4.4 Antibody responses to VOC are reduced relative to the vaccine virus

The breadth of vaccine-induced immunity in older adults was assessed by measuring antibody responses to the Alpha, Delta, and Omicron variants (**Supplemental Table 3**). Anti-S IgG to the Alpha and Delta variants were similar to each other and were both significantly reduced relative to the vaccine virus (2-4-fold lower GMT, p<0.001; **Figure 3A & D**). Titers to Omicron were further reduced relative to the vaccine virus (>5-fold difference in GMT) and the Alpha and Delta variants (2-4-fold lower GMT, p<0.001 for all comparisons; **Figure 3A & D**). Differences between anti-S IgG to the vaccine virus and the VOC were attenuated at 1M_PD3 (fold difference in GMT <1.5 for Alpha and Delta and <4 for Omicron) but remained significant (p<0.0001 for all comparisons). Percent ACE2 inhibition of the Alpha and Delta variants was also significantly lower than for the vaccine strain and functional antibody responses to the BA.1 and BA.2 Omicron variants were undetectable until a third dose was administered (**Figure 3B & D**). nAb responses to the Alpha and Delta variants were significantly reduced relative to the vaccine virus (p<0.001) at all timepoints except for Alpha at 3M_PD2 and nAb titers to the Omicron BA.1 variant were 8-fold lower than to the vaccine virus at 1M_PD3 (**Figure 3C-D)**. In sex-disaggregated analyses, females had higher responses to the VOC than males, and this

difference was significant for anti-Delta S IgG (p=0.038) and anti-Alpha nAb (p=0.048) at 3M_PD2 (**Figure 3E-G & Supplemental figure 4**).

To investigate the cross-reactivity of the vaccine-induced humoral response, IgG titers to seasonal and epidemic β-coronaviruses were measured in the older adult cohort (**Supplemental figure 5**). As reported elsewhere (189, 190), titers of IgG recognizing OC43, MERS-CoV, and SARS-CoV-1 increased significantly in plasma samples collected after SARS-CoV-2 vaccination and remained elevated above baseline levels for 6 months.

3.4.5 Differences between older and younger cohorts are driven by males

To further investigate the sex-specific effects of aging, antibody kinetics against vaccine, Alpha, and Delta antigens were compared between the younger and older adult cohorts during the six-month period following the primary vaccination series. In the whole population, anti-vaccine S IgG was significantly lower in older than younger adults (p<0.001 at 14 days post-vaccination, p=0.004 at 90 days, and p=0.026 at 180 days) (**Figure 4A**). In sex-disaggregated analyses, differences between the older and younger adults were significant among males at all three sentinel points (p=0.004 at 14 days post-vaccination, p=0.005 at 90 days, and p=0.019 at 180 days), but only significant among females at 14-days post vaccination (p=0.004) (**Figure 4B-D**). In addition, the magnitude of the difference between the mean of the older cohort and the mean of younger cohort was consistently larger for males than for females across the three sentinel points (**Figure 4D**). Similar results were observed for anti-Alpha and Delta S IgG (**Figure 4E-L**). There were no significant differences in the rate of waning between older and younger adults, suggesting that antibody kinetics are not age-dependent.

3.5 Discussion

In this longitudinal study, older adult females mounted stronger antibody responses to SARS-CoV-2 mRNA vaccination than older males, and age and frailty were associated with reduced responses in males but not females. While the kinetics of antibody waning in the six months following immunization were not age-dependent, older adults mounted weaker initial responses to vaccination, such that their antibody titers remained lower than younger adults throughout the follow-up period. A sex-specific effect of age was observed, both within the older adult cohort and when comparing younger and older adults, in which age-associated reductions in humoral immunity were greater among males than females. In the older adult cohort, receipt of a third vaccine dose largely eliminated disparities caused by sex, age, and frailty in antibody responses, with the exception of ACE2iAb, which remained lower in frail participants. The effect of age on SARS-CoV-2 vaccine responses has been studied (39, 43, 75-77, 171, 191, 192), but the sex differential impact of age has not been reported previously. Furthermore, studies investigating frailty have not found an effect on antibody responses (42, 104, 105), but have reported that frailty increases the risk of post-vaccination breakthrough infection (50, 51), suggesting that the immunogenicity studies may have been under-powered to observe an effect of frailty, that lack of consideration of biological sex obscured the effect, or that higher levels of antibody are required to prevent infection in frail individuals than in the general population.

The inclusion of four measures of humoral immunity and four SARS-CoV-2 viruses allowed us to capture the breadth and depth of vaccine responses in this vulnerable population. In terms of responses to VOCs, the reductions in anti-S IgG to the Alpha and Delta variants

observed in the older adults were similar to other reports in the general population (193). For the Omicron variant, while reductions in live-virus neutralization in post-vaccination sera from the general adult population have been reported, and were also observed here, there were no reductions in anti-Omicron S IgG (194, 195). Given the importance of neutralizing and non-neutralizing functions of IgG in conferring protection against SARS-CoV-2 (196, 197), the markedly lower anti-Omicron S IgG level in older adults, which persisted after receipt of a third vaccine dose, suggests that this population may be more vulnerable to disease caused by the Omicron variant than younger adults, and that reformulation of vaccines to target the Omicron variant would be beneficial.

Our study has several strengths and limitations. Some of the sex-specific effects observed were differences among males that were absent among females, without statistical evidence of a sex difference (i.e., non-significant sex interaction terms) (198). It is important to note that our findings were generated from *post-hoc* analyses that were not necessarily powered to investigate sex differences, and conclusions are limited by small samples sizes in certain sub-groups. Particularly for age-based analyses, however, the consistency of trends between assays and timepoints, coupled with statistically significant sex differences in the effect of aging at 3M_PD2, lend credibility to the conclusion that the effects of age on antiviral antibody responses are driven by males. Further supporting these findings are similar sex-specific effects of age observed following seasonal influenza vaccination in both younger and older adults (73, 150). While it is important to not over-interpret 'within-sex' differences as 'between-sex' differences (199), there is considerable value in studying differences within males or females (112, 166). This is particularly true given the uniqueness of the community-

dwelling older adult cohort, which represent the 'oldest' old subset, and are distinct from the population of long-term care facility residents that has been the focus of much of the SARS-CoV-2 research in older adults.

There were also missing data in the older adult cohort, particularly at the <1M_PD1 timepoint. These missing data did not, however, depart from the missing at random assumption, and thus multi-level models were used to account for missingness. The timing of sample collection was different in the older and younger cohorts. To account for this, analyses that compared the two cohorts used days post-vaccination as a continuous variable. Finally, although beyond the scope of this manuscript, future work will include measuring cellular immunity post-vaccination.

In conclusion, we report that both age and frailty impair antibody responses to the primary series of SARS-CoV-2 vaccination in older males, and that these disparities are largely eliminated by vaccination with the third dose. Given that male sex is an important risk factor for severe outcomes from COVID-19 (18-20, 22, 172), the finding that older and frail males may be vulnerable to breakthrough infections due to low antibody responses, particularly before a third vaccine dose is administered, is of considerable public health importance. These findings emphasize that increasing third dose coverage among older males is crucial for protecting this vulnerable population from SARS-CoV-2.

3.6 Funding

This work was supported by the NIH/NIA Specialized Center of Research Excellence (U54 AG062333) awarded to S.L.K., the NIH/NCI COVID-19 Serology Center of Excellence (U54 CA260492) awarded to S.L.K, and the NIH/NIAID Johns Hopkins Center of Excellence in Influenza Research and Response (75N93021C00045 and N272201400007C and the CDC (75D30121C11061) awarded to A.P. This work was also supported by funding from the Irma and Paul Milstein Program for Senior Health, the Milstein Medical Asian American Partnership (MMAAP) Foundation of USA, and the Howard and Abby Milstein Foundation awarded to S.X.L, and by the generosity of the collective community of donors to the Johns Hopkins University School of Medicine and the Johns Hopkins Health System for Covid-19 research. J.R.S was supported by a training award from the Fonds de recherche du Québec – Santé (File #287609).

3.7 Acknowledgements

The authors thank the participants, as well as Denise C. Kelly and Eileen Sheridan-Malone for collection of samples. The authors thank Emily Egbert for coordination of samples and healthcare worker patients, and Joseph Shiloach, David Quan, and Yesh Muralidharan for technical assistance.

3.8 Tables

Table 3.1 Older adult participant characteristics.

	All	Male	Female
Included in analysis - n (%)[a]	82	34 (41)	48 (59)
Recruited - n	86	34	52
Excluded - n[b]	4	0	4
Age - median (IQR)	84 (81 - 88)	84 (82 - 88)	83 (81 - 89)
Categories - n (%)[c]			
75-82	32 (39)	12 (35)	20 (42)
83-87	28 (34)	13 (38)	15 (31)
88-98	22 (27)	9 (26)	13 (27)
Frailty - n (%)[c]			
Robust	18 (22)	8 (24)	10 (21)
Pre-frail	53 (64)	20 (59)	33 (67)
Frail	10 (12)	6 (18)	4 (8)
Missing	1 (1)	0 (0)	1 (2)
Vaccine type - n (%)[c]			
mRNA-1273 (Moderna)	24 (30)	8 (24)	16 (33)
BNT162b2 (Pfizer)	58 (70)	26 (76)	32 (67)
Visit participation - n (%)[c,d]			
Pre	82 (100)	34 (100)	48 (100)
<1M_PD1	23 (28)	11 (32)	12 (25)
<1M_PD2	69 (84)	28 (82)	41 (85)
3M_PD2	82 (100)	34 (100)	48 (100)
6M_PD2	80 (98)	33 (97)	47 (98)
1M_PD3	60 (73)	26 (76)	34 (71)

[a] Subset of eligible participants without evidence of prior infection who were included in analysis

[b] Participants with high (> 1:180) nucleocapsid titers, indicating prior infection, were excluded from analysis. One additional participant was excluded due to evidence of severe immune suppression

[c] Percents are based on the number included in analysis in each column

[d] Study timepoints: Pre-vaccination (Pre); 14-30 days post dose 1 (<1M_PD1); 14-30 days post dose 2 (<1M_PD2); 75-105 days post dose 1 (3M_PD2); 165-195 days post dose 1 (6M_PD2); 75-105 days post dose 1 (3M_PD2); 14-60 days post dose 3 (1M_PD3)

Table 3.2 Younger adult participant characteristics

	All	Male	Female
Included in analysis - n (%)[a]	81	32 (40)	49 (60)
Eligible - n[b]	84	32	52
Excluded - n[c]	3	0	3
Age at vaccination - n (%)[d]			
≤29	14 (17)	4 (12)	10 (20)
30-39	32 (40)	11 (34)	21 (43)
40-49	18 (22)	8 (25)	10 (20)
50-59	7 (9)	4 (13)	3 (6)
60-74	10 (12)	5 (16)	5 (10)
Sample 1 - days post dose 2			
Mean (min-max)	33 (16-76)	31 (16-65)	34 (16-76)
Median (IQR)	29 (21-43)	27 (21-41)	29 (21-43)
Sample 2 - days post dose 2			
Mean (min-max)	138 (96-190)	142 (110 - 190)	136 (96-183)
Median (IQR)	137 (125-150)	139 (128-156)	137 (123-147)

[a] Subset of eligible participants without evidence of prior infection who were included in analysis

[b] Eligible participants from affiliated study (ref) were <75 years of age, had remaining serum from 2 samples collected at least 90 days apart 14-200 days following 2 doses of an mRNA SARS-CoV-2 vaccine who did not report prior SARS-CoV-2 infection

[c] Participants with high (> 1:180) nucleocapsid titers, indicating prior infection, were excluded from analysis

[d] Percents are based on the number included in analysis in each column

Supplementary Table 3.1 SARS-CoV-2 antigens for ELISAs

Antigen	Protein name [relevant mutations]	Source
Nucleocapsid	SARS-CoV-2 Nucleocapsid (N) C-terminal domain (247-364)	SeroNet
Vaccine S	ECD ΔFurin HexaPro (200)	JHU
Vaccine RBD	As previously described (180)	JHU
Alpha S	hCoV19/USA/MD-HP01101/2021 (EPI_ISL_825013) [H69del, V70del, Y145del, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H]	JHU
	SARS-CoV-2 B.1.1.7 S-2P(15-1208)-T4f-3C-His8-Strep2x2 [Δ(69-70), Δ144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H]	SeroNet
Delta S	hCoV-19/USA/MD-HP05285/2021 (EPI_ISL_2103264; strain B.1.617.2a) [T19R, G142D, E156G, F157del, R158del, A222V, L452R, T478K, D614G, P681R, D950N]	JHU
	SARS-CoV-2 B.1.617.2 S-2P(15-1208)-T4f-3C-His8-Strep2x2 [T19R, Δ(157-158), L452R, T478K, D614G, P681R, D950N]	SeroNet
Omicron S	SARS-CoV-2-S(1-1208)-2P-3C-His8-Strep2x2 B.1.1.529 [A67V, DEL(69-70), T95I, G142D, Δ(143-145), Δ 211, L212I, ins(214)-EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F]	SeroNet

Supplementary Table 3.2 Anti-vaccine strain IgG geometric mean titers in older adults

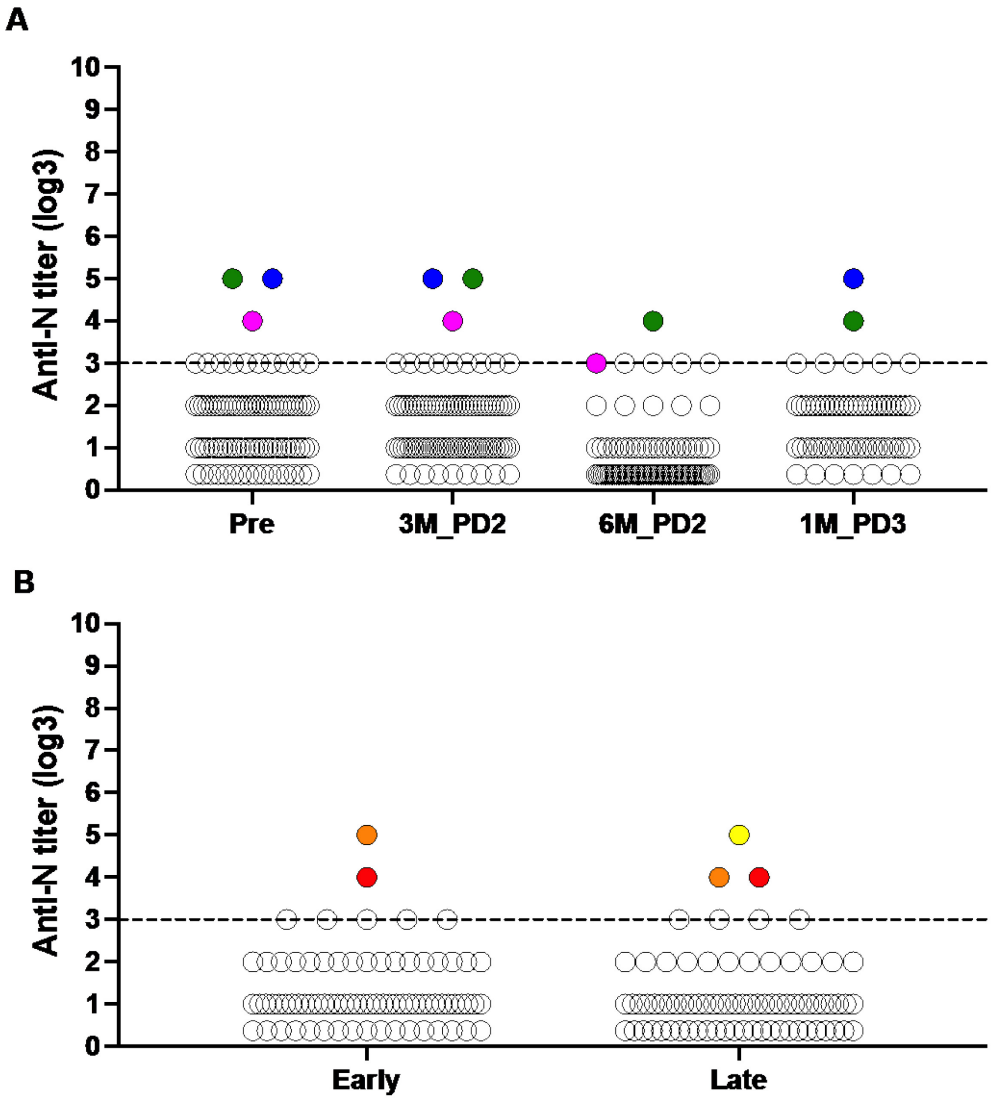
GMT (95% CI)	All	Males	Females
Vaccine S IgG			
Pre	39 (31-49)	43 (30-62)	36 (26-49)
<1M_PD1	3827 (1481-9894)	2186 (463-10313)	6396 (1740-23508)
<1M_PD2	67232 (47320-95524)	45490 (25336-81677)	87790 (56712-135897)
3M_PD2	17354 (12375-24336)	10219 (5956-17532)	25253 (16656-38288)
6M_PD2	5575 (4303-7225)	4115 (2750-6157)	6901 (4927-9667)
1M_PD3	131220 (101585-169501)	120586 (81207-179062)	139980 (98411-199108)
Vaccine S-RBD IgG			
Pre	34 (28-40)	40 (31-53)	30 (24-37)
<1M_PD1	871 (357-2126)	489 (160-1493)	1478 (339-6442)
<1M_PD2	16299 (10293-25810)	11983 (5503-26094)	20110 (11242-35971)
3M_PD2	3041 (2175-4251)	2311 (1247-4283)	3693 (2523-5405)
6M_PD2	1512 (1091-2096)	1123 (679-1857)	1864 (1208-2876)
1M_PD3	57565 (41091-80643)	45628 (25164-82732)	68761 (46015-102751)
Vaccine ACE2-inhibition			
Pre	0.2 (0.2-0.2)	0.2 (0.2-0.2)	0.2 (0.2-0.2)
<1M_PD1	2.3 (1.4-3.7)	1.6 (0.7-3.6)	3.1 (1.7-5.8)
<1M_PD2	13.8 (9.5-19.9)	11.1 (6.1-20.3)	16.0 (9.9-25.9)
3M_PD2	5.6 (4.2-7.4)	3.9 (2.3-6.5)	7.1 (5.2-9.7)
6M_PD2	0.5 (0.4-0.7)	0.4 (0.3-0.7)	0.6 (0.3-0.9)
1M_PD3	93.5 (60.2-145.1)	80.5 (43.3-149.8)	104.7 (55.2-198.8)
Vaccine nAb			
<1M_PD1	36.5 (23.0-58.1)	29.2 (15.4-55.2)	44.9 (21.3-94.8)
<1M_PD2	357.4 (261.9-487.8)	297.1 (175.9-501.8)	405.5 (272.8-602.6)
3M_PD2	61.6 (47.4-80.0)	41.7 (27.4-63.5)	81.2 (58.8-112.1)
6M_PD2	53.2 (41.8-67.9)	38.4 (27.7-53.1)	67.0 (47.8-94.0)
1M_PD3	1236.4 (954.9-1600.9)	1062.1 (717.4-1572.5)	1388.8 (972.7-1982.7)

Supplementary Table 3.3 Anti-Alpha, Delta, and Omicron IgG geometric mean titers in older adults

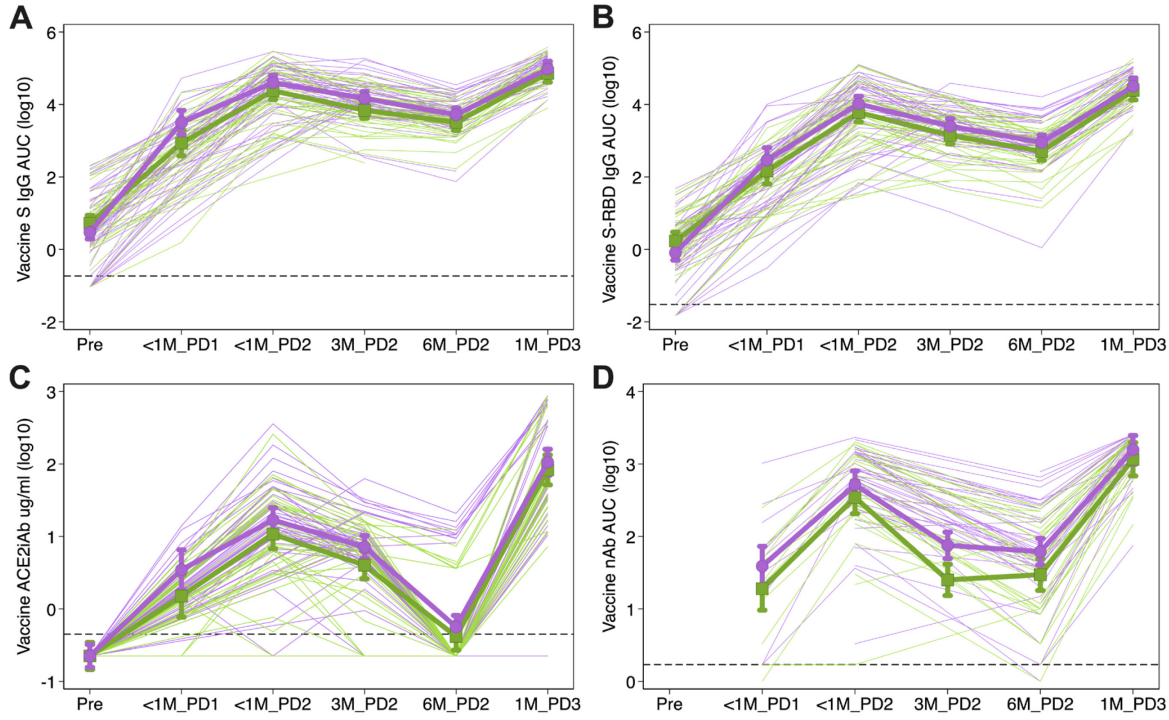
	All	Males	Females
S IgG - GMT (95% CI)			
Alpha			
<1M_PD1	1005 (472-2139)	597 (178-2003)	1620 (578-4542)
<1M_PD2	19112 (12834-28461)	14580 (7894-26928)	22993 (13438-39340)
3M_PD2	7768 (5707-10572)	5531 (3334-9174)	9880 (6713-14542)
6M_PD2	1894 (1398-2566)	1913 (1264-2896)	1880 (1211-2919)
1M_PD3	101108 (78206-130716)	88356 (58551-133334)	111644 (79308-157164)
Delta			
<1M_PD1	1106 (535-2282)	729 (211-2515)	1620 (632-4152)
<1M_PD2	19112 (12935-28240)	12961 (6844-24546)	24917 (15155-40968)
3M_PD2	7768 (5694-10596)	5184 (3093-8689)	10343 (7081-15108)
6M_PD2	1808 (1394-2345)	1418 (952-2113)	2145 (1517-3031)
1M_PD3	80862 (61259-106738)	77443 (50049-119831)	83472 (57148-121920)
Omicron (BA.1)			
<1M_PD1	405 (126-1301)	297 (47-1883)	540 (95-3063)
<1M_PD2	4938 (3386-7201)	3551 (1947-6475)	6185 (3776-10132)
3M_PD2	2118 (1551-2893)	1568 (948-2595)	2620 (1756-3907)
6M_PD2	1231 (898-1686)	951 (578-1564)	1475 (976-2231)
1M_PD3	35761 (26798-47722)	31194 (19580-49697)	39699 (27100-58155)
Percent ACE2 inhibition - mean (95% CI)			
Vaccine			
<1M_PD1	13.0 (6.6-19.4)	7.2 (3.1-11.3)	17.3 (6.6-27.9)
<1M_PD2	54.9 (47.3-62.6)	48.8 (35.5-62.0)	59.2 (49.8-68.5)
3M_PD2	34.0 (28.7-39.3)	30.5 (22.7-38.3)	36.4 (29.1-43.8)
6M_PD2	17.1 (14.0-20.2)	14.7 (11.4-18.0)	18.8 (14.0-23.6)
1M_PD3	84.5 (79.0-90.1)	82.8 (74.0-91.5)	85.9 (78.4-93.5)
Alpha			
<1M_PD1	9.7 (5.1-14.3)	5.3 (2.0-8.7)	12.9 (5.3-20.5)
<1M_PD2	47.0 (40.1-53.9)	41.5 (29.8-53.2)	50.7 (42.0-59.4)
3M_PD2	25.7 (21.2-30.2)	22.1 (16.1-28.1)	28.3 (21.9-34.7)
6M_PD2	14.2 (11.9-16.5)	12.3 (9.5-15.1)	15.5 (12.2-18.9)
1M_PD3	78.9 (72.6-85.2)	76.5 (66.6-86.4)	80.9 (72.3-89.5)

Delta			
<1M_PD1	7.4 (3.0-11.7)	3.8 (-0.4-8.0)	10.0 (2.9-17.1)
<1M_PD2	42.1 (35.2-49.0)	37.6 (25.9-49.2)	45.2 (36.4-54.0)
3M_PD2	25.2 (20.8-29.6)	21.2 (15.4-27.0)	28.0 (21.7-34.3)
6M_PD2	14.4 (12.0-16.8)	12.1 (9.4-14.7)	16.0 (12.3-19.7)
1M_PD3	75.0 (68.5-81.4)	70.6 (60.2-81.0)	78.4 (69.9-86.9)
Omicron (BA.1)			
<1M_PD1	3.1 (1.2-5.1)	1.9 (0.4-3.4)	4.1 (0.6-7.5)
<1M_PD2	1.9 (0.2-3.6)	1.1 (0.0-2.2)	2.4 (-0.4-5.3)
3M_PD2	1.7 (0.9-2.6)	1.5 (0.4-2.5)	1.9 (0.7-3.1)
6M_PD2	4.2 (3.2-5.3)	4.8 (3.0-6.6)	3.8 (2.6-5.1)
1M_PD3	27.4 (19.4-35.4)	24.2 (11.4-36.9)	30.0 (19.2-40.7)
Omicron (BA.2)			
<1M_PD1	1.9 (-0.1-3.8)	1.8 (0.1-3.4)	2.0 (-1.5-5.4)
<1M_PD2	1.9 (0.1-3.8)	0.6 (-0.2-1.4)	2.9 (-0.2-6.0)
3M_PD2	1.2 (0.4-2.1)	0.8 (-0.1-1.6)	1.6 (0.2-2.9)
6M_PD2	1.1 (0.5-1.7)	1.0 (-0.1-2.0)	1.2 (0.4-1.9)
1M_PD3	26.0 (17.3-34.6)	21.5 (7.9-35.1)	29.5 (17.8-41.2)
nAb - GMT (95% CI)			
Alpha			
<1M_PD1	16 (11-23)	13 (9-18)	19 (9-38)
<1M_PD2	135 (98-186)	113 (63-202)	152 (103-224)
3M_PD2	59 (46-75)	43 (28-64)	73 (54-100)
6M_PD2	25 (21-30)	21 (16-28)	29 (23-37)
Delta			
<1M_PD1	12 (9-16)	13 (7-23)	11 (9-14)
<1M_PD2	72 (54-98)	64 (37-111)	79 (55-112)
3M_PD2	17 (15-20)	15 (12-19)	19 (15-24)
6M_PD2	16 (14-18)	15 (12-18)	17 (14-20)
Omicron (BA.1)			
1M_PD3	155 (107-223)	148 (78-281)	160 (102-251)

3.9 Figures



Supplementary Figure 3.1 Anti-nucleocapsid IgG titers in older and younger adults.
Anti-nucleocapsid (N) IgG endpoint titers are plotted on the $\log_3$-scale for the older (**A**) and younger (**B**) adults. Dashed lines indicated the threshold for positivity (titer of 1:180), which was established using pre-pandemic samples. Colored dots indicate individuals who were excluded from further analysis due to positive anti-N IgG indicating previous infection, and dots of the same color are data from the same individual over time.



E

	S IgG (A)					S-RBD IgG (B)				
Significant difference between:	<1M_PD1	<1M_PD2	3M_PD2	6M_PD2	<1M_PD3	<1M_PD1	<1M_PD2	3M_PD2	6M_PD2	<1M_PD3
Visit & baseline	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Visit & <1M_PD2	<0.001		<0.001	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
Visit & previous visit				<0.001	<0.001				<0.001	<0.001
Sexes at visit	0.020		0.026							
Sexes in change since baseline	0.002	0.008	0.001	0.007	0.048	0.013	0.001	<0.001	<0.001	0.014

	ACE2 inhibition (C)					nAb (D)				
Significant difference between:	<1M_PD1	<1M_PD2	3M_PD2	6M_PD2	<1M_PD3	<1M_PD1	<1M_PD2	3M_PD2	6M_PD2	<1M_PD3
Visit & baseline	<0.001	<0.001	<0.001	<0.001	<0.001					
Visit & <1M_PD2	<0.001		<0.001	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
Visit & previous visit				<0.001	<0.001					<0.001
Sexes at visit			0.046					0.001	0.028	
Sexes in change since baseline										

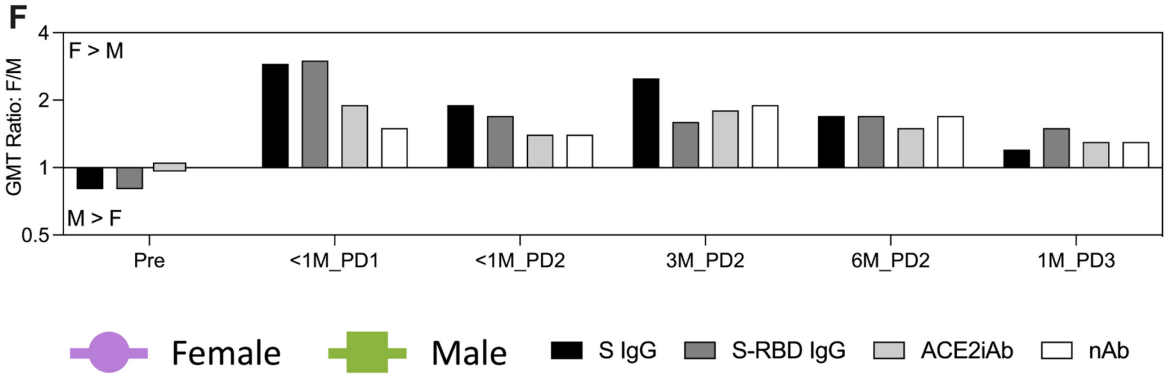
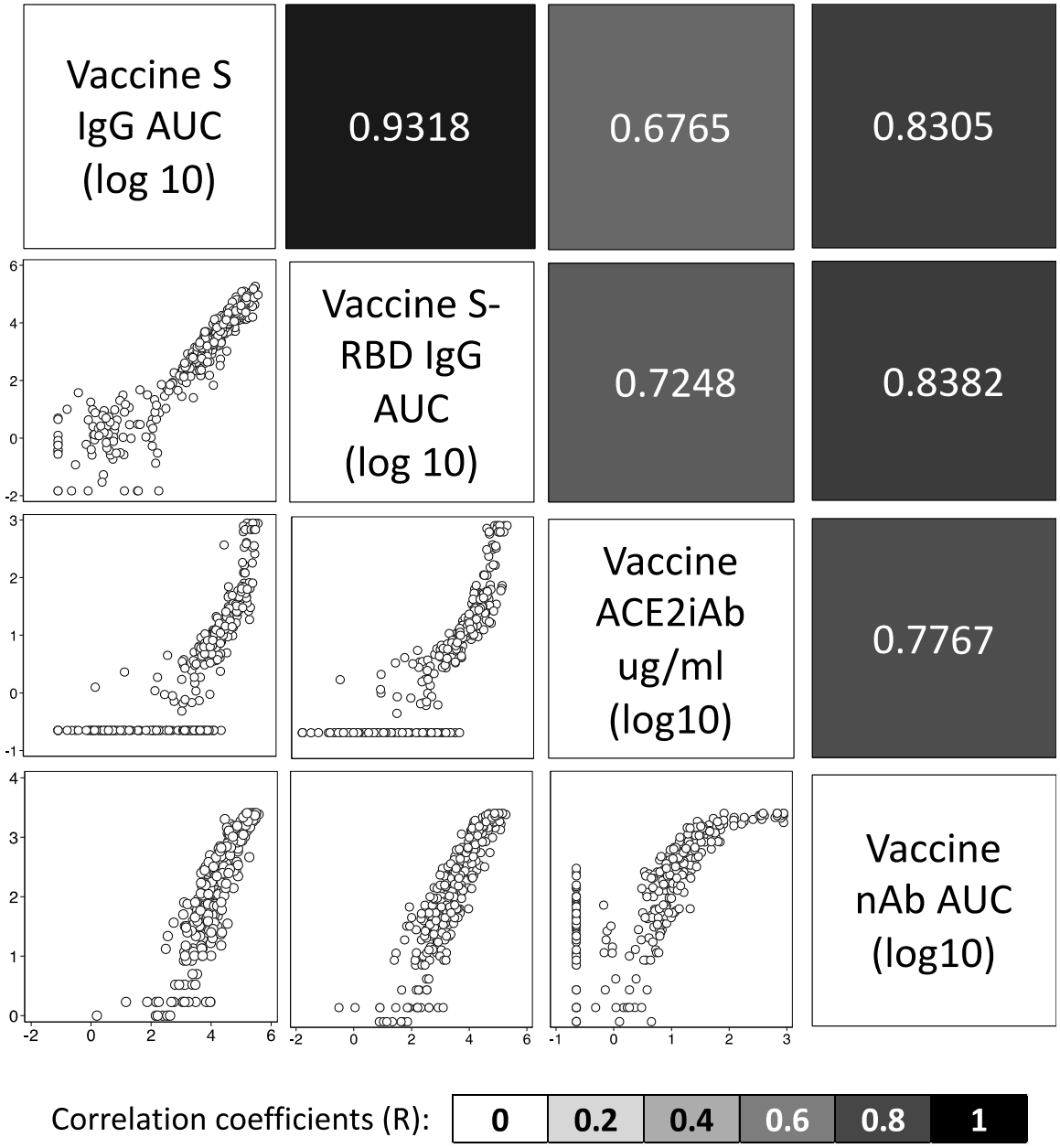


Figure 3.1 Older females mount greater humoral responses to SARS-CoV-2 mRNA vaccines than older males.

Anti-spike (S) IgG (A), S receptor-binding domain (S-RBD) IgG (B), ACE2-inhibiting antibodies (ACE2iAb) (C), and neutralizing antibodies (nAb) (D) against the vaccine strain of SARS-CoV-2 were measured at six timepoints: pre-vaccination (n= 82: 48 females, n = 34 males; nAb not measured), 14-30 days post dose 1 (<1M_PD1; n=23: 12 females, 11 males), 14-30 days post dose 2 (<1M_PD2; n=69: 41 females, 28 males), 3 months post dose 2 (3M_PD2; n=82: 48 females, 34 males), 6 months post dose 2 (6M_PD2; n=80: 47 females, 33 males), and 14-30 days post dose 3 (1M_PD3; n=60: 34 females, 26 males). Differences between timepoints were tested using mixed-effects models with study timepoint as a dummy variable and random intercepts on the individual. Sex differences were tested using an expanded mixed-effects model that included a main effect for sex and an interaction term between sex and study timepoint. All point estimates are shown with error bars indicating the 95% confidence interval. Dashed lines show the limits of detection. All p-values <0.05 are reported in E, where blank cells indicate a p-value >0.05 and crossed out cells indicate that the comparison is reported elsewhere in the table or not tested. The female-to-male ratio of geometric mean titers (GMT) for each assay and each timepoint is shown in F, with the axis on a log2 scale.



Supplementary Figure 3.2 Measures of vaccine-induced humoral immunity to the vaccine strain of SARS-CoV-2 are highly correlated with each other in older adults.
The correlation between anti-S IgG, anti-S-RBD IgG, ACE2-inhbiting antibodies (ACE2iAb), and neutralizing antibody (nAb) in older adults was assessed for all study timepoints together. Scatter plots are shown in the lower half of the matrix, and correlation coefficients (R), color coded by the strength of the correlation, are shown in the upper half of the matrix.

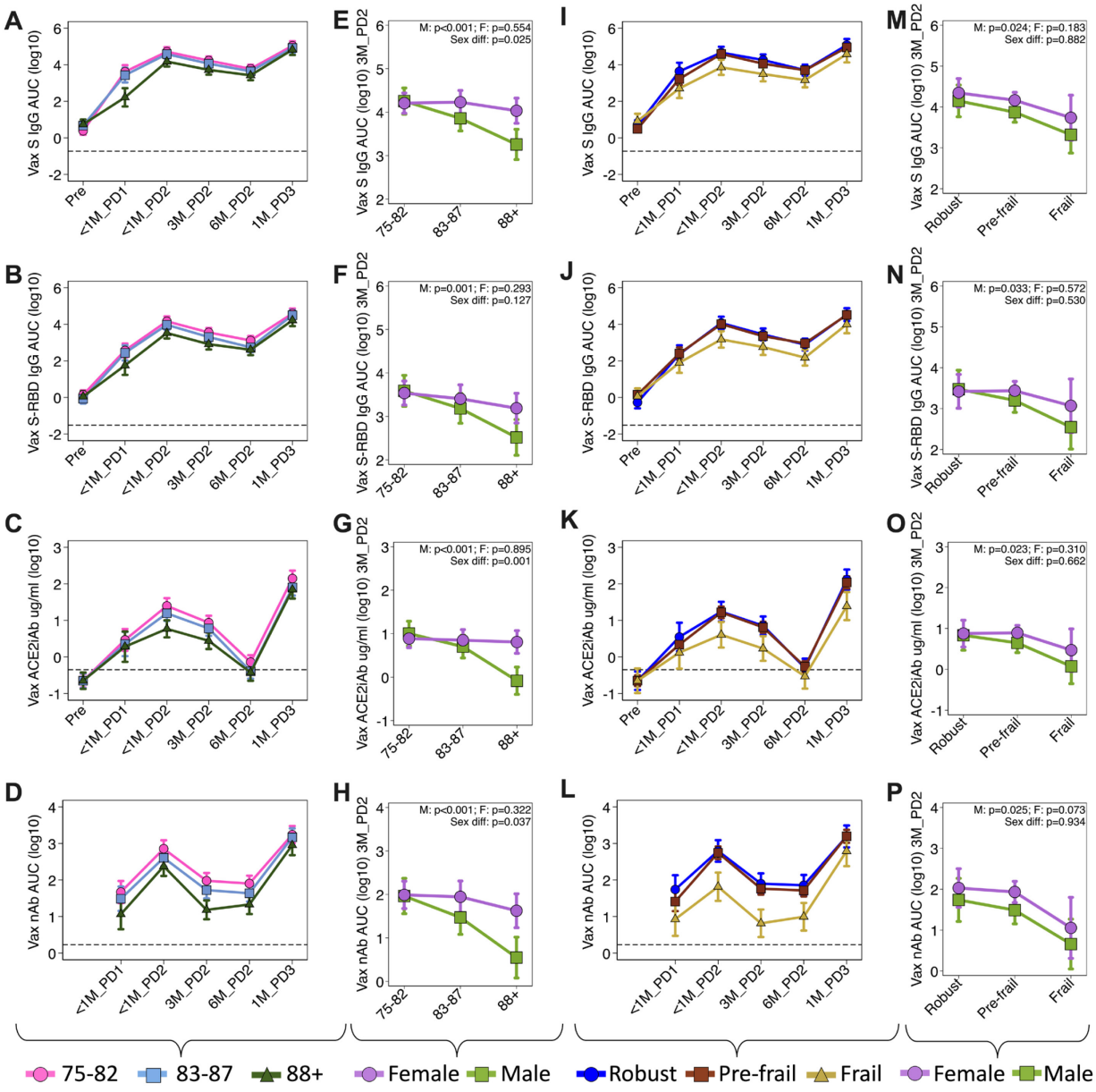
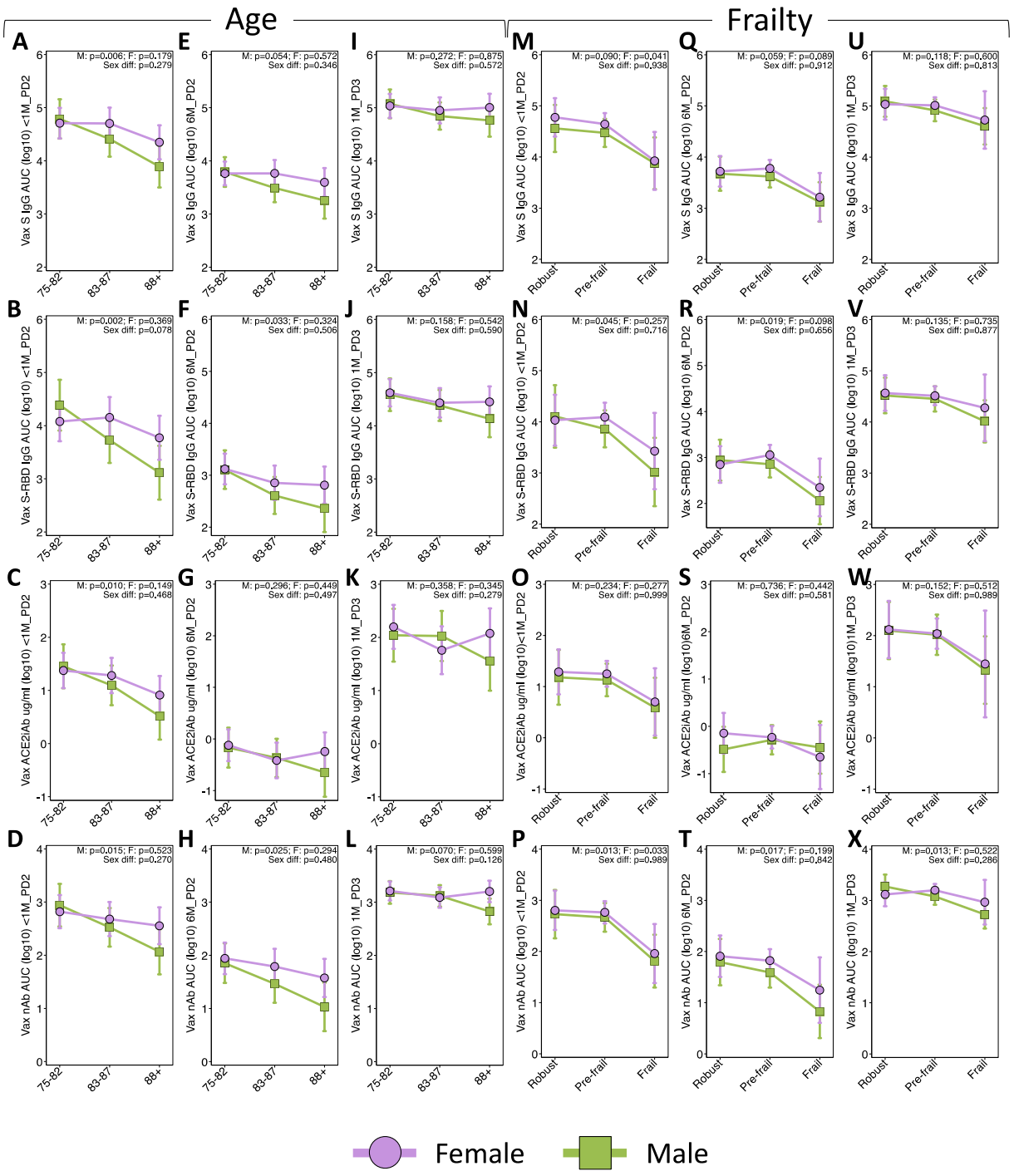


Figure 3.2 Age and frailty impact the antibody response to SARS-CoV-2 mRNA vaccines in a sex-specific manner among older adults.

The effect of age on antibody kinetics is shown for anti-spike (S) IgG (A), S receptor-binding domain (S-RBD) IgG (B), ACE2-inhibiting antibodies (ACE2iAb) (C), and neutralizing antibodies (nAb) (D) against the vaccine strain of SARS-CoV-2. Data are shown for six timepoints: pre-vaccination (n=32 aged 75-82; n=28 aged 83-87; n=22 aged ≥88; nAb not measured), <1M_PD1 (n=10 aged 75-82; n=8 aged 83-87; n = 5 aged ≥88), <1M_PD2 (n=24 aged 75-82; n=25 aged 83-87; n=20 aged ≥88), 3M_DP2 (n=32 aged 75-82; n=28 aged 83-87; n=22 aged ≥88), 6M_PD2 (n=31 aged 75-82; n=28 aged 83-87; n=21 aged ≥88), and 1M_PD3 (n=22 aged 75-82; n=21 aged 83-87; n=17 aged ≥88) (A-D). Sex-specific effects of age at 3M_PD2 are shown separately for

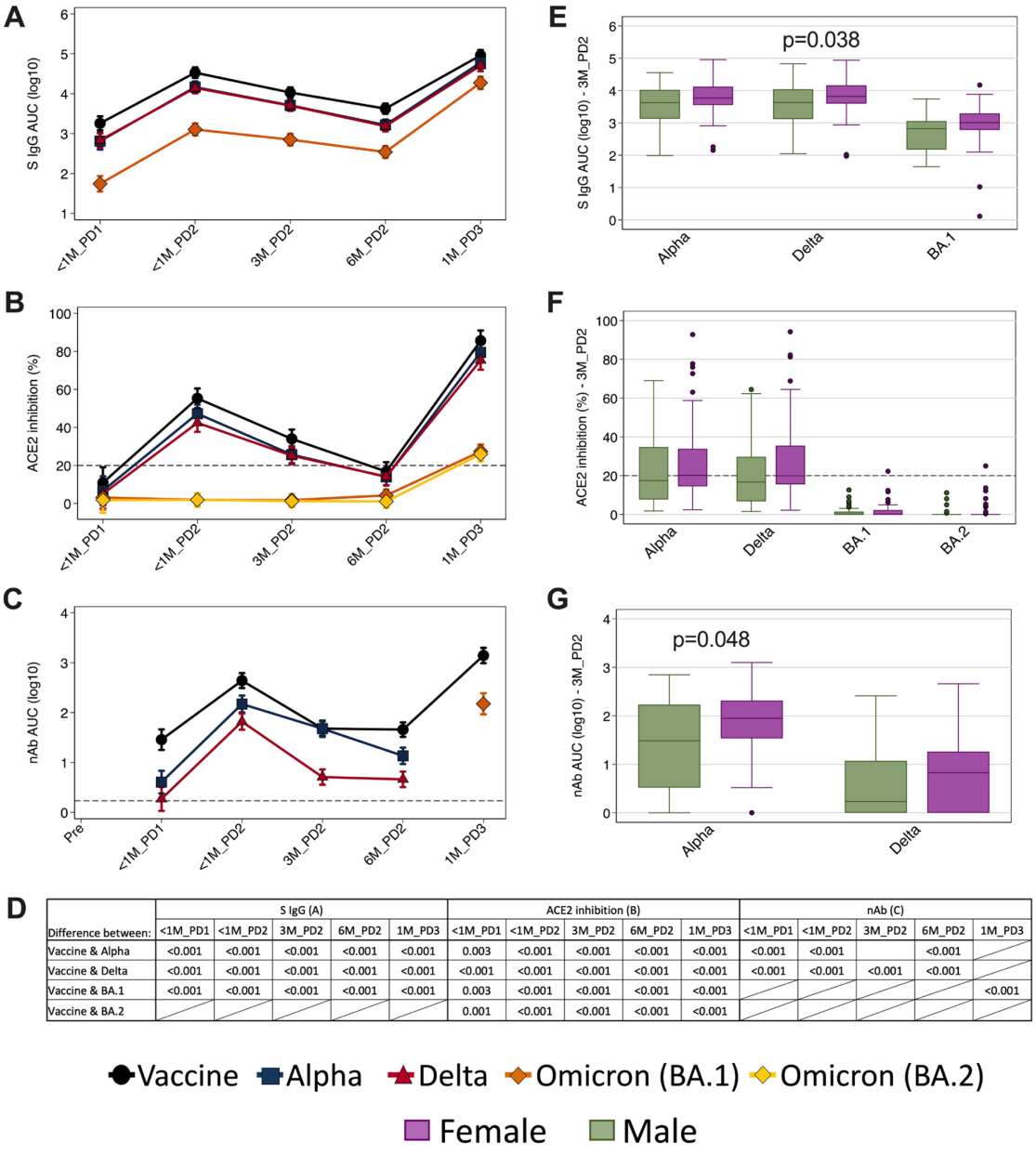
females (n=20 aged 75-82; n=15 aged 83-87; n=13 aged ≥88) and males (n=12 aged 75-82; n=13 aged 83-87; n=9 aged ≥88) (E-H). The effect of frailty on antibody kinetics is shown for the four assays at six timepoints: pre-vaccination (n=18 robust; n=53 pre-frail; n=10 frail; nAb not measured), <1M_PD1 (n=6 robust; n=12 pre-frail; n=5 frail), <1M_PD2 (n=15 robust; n=45 pre-frail; n=9 frail), 3M_PD2 (n=18 robust; n=53 pre-frail; n=10 frail), 6M_PD2 (n=18 robust; n=52 pre-frail; n=10 frail), and 1M_PD3 (n=14 robust; n=39 pre-frail; n=7 frail) (I-L). Sex-specific effects of frailty are shown separately for females (n=10 robust; n=33 pre-frail; n=4 frail) and males (n=8 robust; n=20 pre-frail; n=6 frail) (M-P). The overall effects of age (A-D) or frailty (I-L) at each timepoint were tested using mixed-effects models including a main effect for age/frailty and an interaction term between age/frailty and study timepoint. All p-values <0.05 are shown and dashed lines indicate the limit of detection. At 3M_PD2, the effect of age (E-H) or frailty (M-P) in males and females, and sex-differences in these effects, were tested using linear regression models with interaction terms between sex and age or frailty, and all p-values are shown. Point estimates are shown with 95% confidence intervals.



Supplementary Figure 3.3 Sex-specific effects of aging and frailty 14-30-days and 6-months post dose 2, and 14-30-days post dose 3 in older adults.

The effect of age on anti-S IgG, anti-S-RBD IgG, ACE2iAb, and nAb are shown <1M_PD2 for females (n=15 aged 75-82; n=14 aged 83-87; n=12 aged ≥88) and males (n=9 aged 75-82; n=11

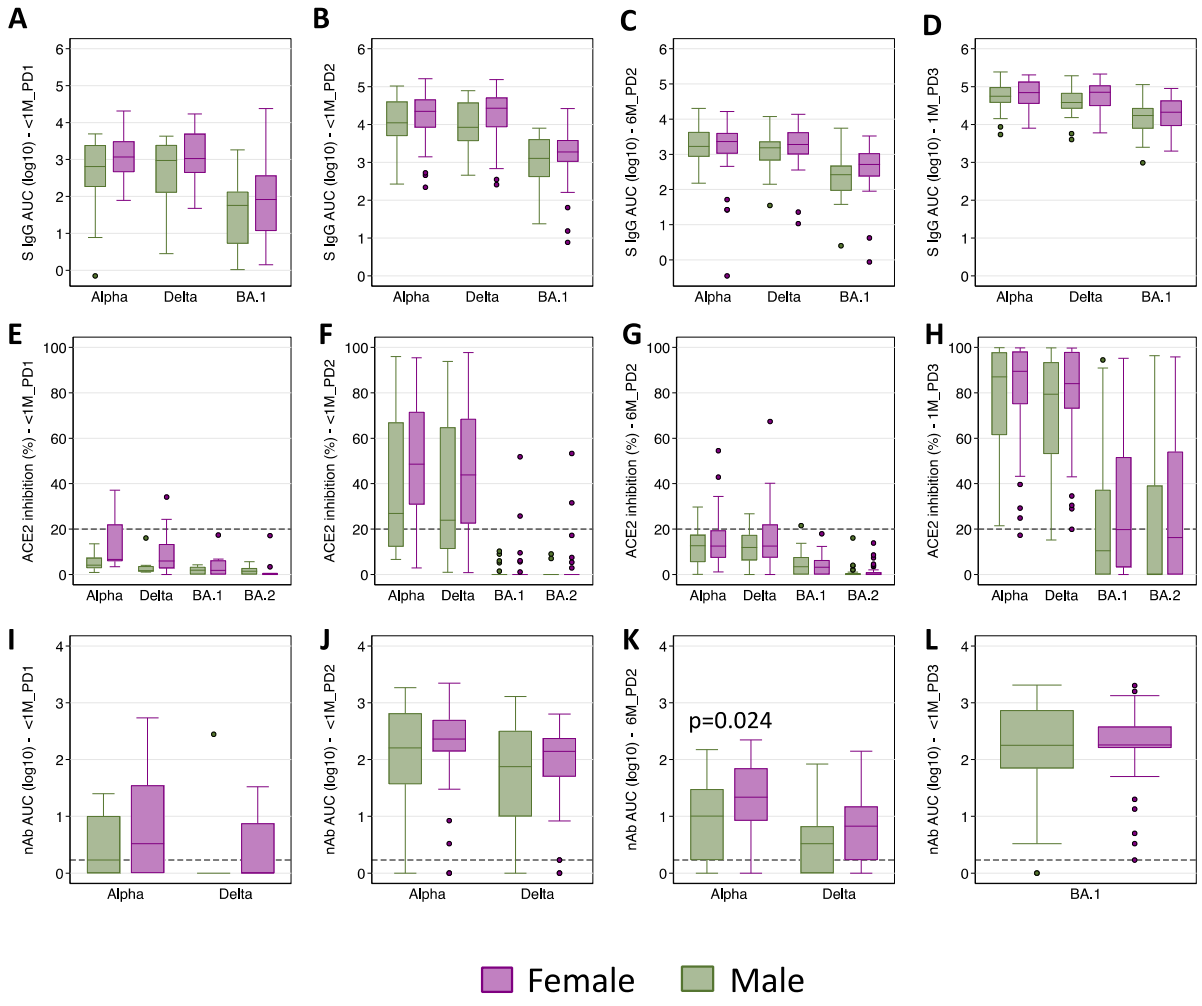
aged 83-87; n=8 aged ≥88) (A-D), 6M_PD2 for females (n=19 aged 75-82; n=15 aged 83-87; n=13 aged ≥88) and males (n=12 aged 75-82; n=13 aged 83-87; n=8 aged ≥88) (E-H), and 1M_PD3 for females (n=13 aged 75-82; n=11 aged 83-87; n=10 aged ≥88) and males (n=9 aged 75-82; n=10 aged 83-87; n=7 aged ≥88)(I-L). Similarly, the effect of frailty on the three measures of humoral immunity are shown for <1M_PD2 for females (n=9 robust; n=28 pre-frail; n=4 frail) and males (n=6 robust; n=17 pre-frail; n=5 frail) (M-P), 6M_PD2 for females (n=10 robust; n=33 pre-frail; n=4 frail) and males (n=8 robust; n=19 pre-frail; n=6 frail)(Q-T), and 1M_PD3 for females (n=7 robust; n=25 pre-frail; n=2 frail) and males (n=7 robust; n=14 pre-frail; n=5 frail) (U-X). The effects of age (A-L) or frailty (M-X) in males and females, and sex-differences in these effects, were tested using linear regression models with interaction terms between sex and age or frailty, and all p-values are shown. All point estimates are accompanied by error bars indicating the 95% confidence interval.



	S IgG (A)					ACE2 inhibition (B)					nAb (C)				
Difference between:	<1M_PD1	<1M_PD2	3M_PD2	6M_PD2	1M_PD3	<1M_PD1	<1M_PD2	3M_PD2	6M_PD2	1M_PD3	<1M_PD1	<1M_PD2	3M_PD2	6M_PD2	1M_PD3
Vaccine & Alpha	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001	
Vaccine & Delta	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Vaccine & BA.1	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001					<0.001
Vaccine & BA.2						0.001	<0.001	<0.001	<0.001	<0.001					

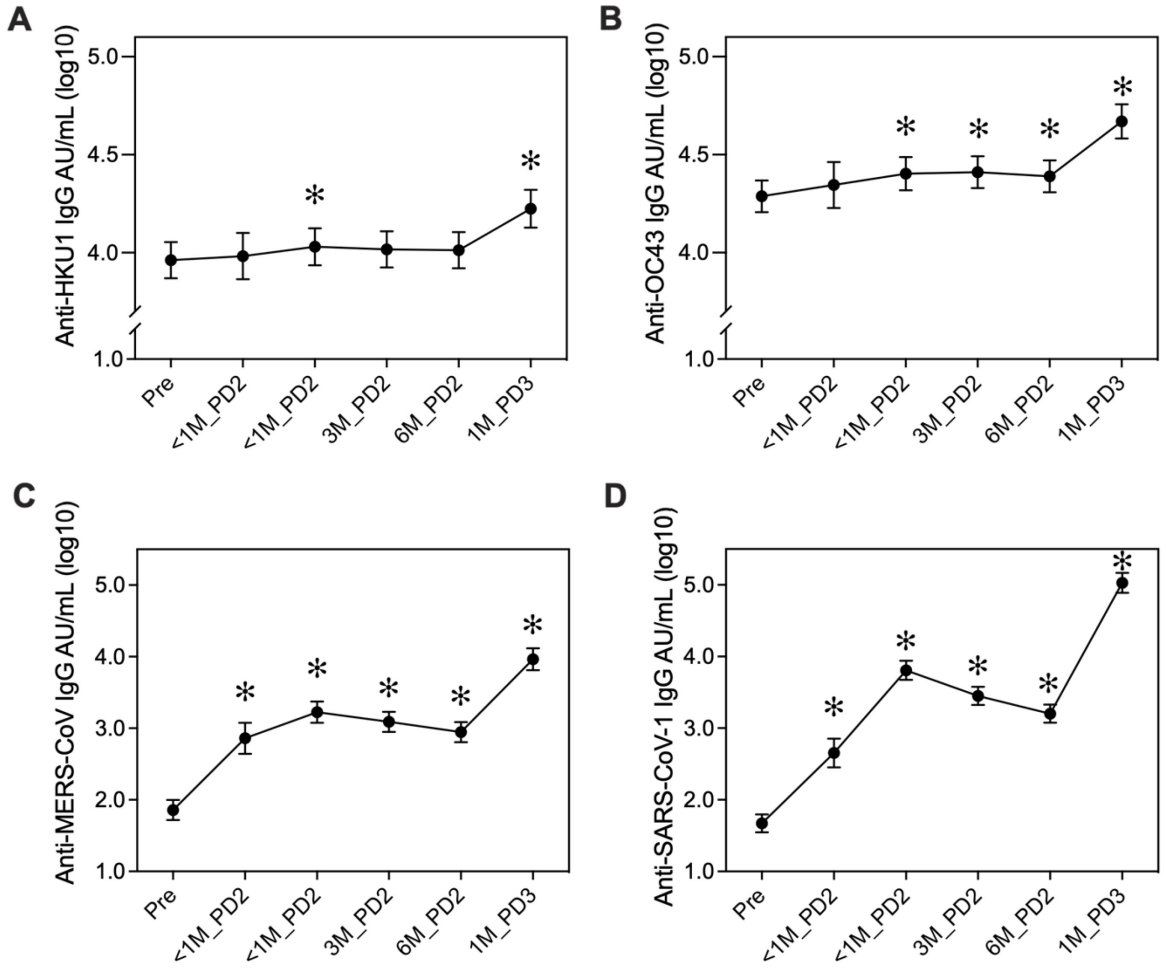
Figure 3.3 Antibody responses to Alpha, Delta, and Omicron variants are reduced relative to the vaccine virus in older adults.

Anti-S (A), ACE2-inhibiting (B), and neutralizing (C) antibodies against the vaccine, Alpha, Delta, and Omicron strains of SARS-CoV-2 were measured post-vaccination, with symbols indicating point estimates and error bars indicating the 95% confidence interval. Differences in the responses between viral strains at each timepoint were measured using paired t-tests, and all p-values <0.05 are shown in D, where empty cells indicate p-values >0.05 and crossed-out cells indicate that the comparison was not tested. Sex-disaggregated data from the 3-month timepoint are shown, and significant sex differences are indicated by p-values (E-G).



Supplementary Figure 3.4 Antibody responses to variants of concern tend to be higher in older females than older males.

Anti-S (A-D), ACE2-inhibiting (E-H), and neutralizing (I-L) antibodies against the Alpha, Delta and Omicron variants of concern are shown for males and females <1M_PD1 (A, E, I), <1M_PD2 (B, F, J), 6M_PD2 (C, G, K), and 1M_PD3 (D, H, L). Dashed lines show the lower limit of detection of the assay. All p-values > 0.05 are shown.



Supplementary Figure 3.5 Antibody responses against seasonal and pandemic β-coronaviruses are boosted by SARS-CoV-2 vaccination in older adults.

IgG specific to the spike proteins of the HKU1 (**A**), OC43 (**B**), MERS-CoV (**C**), and SARS-CoV-1 (**D**) were measured before and at five timepoints after receipt of a SARS-CoV-2 mRNA vaccine. Differences between timepoints were tested using multi-level models with study timepoint as a dummy variable and random intercepts on the individual to account for repeat measures. All point estimates are shown with error bars indicating the 95% confidence interval and asterisks indicate significant ($p<0.05$) increases relative to the pre-vaccination vaccination timepoint.

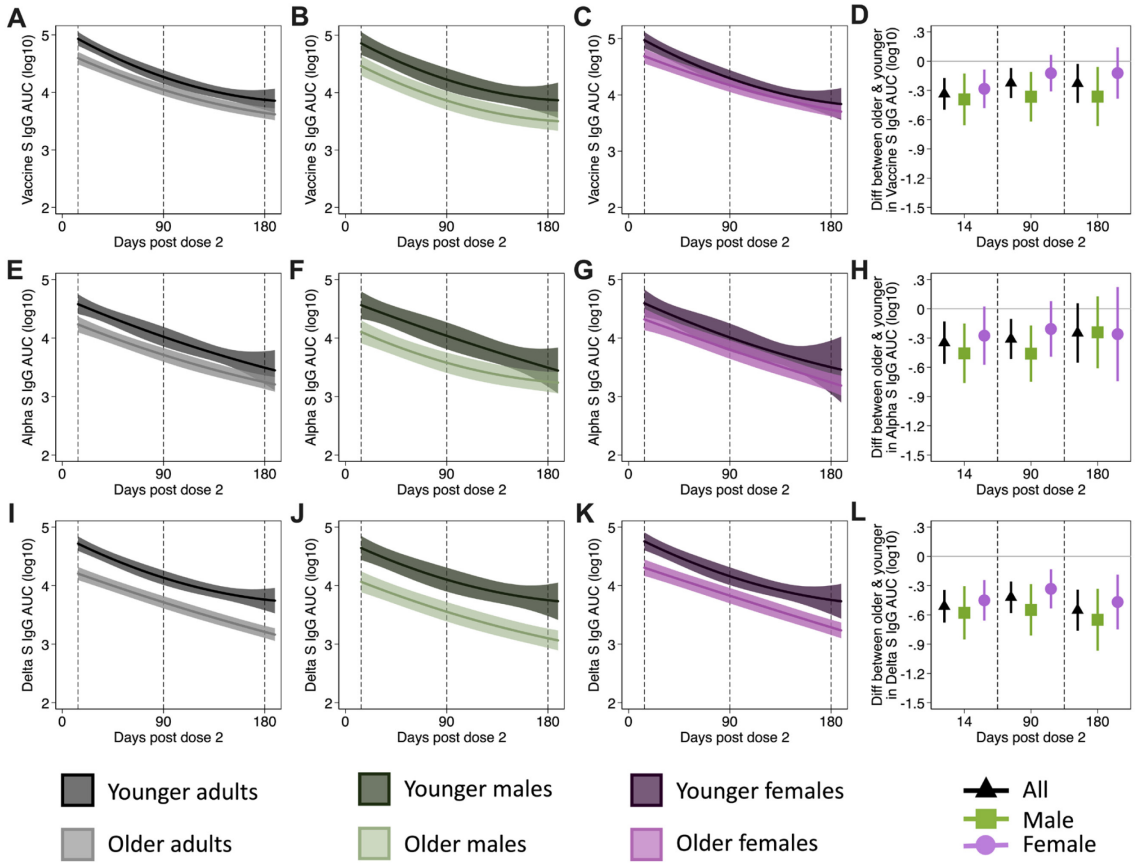


Figure 3.4 Differences between younger and older adults in antibody responses to the vaccine strain of SARS-CoV-2 are sex-dependent.

Plasma samples were collected from older adults at 3 timepoints after the primary vaccination series and from younger adults at 2 post-vaccination timepoints: 16-76 days post dose 2 (early) and 96-190 days post dose 2 (late). Differences in anti-vaccine strain S IgG levels over time were compared between all younger and older adults (A), males (younger: n=27 early; n=30 late) (B), and females (younger: n=48 early; n=48 late) (C), and summarized at three sentinel points (14-, 90- and 180-days post-vaccination) (D). Comparisons of the anti-Alpha S IgG response between the younger and older groups are shown for the whole population (E), males (younger: n=27 early; n=26 late) (F), and females (younger: n=39 early; n=34 late) (G), with differences summarized at 14-, 90- and 180-days post-dose 2 (H). Comparisons of the anti-Delta S IgG response between the younger and older groups are shown for the whole population (I), males (younger: n=27 early; n=26 late) (J), and females (younger: n=47 early; n=42 late) (K), with differences at summarized 14-, 90- and 180-days post-dose 2 (L). Kinetics were analyzed using mixed-effects models with fixed effects including days post-dose 2 as a continuous predictor with cubic B-splines (knots at 30-, 100-, and 160-days post-vaccination). Shaded areas indicate 95% confidence intervals (A-C, E-G, I-K). In D, H, and L, point estimates for the difference between cohorts are shown with 95% confidence intervals, such that confidence intervals that do not span the null value of zero are statistically significant.

CHAPTER 4

THE INTERSECTION OF GENDER AND RACE IN OLDER ADULTS' DECISION TO RECEIVE COVID-19 VACCINES

Janna R. Shapiro, Lois Privor-Dumm, Erica N. Rosser, Sean X. Leng, Sabra L. Klein, and Rosemary Morgan

Manuscript in preparation

4.1 Abstract

COVID-19 vaccines are essential public health tools for protecting older adults, who are at high risk of severe outcomes associated with COVID-19. Little is known, however, about how older adults approach the decision to receive a COVID-19 vaccine. We hypothesized that intersections between gender and race may provide unique insight into the decision-making process and the factors that lead to vaccine uptake among hesitant individuals. We performed in-depth interviews with 24 older adults who had been vaccinated against COVID-19 and used the framework approach with an intersectional lens to analyze data. Two typologies emerged: eager compliers did not question the need to vaccinate, whereas hesitant compliers were skeptical of the vaccine and underwent a thorough decision-making process prior to vaccination. For eager compliers, the vaccine offered protection from a disease that posed a serious threat, and few risks were perceived. In contrast, hesitant compliers perceived risks associated with the vaccine product or mistrusted the infrastructure that led to rapid vaccine development. Hesitancy was greater among Black participants, and only Black participants reported mistrust in vaccine infrastructure. At the intersection of gender and race, a 'White male effect' was observed, whereby White men perceived the fewest risks associated with the vaccine, and Black women were the most fearful of serious side effects. Nearly all hesitant compliers ultimately got vaccinated due to the threat of COVID-19. Convenient access through vaccine clinics in senior's buildings was pivotal for hesitant compliers and external and internal influences had differential impacts by race and gender. Emphasizing the risk of COVID-19, convenient and accessible opportunities for vaccination, and messages that are targeted to specific groups are likely to increase vaccine uptake among older adults.

4.2 Introduction

Due to the elevated risk of severe health consequences following COVID-19 in older adults (16, 17, 142), vaccination programs that meet the needs of this vulnerable population are of considerable public health importance. Much of the existing research measuring the success of vaccine programs is rooted in coverage rates, often ignoring the complexities of how people make decisions about vaccines, and barriers to accessing recommended vaccines. Vaccine behavior (i.e., whether an individual accepts a vaccine), however, does not reveal the true scope of vaccine hesitancy, defined as a continuum between individuals who accept all vaccines with no doubts and those who refuse all vaccines with no doubts (201). Individuals who accept all vaccines according to recommendations, or who delay vaccination, may be hesitant despite their observed behavior (202). While the immediate public health concerns surrounding vaccine hesitancy involve those who refuse or delay vaccination, those who accept vaccines but have concerns may be particularly vulnerable to misinformation and are at risk of refusing vaccines in the future (202). Individuals who accept vaccines despite hesitations are also valuable sources of information regarding factors that contribute to vaccine acceptance.

The decision to receive a vaccine is complex, with many contributing factors, including perceived importance of the vaccine, risk perception, trust, and past experiences (203, 204). The influence of gender norms, roles, and relations is often over-looked, yet there is substantial evidence from other areas of health research that men and women are likely to approach vaccine decisions differently (4). For example, men's vaccine decision-making process may be affected by masculine norms prescribing independence and self-reliance, which translate into lower likelihood of seeking healthcare and increased likelihood of partaking in risky behaviors

(205, 206). There are also gender differences in risk perception, with women and men perceiving the same risks differently or perceiving different risks altogether (207). For example, during the COVID-19 pandemic, women surveyed in the U.S. perceived a greater risk of disease, while men perceived a greater risk of financial hardship (208). In the case of vaccination, differences in risk perception are compounded, as vaccine acceptance can be the result of weighing the competing perceived risks of the disease prevented by the vaccine and the vaccine itself (209).

Gender norms, roles, and relations must be considered in the context of their intersection with other social stratifiers, such as race (210). Following the theory of intersectionality, the relationships between factors and differences within groups can explain and resolve inequities in health outcomes (211). Racial disparities in vaccine uptake have been most extensively studied in the context of seasonal influenza vaccination, where the perceived risks of both the disease and the vaccine, beliefs, attitudes, and trust in healthcare have been identified as contributing factors (212-218). The direct impact of lived experiences of race and racism in healthcare has also been investigated, with perceived racial fairness emerging as a powerful predictor of vaccine attitudes, such that experiences of unfair treatment by a healthcare professional can discourage vaccination (219). While racial disparities in vaccine attitudes and uptake have been observed during COVID-19 (220), little is known about the intersection of race and gender.

In the context of the COVID-19 pandemic, it is increasingly important to understand how older adults make decisions about vaccines. Much of the literature available on this topic has

focused on the seasonal influenza vaccine, but there is evidence to suggest that the decision-making process may be substantially different for the COVID-19 vaccines, given the novelty and rapid development of the vaccines, the politically charged environment and other circumstances of the pandemic (221, 222). We hypothesized that previously unexplored links between gender and race may provide unique insight into the decision-making process and what factors ultimately lead to acceptance among hesitant individuals. Through qualitative analysis of in-depth interviews (IDI) with older adults, we aimed to understand how these factors could be leveraged to design more effective public health messaging and vaccine programs for this diverse group of individuals.

4.3 Methods

4.3.1 Context

This study took place in the greater Baltimore area, where the population is 30% White, 62% Black, and 5% Hispanic or Latino, and 14% of city residents are over the age of 65 (223). Median household income in 2019 was $50,177 and an estimated 17% of the population over 65 years of age lived below the federal poverty line, compared to national averages of $65,712 and 9%, respectively (224). As of this writing, 81% and 91% of those over 65 are fully vaccinated against COVID-19 in Baltimore City and County, respectively (225). Racial inequities in vaccine coverage were prominent among older adults early in the vaccination campaign, however, concerted efforts by the Baltimore City Health Department, partners, and the state's Vaccine Equity Task Force led to significant improvements in vaccine coverage equity (226-228). Data

disaggregated by both age and race at the city level are limited, but state-level data suggest uniformly high coverage among older adults by the end of 2021 (229).

4.3.2 Participants and recruitment

Individuals were eligible to participate if they were over the age of 70 and resided in the Baltimore area. Although the age of 65 is often used as the cut-off for older adults, in pilot data, we found that many individuals between the ages of 65 and 70 were not fully retired, and thus had different experiences. We therefore restricted analysis to those over the age of 70. Participants were either recruited from the community or selectively sampled from an existing cohort of older adults (73). Community recruitment included distributing flyers in seniors' buildings, snowball sampling, and referrals from Baltimore's Vaccine Acceptance & Access Lives in Unity Education and Engagement (VALUE) ambassadors. Purposive sampling from the existing cohort was based on approximately matching ages to those who had been recruited from the community. Recruitment efforts focused on obtaining a sample with approximately equal numbers of participants by gender and race. Most White participants came from the existing cohort (73), and most Black participants were recruited from the community. Recruitment continued until saturation was achieved, in that interviews no longer yielded new information.

4.3.3 Data collection

Semi-structured IDI were conducted from October 2021 – February 2022. Due to the on-going COVID-19 pandemic and the high-risk nature of the study population, all interviews were conducted over the phone. After collecting basic demographic information, interviews focused

on five key themes: (1) Experience of the COVID-19 pandemic; (2) Sources of information regarding vaccines; (3) Decision-making process for the COVID-19 and seasonal influenza vaccines; (4) Experiences receiving the COVID-19 and seasonal influenza vaccines; (5) Lived experiences of infectious diseases and vaccination. Participants received a Visa gift card upon completion of the interview. Interviews were 20-60 minutes in duration, and audio recordings were professionally transcribed.

4.3.4 Data analysis

Data were manually analyzed using the framework approach (230, 231). Following a familiarization stage, a thematic framework was developed that largely followed the themes of the IDI. The thematic framework was then systematically applied to all transcripts, and key quotes were abstracted and categorized into a series of charts. The first set of charts categorized data by individual, to provide an overview of each participant. The second set organized information by theme, allowing for analysis across participants, and identification of similarities and differences. For the final set of charts, key quotes were categorized by increasingly specific sub-codes, which were deduced from thematic text-based analysis.

An intersectional lens was applied throughout analysis. Instead of focusing on individual factors, analysis focused on how factors interacted at multiple levels and on differences between and within groups (211). Our primary interest was at the intersection of gender and race. To facilitate this, data were grouped into four key demographic groups (Black women (BW), Black men (BM), White women (WW) and White men (WM)) at all charting steps. The intersection of gender and race was also considered in the greater context of other key socio-

economic factors. Accordingly, as a proxy for socioeconomic status, participant's ZIP codes were linked to Census data to determine the median household income and percent of residents over the age of 65 living below the federal poverty level.

The lead author, who collected data and did much of the analysis, is a young adult White woman. R.M. and L.P.D., who contributed to design of data collection tools and guided analysis, are also White women, although L.P.D. leads the VALUE Peer Ambassador Education program working primarily in the Black community. S.L.K., a White woman, and S.X.L., a man of Asian descent, provided guidance and expertise on the study population, while E.N.R, a Black woman, provided significant editorial contributions. The composition of the study team may have influenced the type of data that were collected and the themes that emerged during analysis.

4.3.5 Ethics

All participants provided oral consent and the study protocol was reviewed and deemed as exempt research by the Johns Hopkins School of Medicine Institutional Review Board.

4.4 Results

4.4.1 Study participants

Twenty-four adults over the age of 70 were interviewed, with an approximately equal distribution among the four race/gender categories (**Table 1**). Ages were similar in each of the four core groups, but based on ZIP codes, the Black participants lived in neighborhoods with lower median household incomes and higher levels of poverty in those over 65. Levels of education also varied by group, with White men being the most educated. Most Black participants and some White participants lived in seniors' buildings. This had important

implications for vaccine access, as the Baltimore City Health Department offered in-house vaccine clinics in many seniors' buildings in the spring of 2021.

4.4.2 Typologies

While all participants received the primary series of COVID-19 vaccines (either one or two doses), two distinct typologies emerged in how participants approached the decision to get the vaccine: eager compliers (EC) and hesitant compliers (HC). Eager compliers actively sought out opportunities for vaccination and did not question the need for or the validity of the vaccine. In contrast, hesitant compliers were skeptical of the vaccine and underwent a thorough decision-making process. More of the Black participants, particularly women, were characterized as HC. Only two White participants, one man and one woman, were HC. In the sections below, we discuss the factors that contributed to eager and hesitant compliers' decision to receive a COVID-19 vaccine, with an emphasis on differences that emerged at the intersection of race and gender.

4.4.3 Eager compliers

For the EC, the vaccine was seen as an obvious way to protect themselves from the risk posed by COVID-19. Coupled with low perceived risk of the COVID-19 vaccine and positive past experiences with vaccines, there was little debate as to whether to receive the vaccine.

4.4.3.1 Vaccine as solution to COVID-19

All the EC felt that they were at significant risk of getting COVID-19, and that the consequences of disease might be severe. For many, age and comorbid conditions contributed

to feelings of risk. Faced with the prominent threat of COVID-19, the vaccines were enthusiastically received as a strong source of protection.

> *As soon as they said that COVID was respiratory, me with COPD and heart problems, I knew right away that I was not going to be staying on side lines talking about "I'll wait". (119_BM)*

One participant who had been hospitalized with COVID-19 early in the pandemic was particularly desperate to get the vaccine to avoid further illness. In addition to viewing the vaccine as critical to protecting their own health and well-being, several women noted the benefits of vaccination for their loved-ones and community. This included being able to spend time with grandchildren and protecting medically vulnerable family members.

> *Part was my husband's health. He's a lung cancer survivor… and I always thought if God forbid he got it, you know, it would be the end. (1245_WW)*

The perceived need of the vaccine is perhaps best exemplified by how many EC persisted to get a vaccine as soon as possible, despite a range of a barriers, including lack of knowledge about where to receive it, difficulties booking appointments online due to low computer literacy, and physical barriers to accessing mass vaccination sites. Physical barriers were particularly prominent for women.

> *I was being pushed in a wheelchair. I could not walk. My daughter took me up there… I can do nothing by myself. I have to depend on somebody taking me somewhere. (104_BW)*

Overall, COVID-19 posed a serious threat for the EC and receipt of the vaccine was an obvious choice that did not require in-depth deliberations.

4.4.3.2 Low perceived risk of the vaccine

The EC either did not perceive any risks associated with the vaccine or were not deterred by the risks they were aware of. Participants had trust in the systems that led to the development and emergency use authorization of the vaccines, and in some cases, the speed at which the COVID-19 vaccines were developed reinforced this trust.

> *It just reinforces my trust in the medical establishment that, you know the medical establishment has managed to find a vaccine that is as effective as these are. (118_WM)*

Many noted that with so many people vaccinated, unknown side effects were unlikely. When EC did perceive risks associated with the vaccine, they were of minor side effects, a general fear of the unknown, or were immediately qualified with an acknowledgement that severe risks are exceedingly rare. Several White men were readily willing to assume the risk of a rare adverse event given the tremendous perceived benefit of the vaccine.

> *I know there is a risk. It can kill, it can cause permanent disability, but that is very rare, and that it is risk I am willing to take, because the chances are so small compared to the benefits of the vaccine. (1085_WM)*

Taken together, the risks that the EC associated with the vaccine were perceived as minimal, and largely did not influence their decision to get the vaccine.

4.4.3.3 Positive experiences with vaccines

Many EC expressed general pro-vaccine sentiments, had a history of compliance with vaccine recommendations, and were willing to receive any future COVID-19 vaccines (e.g., boosters) that become available. Participants reported that most of their families and communities were vaccinated, with the notable exception of some participants' children or grandchildren who refused the vaccine, which lead to significant frustration and conflict within the family. In other cases, mainly for White women, a family-based decision-making process contributed to confidence in the vaccine.

> *The family had talked about it, and everybody, the older people in my family, it was not a question. It was yes, of course, we're going to get the vaccine. And so, I didn't question it, I knew I would get it. (109_WW)*

Many participants had positive memories of getting vaccines as a child, or ensuring that their own children were vaccinated, leaving them no reason to doubt the COVID-19 vaccines. One Black man linked his experience during the pandemic, and willingness to be vaccinated, to past experiences with infectious diseases.

> *I had a partner who had been exposed to Syphilis… I have lived through AIDS ... So that really raised my awareness about the trans-social diseases. So, I was on board when COVID-19 came along… I am savvy about the trans-social diseases (117_BM)*

Three White women discussed how they were confident that the COVID-19 vaccines would have the same effect that as the polio vaccine.

I am confident that it [the vaccine] is still working… Like when we had… polio years ago and when you took the pills or the polio shots, they worked. I think this is going to be the same thing (1185_WW)

One notable exception to most EC's positive history with vaccines, was one Black man's story of becoming ill after receiving an influenza vaccine many years ago, resulting in refusal of the vaccine since. These feelings were, however, restricted to influenza, as the participant was eager to receive the COVID-19 vaccine after hearing a friend's story of severe disease following infection. Taken together, the EC either had positive experiences with vaccines or viewed the COVID-19 vaccine as distinct from other vaccines, such that they readily complied with recommendations.

4.4.4 Hesitant compliers: Sources of hesitation

As opposed to EC, who readily accepted vaccination, HC had a variety of concerns about the vaccine and the system providing it.

4.4.4.1 Risks associated with the vaccine product

For both White and Black HC, vaccine hesitancy stemmed from perceived risks associated with the vaccine product. For the Black women HC, concerns were primarily focused on unknown long-term consequences. Many suggested that accepting the vaccine required assuming some degree of risk.

It will take a long time before we find out exactly...what benefits the vaccine has and what benefits it does not have, and what side effects it has. (101_BW)

Comorbid conditions also contributed to perceived risk associated with the vaccine. One participant suggested that people with underlying conditions, like her diabetes, need to make sure that the vaccine is appropriate for them.

Unless they have underlying sickness and have to ask a lot of questions, think twice… They need to check it out first. Vaccines don't work for everyone. (116_BW)

For three Black HC, negative experiences with influenza vaccines contributed to their perception of risk associated with the COVID-19 vaccine. Two women fell ill after receiving an influenza vaccine years ago, which prompted them to stop taking the vaccine for several years. One man reported an allergic reaction to the influenza vaccine, such that in consultation with his doctor, he no longer receives it. Although the influenza vaccine was largely seen as separate from the COVID-19 vaccines, these negative experiences did contribute to general feelings of skepticism about the unknown side effects of vaccination.

The two White HC also perceived risks associated with the vaccine product. The White man who was hesitant referred to perceived lack of efficacy of the vaccines and questioned the need for vaccines altogether.

Most of the arguments that I'm hearing… are that this is going to be like every other flu or virus. It will burn itself out. And it's not that inoculating people is causing it to burn out. (1225_WM)

The White woman, on the other hand, was concerned about the mRNA technology because it was different than vaccines that she had received in the past. Despite having clear questions about the vaccine, she did not want to be viewed as vaccine hesitant.

I wanted to wait and see, because I did not know what the mRNA vaccine was. Nobody knew… I would not describe myself as vaccine hesitant. I just wanted to know what I was getting. (1023_WW)

Although the types of risks identified in relation to the vaccine product differed by race, for both White and Black HC, they figured heavily in deliberations about the vaccine.

4.4.4.2 Mistrust in vaccine infrastructure

For two Black HC, but for no White HC, uncertainty stemmed from mistrust in the system that made and provided the vaccine, rather than the vaccine itself. They questioned the motives for making the vaccine and the speed of development, leading them to believe that the vaccines were not adequately tested. For one woman, this mistrust was mainly focused on the pharmaceutical industry. In contrast, one man's long-standing mistrust in the government supported the notion of collusion between the government and pharmaceutical companies.

I just have a question as to the validity of the testing for the vaccines and how quickly they came out… In order for them to have that ability to get something

that quickly, they had to have the information from the Government who created it... If you know the history of this country, it would not be the first time that the Government put something on people (102_BM).

For these participants, distrust in the system providing the vaccines stemmed from existing misgivings with the government and pharmaceutical industry, which were heightened by the novelty of COVID-19 and speed at which the vaccines were developed.

4.4.5 Hesitant compliers: Decision to vaccinate

Despite the uncertainty surrounding the COVID-19 vaccine, all the HC in our sample ultimately decided to receive the vaccine. Below are the factors that were pivotal in the decision to vaccinate.

4.4.5.1 High perceived risk of COVID-19

For all the Black HC, while risks associated with the vaccine or distrust in the vaccine infrastructure remained prominent, the threat of COVID-19 made the vaccine seem necessary for protection. They ultimately decided to get vaccinated because the risk of COVID-19 out-weighed the perceived risks associated with the vaccine.

With the number of people dying going up, there was no way to say that was fake news. They showed tractor trailers full of bodies. So, it is like you had to have a come to Jesus moment and go and grin and bear it…You are just rolling the dice when you walked out of your door, and so, I decided to stop rolling the dice. (102_BM)

In contrast, one White HC did not feel at great risk due to COVID-19 due to his rural residence, such that the risk of disease did not figure into his decision to get the vaccine. For all other HC, regardless of race or gender, the risk of COVID-19 was the primary factor in their decision to receive the vaccine.

4.4.5.2 Convenience & ease of access

For those who lived in senior's buildings, access to in-house vaccine clinics was a major facilitator and directly contributed to the decision to get the vaccine for some Black HC. Participants listed many benefits of these vaccination clinics, including feeling that they were safer and cleaner than mass vaccination sites, convenience due to the absence of lines or long wait-times, and privacy when getting the vaccine.

> *I have more faith having it in this building. I may not have gone had it been down at one of the centers that's close to us…. the centers were not that clean. (105_BW)*

Convenience and ease of access were not, however, motivators for all participants. One particularly skeptical Black man refused the vaccine that was offered to him in his building.

> *So, I did not get a vaccination until June, and it was after they had come into the building … I wanted to make sure that when I made a decision, it was not a hurried decision, and I went jumping the line to get a needle in my arm before I knew anything about it. (102_BM)*

4.4.5.3 Fostered trust in the vaccine

External and internal influences fostered trust in the vaccine for both White and Black HC (see representative quotes in **Table 2**). In terms of external influences, most of the Black HC trusted their doctors and consulted them regarding the vaccine. These recommendations were most influential for those who had safety concerns about the vaccine due to their allergies or underlying conditions. In contrast to consulting his personal physician, for the one White man who was a HC, being contacted by Veterans Affairs motivated him to receive the vaccine. Several White participants (both EC and HC), mostly men, reported being contacted by their healthcare system regarding opportunities to get the vaccine, while none of the Black participants reported this. For a Black woman, the recommendation from the governor was pivotal in her decision to receive the vaccine. Across gender and race groups, media coverage of the vaccine was important in the decision-making process. For one White man, the sheer volume of coverage, compared to how rarely other topics, such as influenza, are discussed, lent credibility to how serious COVID-19 was. For a White woman, information about the mRNA vaccine platform from trusted news sources addressed her hesitations. For a Black woman, on the other hand, seeing an older Black woman get vaccinated on the news was influential. Finally, several participants discussed the role of community. Three of the Black HC, two women and one man, discussed how their families contributed to their decision-making process. Vaccination was seen as a way to protect their communities, with all of them specifically discussing their grandchildren. In contrast, the one White man who was a HC stated that he was not influenced by the anti-vaccine opinions being discussed in his community.

In terms of internal influences, for many in our sample, the concept of vaccines was familiar. Participants noted that they had been receiving vaccines all their lives and were comfortable with them. Several Black women also referred to their faith in fostering trust in the vaccine. Taken together, unlike the near-unanimous perceived risk of COVID-19 as a motivator to vaccinate, external and internal influences had heterogeneous effects. Each resonated with certain participants, according to their specific concerns about the vaccine or lived experiences.

4.4.6 Gender, race, and their intersection

The ways in which gender, race, and their intersection impacted the vaccine decision-making processes described above are summarized in **Table 3**. The most prominent impact of gender norms, roles, and relations manifested in how women discussed the impact of their decision to get vaccinated on their communities, acknowledging the role the vaccine could play in protecting themselves and loved ones. In terms of race, it is notable that many of the HC were Black, particularly Black women. In addition, the sources of hesitation varied by race in that several Black HC but no White HC expressed mistrust of the system that developed and provided vaccines. Finally, at the intersection of gender and race, notable differences in risk perception emerged. The Black women in our sample were particularly concerned about unknown long-term consequences associated with the vaccine whereas the White men knew that rare adverse events were possible, but did not think they would be affected.

4.5 Discussion

Through IDIs with older adults in the Baltimore area, we found that the risk of severe illness following COVID-19 infection was the primary reason for deciding to receive the COVID-19 vaccine. This was true for both eager and hesitant compliers, even though the two groups approached the decision-making process differently (**Figure 1**). For EC, the role of vaccines in mitigating the risk of disease was clear, and the decision to vaccinate was as an obvious conclusion. For the HC, however, this conclusion was the result of assessing the competing risks of the vaccine and the disease, and consideration of a variety of external and internal influences. In addition, the convenience of in-house vaccine clinics was pivotal for some.

Our findings on how individuals approached the decision to receive the vaccine cannot be dissociated from their lived experiences, which are fundamentally shaped by gender and race. These observations can be interpreted through existing literature. For example, the role that community played in the decision to vaccinate for many women is consistent with the traditionally feminine roles of caregiving and promoting health (232). Furthermore, the finding of increased vaccine hesitancy and mistrust among Black participants is likely rooted in the long history of unethical treatment and racism in healthcare settings and should not be viewed as an individual lack of trust, but rather as a failure of the healthcare system (233, 234). Our finding of hesitant or delayed vaccine acceptance among many of the Black participants is mirrored in national immunization coverage data, where a significant gap in coverage between White and Black Americans was evident in the early stages of the vaccine campaign but largely disappeared by the end of 2021 (235). Finally, the observation that Black women were concerned about long-term side effects, but White men were not, is consistent with the 'White

Male Effect', whereby White men perceive the lowest levels of risk and women of color perceive the greatest levels (236). Researchers have hypothesized that because White men are traditionally in positions of power and control, they feel protected from dangers and are thus more willing to take risks, whereas other groups feel more vulnerable to risks (236). Along with the observed differences at the intersection of race and gender, we acknowledge that the decision to vaccinate is the result of interactions between various social processes, such that it is difficult to untangle the complex causes of the phenomena observed.

This work has several important implications for public health messaging and the design of vaccine programs. Above all else, highlighting the risk of disease is likely to increase vaccine uptake. Furthermore, the racial and gender differences in the vaccine decision-making process suggest that a 'one size fits all' approach to vaccine promotion is likely to be ineffective (112). Instead, different types of messages may resonate with different groups. For example, emphasizing the link between vaccination and community is likely to resonate with women more than men, and vaccine promotion at faith-based institutions may have an important effect among Black women. In terms of the design of vaccine programs, holding vaccine clinics in seniors' buildings was a highly effective tool for improving vaccine coverage among hesitant older adults, both men and women alike. Such programs should be expanded to community-dwelling older adults and to include other vaccines recommended for this population.

This work also has several limitations. Based on participant ZIP codes, Black participants were likely of lower socio-economic status than White participants, such that some of the findings attributed to race may be influenced by socio-economic factors or education levels. In

addition, interviews were conducted several months after most participants were vaccinated, so it is possible that attitudes may have shifted over time. Because availability of booster vaccines changed substantially over the period of time that interviews were conducted, we were also unable to systematically assess attitudes towards booster vaccines. Finally, the positionality of our research team must also be noted. White women led this research, which likely impacted how data were interpreted.

In conclusion, we find that vaccine acceptance obscures true levels of vaccine hesitancy, and that many who comply with recommendations have unresolved concerns about vaccines. For those who were hesitant, messages that emphasize the risk of COVID-19, along with convenient and accessible opportunities for vaccination, were the most important factors in the decision to ultimately receive the vaccine. Sources of hesitation and the role of external and internal influences on vaccine attitudes varied by gender and race, such that more targeted approaches to vaccine promotion would increase vaccine uptake and better serve this population.

4.6 Acknowledgements

The authors would like to thank the study participants, as well as the VALUE ambassadors and service coordinators who helped with recruitment. This work was supported by a NIH/National Institute on Aging Specialized Center of Research Excellence U54 AG062333 awarded to S.L.K. J.R.S was supported by a training award from the Fonds de recherche du Québec – Santé (File #287609).

4.7 Tables

Table 4.1 Participant demographics

	Black Women	Black Men	White Women	White men
N	6	5	6	7
Age - mean	80	79	83	80
Resident of senior's building - N	5	3	2	3
Location of COVID-19 vaccine - N				
Senior's building	5	2		
Mass vaccination cite	1	2	6	5
Pharmacy		1		
Veterans Affairs				2
Median household income[a] - mean	38,651	47,848	83,264	79,721
% 65+ living in poverty[a] - mean	20.9	18.9	8.5	11.2
Highest level of education - N				
Some high school		1	1	
High school/GED		1	2	2
Some college/post-secondary	3	1		1
Associates/vocational degree	3			
College		1	2	1
Some post-graduate			1	
Post-graduate				3
Unknown		1		
Typology				
Eager complier (EC)	2	3	5	6
Hesitant complier (HC)	4	2	1	1

[a] Estimated by linking zip codes to census data

Table 4.2 Factors that fostered trust in the vaccine among hesitant compliers

Recommendation from healthcare professional
I had to ask my doctor, do you think I should take it, because my other shots didn't work out. And he said, no, it doesn't have the same things in as the flu shot [has] in it. He said it had different medication in it or whatever. So, I said, "well, I'll try it". (120_BM)
Recommendation from government
I would say more that Hogan [the governor] made the difference… how he cared about his people… he was so adamant with making sure that the people of Maryland got the shot and took care of themselves with it. (103_BW)
Media coverage
Well, they certainly publicized it more, for one thing. I mean, you never see the television monopolize every single day by one thing like this. You never see the flu…so it definitely had me concerned somewhat if it's that serious, if it's something to pay attention to. So, it has its effect. You are reminded of it every day. (1225_WM)
For a while, I said I wasn't going to get it and then, I saw an old Black lady on TV... She was an elderly lady, older than me. I believe she was in her nineties, and she was getting, I think they said she got the first shot, I think. And she gave me courage and I said wow. If she is going through with it, I think I can do it too. (101_BW)
Community
Well, I have been fortunate enough to have great grandchildren and I love them to death. I wanted to be able to see them and I wanted them to be able to visit me. So, any precautions I can do to help them, I am going to do. (101_BW)
Lived experiences
Well, I am a child of the fifties and sixties. So, we received vaccinations on the regular for school, etcetera. So, I already had a mindset that vaccines were good. (102_BM)
Faith
It is an unknown thing but step out on your faith. Believe that the technicians and everybody that has handled it before… At least one of them got to know something about the good lord. (101_BW)

Table 4.3 The impact of gender, race, and their intersection on vaccine decision-making

Gender
• The women in our sample were more likely to see the vaccine as beneficial to their community and families than the men. • Women were more likely to note physical barriers to accessing the vaccine (i.e., being wheelchair-bound and dependent on others, being unable to stand in line).
Race
• More Black participants were classified as HC than White participants. • The Black HC noted more personal reasons for hesitancy (i.e., fear of side effects, interaction with their comorbidities), whereas the White HC presented external reasons for hesitancy (i.e., concerns about mRNA technology, lack of need for the vaccine) • For the White HC in our sample, perceived risks of the vaccine were entirely associated with the vaccine product, while for some of the Black HC, risks were associated with the systems that developed and provided the vaccines. • For all the Black HC in our sample, risks associated with the vaccine remained prominent, despite their ultimate decision to get the vaccine. • More Black participants consulted their doctors about receiving the vaccine, while White participants were more likely to make the decision without their doctor's input. • Several White participants reported that their healthcare system had reached out to them with information about the vaccine and opportunities to receive it, while none of the Black participants reported this.
Intersection of gender and race
• Black women in our sample were particularly concerned about unknown long-term consequences associated with the vaccine. • The White men who participated were more readily willing to assume the risk of a rare adverse event associated with the vaccine. • Faith was important in fostering trust in the vaccine for several of the Black women. • Several white women had confidence in the COVID-19 vaccine because of their positive experience with the polio vaccines.

Abbreviations: EC: eager complier; HC: hesitant complier.

4.8 Figures

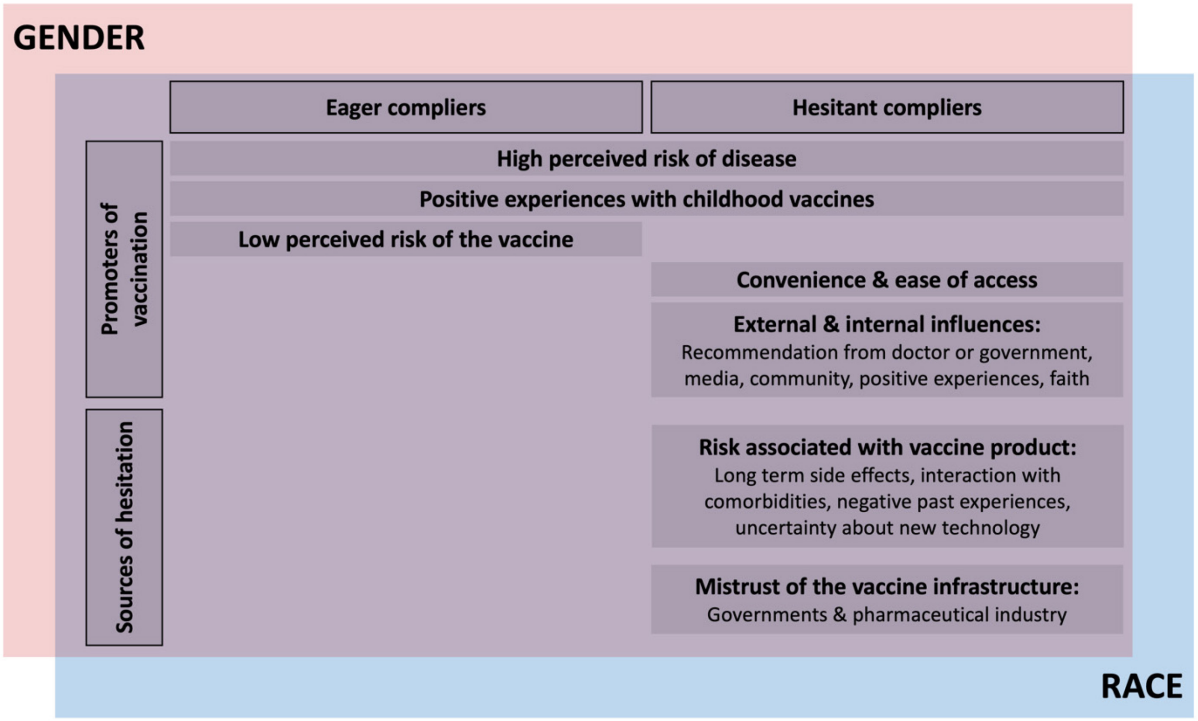


Figure 4.1 Factors that contributed to the decision to vaccinate for eager and hesitant compliers.
The promoters of vaccination and the sources of hesitation are summarized for the eager and hesitant compliers. The diagram is positioned at the intersection of gender and race to demonstrate that the process through which individuals approach the decision to vaccinate cannot be disassociated from their lived experiences, which are fundamentally shaped by gender and race.

CHAPTER 5

GENERAL DISCUSSION

Janna R. Shapiro

5.1 Introduction

The research in this dissertation illustrates the importance of considering sex and gender in vaccine research and highlights the benefits of an intersectional approach to studying vaccines in older adults. This type of work requires inter-disciplinarity, as well as thorough consideration of how to recruit and retain representative populations of older adults in clinical research. The findings presented here have important implications for measuring the success of vaccination – whether it be from an immunological perspective or in terms of the design of vaccine programs – and suggest that more tailored approaches to vaccinology would better serve older adults.

5.2 Methodological considerations

5.2.1 Inter-disciplinarity

Research is often siloed, yet there is tremendous added value to an inter-disciplinary approach, especially in the context of vaccinology. Vaccines sit at the intersection of science and society, in that the success of any vaccine program relies on effective vaccines for the target population, accessible delivery systems, and willing recipients. Thus, to comprehensively study vaccines, one must consider a diverse array of techniques and approaches. Accordingly, this dissertation employed laboratory, quantitative, and qualitative methods to understand the role of sex and gender in vaccine outcomes in older adults. While inter-disciplinarity adds richness and depth to research, combining methodologies also requires careful consideration.

For Chapters 2 and 3, analyzing laboratory data with complex statistical models allowed for extracting the full value from the longitudinal study designs. For example, in Chapter 2,

mixed effects models allowed us to combine data from repeatedly vaccinated participants over a six-year period, while accounting for the lack of independence between repeat measures of humoral immunity and missing data from individuals who did not participate in all six years. In Chapter 3, the humoral response to vaccination was measured in multiple ways (i.e., antibody binding, functional inhibition of ACE2, breadth relative to variants of concern and seasonal coronaviruses), while longitudinal data analysis allowed for interrogation of the effect of host factors at each timepoint and in trajectories over time. In both cases, the combination of immunological data and statistical methods yielded rich findings.

The qualitative work, presented in Chapter 4, was not directly integrated into the laboratory and quantitative findings, but allowed us to address a different aspect of vaccinating older adults. For a vaccine to have the desired effect, the target population must be able to access the vaccine and want to receive it. In other words, understanding sources of heterogeneity in immune response to vaccination is only useful if people are willing and able to receive the vaccine. Vaccine attitudes are complex to measure, as they are shaped by a variety of sources of information, depend on each individual's lived experiences, and are not necessarily represented by vaccine behavior (i.e., individuals who receive vaccines may have significant hesitations) (202, 203). Thus, qualitative methods are needed to obtain a nuanced and comprehensive understanding of how vaccine decisions are made. Importantly, qualitative methods also allowed for a more detailed study of how gender norms, roles, and relations contribute to vaccine decision-making. Therefore, although not directly integrated with the quantitative and laboratory work, the qualitative research presented in Chapter 4 complements the findings presented in Chapters 2 and 3.

5.2.2 Intersectionality

The theory of intersectionality proposes moving beyond individual factors, and instead, assessing how the interactions between factors create within-group differences that shape health outcomes (211). By focusing on both inter-group and intra-group differences, we can better understand the causes of inequities in global health (211). Intersectionality has been widely applied in social epidemiology, for example in studies of how race and gender interact to create different experiences of social privilege, and its utility in many health-related fields is increasingly recognized (237, 238). The few examples of the application of intersectionality to vaccinology have been in research on disparities in vaccine coverage. For example, in considering the intersectional roles of gender, race/ethnicity and sexual orientation, black lesbian women and girls were the least likely to initiate HPV vaccination in the US, suggesting a compounding effect of sexual orientation and race/ethnicity (239). Furthermore, a study by UNICEF proposed that gender inequities in immunization can be best understood and addressed using an ecological framework that takes into account the interaction of individuals, households, communities and systems (240). This methodology, however, has not been widely implemented in studies of vaccine behavior, and is rarely applied in vaccine immunology.

Intersectionality was a central premise of the three papers presented in this dissertation, but this lens was implemented differently depending on research methodology. In Chapters 2 and 3, intersectionality was implemented quantitatively, using interaction terms in regression models to determine if there were sex-specific effects of age or frailty. This allowed for interrogation of how outcomes differed not only between males and females, but also among males and females. In both cases, the most notable sex differences were in the effects

of age, rather than in the direct comparison of outcomes between males and females. In chapter 4, intersectionality was applied qualitatively, using thematic analysis to understand how interactions between gender and race contributed to vaccine decision-making. Following the theory of intersectionality, however, it must be noted that many factors beyond gender and race, such as socio-economic status and education level, likely also contributed to the processes under investigation. Across the three papers, intersectionality guided the design of research questions and research methodology.

5.2.3 Not controlling for sex

In much of the published literature, sex and/or gender are 'controlled' or 'adjusted' for in analyses. Controlling for sex or gender means treating these variables as confounding factors, rather than variables of importance to the research question. Technically, this usually means that a term was included in a regression model to account for the fact that sex, gender, or both might influence the predictor and the outcome, and possibly confuse the relationship under investigation. While this allows for sex or gender differences in the outcome at baseline, it also forces this difference to be the same at all levels of the predictor.

As demonstrated by the work in this dissertation, however, the true relationships between our predictors and outcomes can be modified by both sex and gender, such that there is considerable danger in ignoring sex and gender differences by controlling for them statistically. For example, in Chapter 2, analyses that controlled for sex revealed no significant association between age and pre-vaccination HAI titers. Analyses that allowed the effect of age to vary by sex, however, revealed that titers did decrease with age, but only in males.

Furthermore, models that included an interaction between sex and age were more robust and of higher statistical quality than those which controlled for sex. In this case, assuming that the effect of age was the same in males and females obscured a meaningful effect of age in males and would have led to the false conclusion that age was not important to consider when assessing the durability of vaccine-induced immunity. The approach of treating sex/gender as variables of importance, rather than variables that confuse research, was a central tenet of the research presented in this dissertation.

5.3 Sex differences in the aging immune system

In Chapters 2 and 3, the effects of aging on the immune response to vaccination were greater in males than females for both the seasonal influenza and COVID-19 vaccine. Since the influenza vaccine is inactivated and the COVID-19 vaccines were mRNA-based, the consistency of this finding across the diverse vaccine platforms points to fundamental sex differences in aging of the immune system. This observation is supported by several lines of basic immunology research. For example, age-related changes in the frequency of T and B cells occur more slowly in females than in males (69), and the effect of aging on the composition of the T cell compartment differs by sex (70). In addition, decreases in T cell production of inflammatory cytokines IFNγ and IL-17 that occur with age in males are not observed in females (71), and older females have heightened levels of GM-CSF, CRP and IL-5 relative to older males (72). In perhaps the most comprehensive study to date, an accelerated aging phenotype was observed in males, characterized by inactivation of B cell loci, a decline in B cell frequency, lower adaptive immune cell activity, and increases in inflammatory pathways associated with 'inflammaging' (67). Taken together, the literature suggests that the process of immune aging is more

pronounced and faster in males than in females and provides potential underlying mechanisms for the outcomes observed in Chapters 2 and 3.

5.4 Vaccinating older adults

5.4.1 Changing landscape

Immunization programs have traditionally focused on infants, particularly in the first year of life (241-243). These programs were conceptualized at a time when the global population was younger and there was a tremendous burden of vaccine-preventable diseases (VPD) in children under five (244). With population aging, however, the number of people above 65 years of age is expected double by 2050, and the number of people above 80 years of age is expected to triple (115). The WHO estimates that there will be 1.5 billion people 65 years or older by 2050, out-numbering children under five, adolescents, and youth (115). In addition to a growing population, the number of vaccines recommended for older adults has also increased (245), resulting in the global incidence of vaccine preventable diseases being higher in adults than children (244). The COVID-19 pandemic has amplified the burden of vaccine-preventable diseases in this population, and further highlighted the need for effective vaccines (246).

Vaccinating older adults has many benefits in addition to the direct prevention of infection. For example, the term 'vaccine-preventable disability' has been coined to refer to the role that vaccines play in protecting functional ability and quality of life in older adults (246). Accordingly, one of the priorities of WHO's immunization agenda 2030, a document outlining the global vaccine strategies for the coming decade, is to address immunization across the life course, with the objective of people of all ages receiving recommended vaccines (242).

Centered within the changing landscape of a growing older adult population, more available vaccines, and greater emphasis on vaccine programs, this dissertation provides insight into the approaches needed to recruit and retain older adult research participants, as well as innovative methods to measure the effects of aging.

5.4.2 Recruitment and retention of 'hard-to-reach' older adults

Despite the clear public health need to develop and implement vaccines for older adults, there are many challenges associated with vaccine research in this population. These challenges are perhaps best exemplified by the lack of representation of the 'oldest old' in many COVID-19 vaccine trials, despite a tremendous need for an effective vaccine in this population (247). For example, the initial efficacy estimate for BNT162b2 (the Pfizer vaccine) in those over 75 years of age was based on five cases in the placebo group compared to no cases in the vaccinated group, resulting in a confidence interval that spanned from -13.1% to 100% and significant uncertainty in the impact the vaccine would have in this vulnerable population (48). Many reasons have been cited in the literature to explain the underrepresentation of older adults in clinical research, including deliberate exclusion due to the presence of comorbidities and polypharmacy, access and mobility issues, and cognitive impairments interfering with informed consent (248, 249). Another challenge, that was particularly relevant to this dissertation, is that there are more older females/women than older males/men, due to sex differences in lifespan (250). For these reasons, older adults, particularly the oldest old and racial/ethnic minorities, have been defined as a 'hard-to-reach' population (251). The challenges associated with including older adults in research have been heightened during the pandemic, with many older adults fearful of interacting with others.

In the research described in this dissertation, multiple techniques were used to recruit and retain older adults as research participants. For the larger cohort used in Chapters 2 and 3, home visits were a highly effective tool to allow for representation of older and frailer participants who may have been unable to travel to study visits at a medical center. For the influenza vaccine study, providing the vaccine during a home visit was an added incentive. Furthermore, the longitudinal nature of the studies meant participants developed meaningful relationships with study staff. Particularly during the pandemic, when many older adults were isolated, the social contact provided by study visits helped with retention for some. For others, the pandemic deterred participation due to the risk of close contact with study staff. To accommodate this, in the 2020-2021 season, study procedures were modified to collect as much data as possible over the phone and limit in-person contact. While these modifications were successful in limiting the risk of COVID-19, they introduced additional challenges. For example, it was difficult to interact with participants with hearing deficits over the phone, and some grew frustrated and unengaged due to the length of the phone interviews and the increased contact with the study (i.e., what was previously one long visit was split into a shorter visit and a phone call). Despite these challenges, recruitment and retention remained relatively high for the 2020 and 2021 influenza seasons, as well as for the COVID-19 sub-cohort.

For the qualitative work, an emphasis was placed on recruiting approximately equal numbers of men and women and of Black and White participants. This proved to be quite difficult, however, due to unequal interest in the study. Upon sending flyers to several senior's homes in Baltimore, multiple women immediately expressed interest in the study, while few men did. This was particularly true of Black men, who were very difficult to recruit. Recruitment

was further complicated by the fact that senior's buildings were closed to visitors during the pandemic. To address these challenges, we continued to send flyers to residences and senior's centers and built relationships with the service coordinators at some buildings so they could help promote the study to residents. Finally, connections through Johns Hopkins' involvement in Baltimore's VALUE (Vaccine Acceptance & Access Lives in Unity, Education & Engagement) program facilitated referrals of participants.

In addition to recruitment efforts, in-person interviews were replaced with phone interviews in the context of the ongoing COVID-19 pandemic. Traditionally, phone interviews were not the preferred method for collection of qualitative data, due to the absence of visual and non-verbal cues, lack of depth in data collection, and issues with phone coverage and clarity of recordings (252). In the context of the pandemic, however, many have noted the need to switch to alternate methods of data collection to protect both study participants and staff, despite potential complications (253, 254). While there were occasionally issues with phone signal and poor connection quality making it difficult to understand the participants, being able to complete the study from the comfort of their homes largely acted as an incentive to participate. Nearly all participants reported feeling vulnerable to COVID-19 and were apprehensive of in-person interactions. Furthermore, while it is impossible to know what data could have been collected during in-person interviews, the data collected by phone was rich and allowed for in-depth analysis.

Together, the recruitment and retention strategies employed constitute a comprehensive effort to include older adults in vaccine research, particularly certain under-

represented subsets of older adults, such as the oldest old (i.e., aged over 80 years), men, and racial minorities. While the cohorts employed in Chapters 2 and 3 lacked racial diversity, they did include many participants above 80 years of age, at various levels of frailty, and with diverse histories of comorbidities and polypharmacy. For Chapter 4, best efforts were made to obtain a sample that was more racially representative of the Baltimore area. Importantly, protocols were adapted to protect the health of study participants and staff during the pandemic, allowing for important research on diseases that are particularly lethal for older adults.

5.4.3 Frailty: moving beyond chronological age

There is increasing acceptance that chronological age does not always accurately reflect the functional status of older adults (255, 256), highlighting the need to measure the biological process of aging through biomarkers, epigenetic markers and deficit or frailty indices (256). These measures allow us to capture the heterogeneity of the aging process between individuals of the same chronological age (257). Frailty is conceptualized as a state in which older adults experience declines in physiological function across multiple organ systems, leading to increased vulnerability and decreased ability to cope with stressors (89). An estimated 15% of older adults (≥65) in the U.S. are considered frail, with an additional 45% considered pre-frail (258). Based on the prevalence of frailty and its association with poor health outcomes, the benefit of including frailty assessments in clinical research is increasingly clear (259).

There is active debate in literature about the relationship between frailty and vaccine responses. Frailty is associated with substantial changes to the immune system, including chronic low-grade inflammatory phenotype (CLIP) and alterations to the adaptive immune

system (90, 260, 261), suggesting theoretical mechanisms through which frailty may impair vaccine response. In Chapter 2, frailty was not associated with pre- or post-vaccination HAI titers in either males or females, nor was a sex difference in the impact of frailty observed. In contrast, in Chapter 3, there was a strong association between frailty and multiple measures of antigen-specific antibody responses, and this effect was greater in males than in females. The discrepancy between the two vaccines may be due to immune history. While there was substantial pre-existing immunity to influenza through a lifetime of exposure to infection and vaccination, there was no pre-existing immunity to SARS-CoV-2. This suggests that the effects of frailty are more prominent in *de novo* immune responses, such as the ones stimulated by the SARS-CoV-2 vaccines, than in the recall responses stimulated by the influenza vaccine. This hypothesis is also consistent with the sex difference observed in the effect of frailty. There is substantial evidence that females mount stronger *de novo* immune responses than males (5), suggesting that the robustness of the immune response in females may compensate for the effects of frailty. More research, with diverse vaccine platforms and antigens and large, representative study populations, is needed to better understand the role of frailty in vaccine responses.

5.5 Impact & implications

5.5.1 Precision vaccinology: One size does not fit all

A central finding of this dissertation is that older adults are a heterogeneous group that should not be treated as a single entity when it comes to vaccines. This was true for vaccine immunogenicity and vaccine uptake, with the overarching policy implication being that a ‘one

size fits all' approach to vaccinology does not equitably prevent disease in this population. Instead, a more precise approach would be beneficial.

In terms of the immunological response to vaccination, the data presented in Chapters 2 and 3 suggests that the effects of aging on vaccine responses are significantly greater for males than for females. Based on these findings, alternate vaccination strategies should be considered for older males. For influenza, although more data is needed, decreasing pre-vaccination titers with age in males suggest that antibody response induced be the previous year's vaccine are not sufficiently durable. A solution to this could be to offer older males mid-season influenza boosters, which would provide a boost in protection for the second half of the influenza season and prime males to respond better to the subsequent year's vaccine. Furthermore, this additional vaccine dose may contribute to eliminating the male-bias in influenza B infection and hospitalization observed at older ages (13). For COVID-19, older and frail males had lower antibody titers after the second vaccine dose, suggesting they may be vulnerable to breakthrough infections. These disparities were largely resolved through receipt of a third vaccine dose, highlighting the tremendous public health value of third vaccine doses for older adults, particularly older males.

The concept of tailoring vaccine strategies to specific sub-groups is not new. Precision vaccinology posits that instead of the traditional "isolate-inactivate-inject" paradigm, specific vaccines or vaccination strategies be employed to overcome factors associated with poor immunogenicity (262). The advent of vaccine products specifically for those above 65 years of age has followed this model to achieve better outcomes (262). The same cannot be said,

however, for host factors beyond age, although it has been suggested that vaccine doses and schedules for be altered for males and females (263). While development of new vaccine products for sub-groups is logistically complicated due to the costs of research and development, utilizing existing products in novel ways could have a large benefit.

5.5.2 Tailoring vaccine messages and programs to promote uptake

The investigation into how older adults made the decision to receive the COVID-19 vaccine presented in Chapter 4 also yielded several findings following the precision vaccinology philosophy. Importantly, many of the participants who had hesitations about the vaccine were in part persuaded by in-house vaccination clinics in their seniors' buildings. This program, implemented by the Baltimore City Health Department, was tailored to the needs of older adults, by removing the need to book vaccine appointments online and eliminating barriers caused by lack of transportation or reduced mobility. Importantly, participants noted that in addition to removing barriers, getting vaccinated in their buildings felt cleaner and safer. The success of this program in promoting vaccination is notable and suggests that expansions to deliver other vaccines recommended for older adults and to include those who do not reside in retirement communities or long-term care facilities would yield significant returns.

In addition, intersectional analyses revealed that sources of hesitation regarding the vaccine, as well as motivators to ultimately accept the vaccine, varied by both race and gender. This was particularly true for the of role external influences in the decision to vaccinate. For example, the role that getting vaccinated could play in protecting loved ones, as well as protecting themselves from acquiring COVID-19 from grandchildren, was valued more by

women than by men. Furthermore, Black women were the only sub-group that reported that their faith played a substantial role in fostering trust in the vaccine. These findings suggest that to increase vaccine uptake, targeted messages that address different types of hesitations surrounding the vaccine and leverage motivators that resonate with different sub-groups would be more effective than a general approach.

5.5.3 Incorporation of sex and gender in vaccine research

Historically, biological sex was not considered as a variable of importance in clinical research, and women/females were often excluded as research participants (264). While regulations stemmed from attempts to prevent fetal harm, and were restricted to women of child-bearing age, policies quickly resulted in women of all ages being excluded from clinical research for much of the 1970's and 1980's, until NIH policies began to change in the early 1990's (264, 265). Several decades later, however, challenges remain to inclusion of sex as a biological variable, and considerations of gender are even further behind (266). This is evident in much of the published vaccine research, where sex/gender are either controlled for or ignored altogether (267). The research presented in this dissertation, however, validates the importance of considering sex and gender as fundamental modifiers of vaccine outcomes. In all chapters, the inclusion of sex/gender added depth, nuance, and novelty to the research.

These lessons can be widely applied to all areas of public health research. Instead of controlling for sex and gender – be it statistically or in the application of an intervention – the quality of our work can be improved by considering sex and gender as variables of importance that can explain, rather than confuse, research. A first, and necessary, step is to disaggregate

data to interrogate how sex and gender intersect with each other or with the predictors and outcomes under investigation. Disaggregation of data is a trigger for sex- and gender-responsive research that allows for understanding how the true relationship between a predictor and outcome differs between males and females or between men, women, and gender minorities (268). This avoids the pitfalls and unintended consequences of ignoring sex as a biological variable and gender as a social variable and adds richness and depth to the field of global health, which undoubtedly benefits the populations we serve.

5.6 Limitations

This work is not without limitations. While the study population in Chapters 2 and 3 were diverse in terms of age and frailty, they were not racially diverse. The overwhelming majority of participants were White, in stark contrast to the population of Baltimore city, which is 30% White, 62% Black and 5% Hispanic or Latino (223). Significant effort was spent on addressing this issue in Chapter 4, but difficulties recruiting Black men resulted in continued under-representation of Black Americans. Lack of racial diversity limits the generalizability of conclusions drawn from the data collected.

Chapter 2 was a secondary data analysis, meaning that the study was not designed to investigate sex differences or sex-specific effects of aging. Therefore, the sample size was not powered to test these hypotheses. While sex differences in the effect of aging did emerge despite potential issues with sample size, no sex-specific effects of frailty were observed. It is possible that sex differences do exist but were not observed in this study due to lack of statistical power and small sample sizes, particularly of frail participants. While the COVID-19

vaccination study presented in Chapter 3 was conceptualized with the aim of understanding sex differences in vaccine responses, the sample was one of convenience drawn from the larger influenza study cohort, meaning that many of the same issues persisted. Due to the nature of this work, conclusions should be viewed as hypothesis-generating, and additional studies that are specifically designed to test these hypotheses are needed to validate findings.

Furthermore, Chapters 2 and 3 relied on serological measures of the vaccine response. For influenza, HAI titers are the gold standard, but lack the functional quality of neutralization assays (164). In addition, measures of cell-mediated immunity were not included. As opposed to circulating antibodies, PBMC can provide deeper insight into vaccine-induced immunological memory and predict the magnitude and quality of the response upon viral challenge (269). While the available humoral data provides important insight into vaccine-induced responses, other sources of data are needed to understand which sub-groups are at elevated risk of breakthrough infections following vaccination.

For the qualitative work presented in Chapter 4, an important limitation is that all participants were vaccinated against COVID-19. This allowed us to study the factors that led to the decision to receive the vaccine among hesitant individuals and to document vaccine hesitancy that remains despite being vaccinated, but did not allow us to investigate sources of hesitation among individuals who refused vaccination. Interviewing unvaccinated individuals could have provided additional insight, particularly in understanding the perceived risks associated with the vaccine and mistrust in the vaccine infrastructure.

5.7 Recommendations for future research

Based on the findings and limitations of the work presented in this dissertation, there are several avenues for future research. These include running additional assays and analyses on the samples and data collected from the studies described, and additional studies to validate and expand on the conclusions.

In terms additional assays, the data presented in Chapter 2 should be accompanied by neutralizing antibody data, to determine if the patterns observed hold true for functional humoral responses. Furthermore, there are many avenues that could be pursued using the peripheral blood mononuclear cells (PBMC) collected during the influenza and COVID-19 studies to determine if other aspects of the immune response follow similar trends by sex and age. Since the inactivated influenza vaccines used in the study have a low capacity to induce CD8+ T cell responses (270, 271), flow cytometry analysis of PBMC should focus on quantifying the frequencies of diverse B cell populations (i.e., plasmablasts, memory B cells and age-associated B cells), which would help uncover the mechanisms for the greater effects of age seen males. It may also be of interest to study CD4+ helper T cell subsets, including memory CD4+ T cells, which are important in promoting the B cell response (272). For the samples collected post SARS-CoV-2 vaccination, B cell, CD4+ T cell, and CD8+ T cell populations are all of significant interest due to the broad immune response elicited by mRNA vaccines (273-275) and may be age-dependent (77). Techniques could include IFNγ ELISpots to measure the frequency of T cells secreting IFNγ following *ex-vivo* stimulation, or flow cytometry to measure frequencies of antigen-specific memory B cell and T cell subsets. For both studies, identification of age-associated B cells is of particular interest, as they may play a role in abnormal B cell

responses observed at older ages (276). Little is known, however, about sexual dimorphisms in the development and impact of age-associated B cells in humoral responses to vaccination.

Additional samples could also be collected from existing cohorts. For the influenza study, a potential mechanism to explain decreasing pre-vaccination titers decrease with age in males is that vaccine-induced immune responses become less durable with age in males. To test this hypothesis, samples could be collected monthly following vaccination to study sex differences in the kinetics of waning immunity. For the COVID-19 study, following participants for several months after receipt of the third vaccine dose would reveal whether the declines in immunity observed after the second dose reoccur and support policy decisions regarding the need for a fourth vaccine dose in this population.

In terms of new study designs, for both influenza and COVID-19, it would be of interest to determine if the sex-specific effects of age and frailty hold true across the lifespan. To answer these questions, a larger cohort of adults at all ages would be needed. In this context, it would be particularly interesting to use non-linear models to study sex differences in how vaccine-induced immunity changes with age and to determine if the pattern of abrupt age-related changes occurring earlier in men that was observed in PBMC profiling holds true in a more applied scenario (67). In terms of the effects of frailty, studies of vaccines with diverse platforms and for which the role of pre-existing immunity is understood (i.e., the herpes zoster or pneumococcal vaccines) would contribute to elucidating the mechanisms through which frailty impacts vaccine responses and explain the discrepancy between the influenza and COVID-19 vaccines.

Following the qualitative work on the vaccine decision-making process described in Chapter 4, there are several directions for future research. First, although the analysis presented above focused on the decision to vaccinate, other important themes emerged from the interviews, including where older adults sought out information about vaccines and public health measures during the pandemic, and how older adults confronted vaccine hesitancy in their families. Understanding the intersectional roles of gender and race in these contexts would provide valuable insight into how to best reach older adults with public health messaging. Beyond the data already collected, additional qualitative studies could expand to include other racial or ethnic groups, unvaccinated individuals, and participants from other geographical areas. Using a mixed-methods approach, the findings presented above could also be used to inform the design of surveys to validate and better understand how findings could be implemented in public health programs. For example, this could include a formal evaluation of the in-house vaccination clinics offered in senior's buildings by the Baltimore City Health Department, or quantitative research to understand if the factors determined to contribute to the decision to vaccinate among hesitant individuals in our sample hold true in other contexts.

5.8 Conclusion

In conclusion, the work presented in this dissertation demonstrates the importance of untangling heterogeneity in vaccine outcomes to achieve equitable prevention from infectious diseases. This pursuit requires inter-disciplinarity, and consideration of factors that are often ignored in vaccine research. For both the influenza and COVID-19 vaccines, previously overlooked effects of sex and gender on vaccine uptake and immune responses could be

leveraged to design better vaccines, vaccine strategies, and vaccine programs that meet the needs of all older adults.

APPENDICES: PREFACE

The following section includes published manuscripts to which I made substantial contributions as first or co-first author, but that fell outside the scope of this dissertation. This includes:

AP1. Sex-specific effects of age and body mass index on antibody responses to seasonal influenza vaccines in healthcare workers. Research article, *Vaccine 40(11)*

AP2. Adaptive immune responses in vaccinated patients with symptomatic SARS-Co2-2 Alpha infection. Research article, *JCI Insight 7(5)*

AP3. Stop 'controlling' for sex and gender in global health research. Commentary, *BMJ Global Health 6(4)*

AP4. COVID-19: Use intersectional analyses to close gaps in outcomes and vaccination. Correspondence, *Nature 591(7849)*

APPENDIX 1

SEX-SPECIFIC EFFECTS OF AGE AND BODY MASS INDEX ON ANTIBODY RESPONSES TO SEASONAL INFLUENZA VACCINES IN HEALTHCARE WORKERS

Helen Kuo*, Janna R. Shapiro*, Santosh Dhakal, Rosemary Morgan, Ashley L. Fink, Hsuan Lui, Jason W. Westerbeck, Kristyn E. Sylvia, Han-Sol Park, Rebecca L. Ursin, Patrick Shea, Kathryn Shaw-Saliba, Katherine Fenstermacher, Richard Rothman, Andrew Pekosz, and Sabra L. Klein

*Co-first authors

Vaccine 2021

AP1.1 Abstract

Healthcare institutions with mandatory influenza vaccination policies have over 90% vaccination rates among healthcare workers (HCWs) resulting in a population that has received the influenza vaccine in many, consecutive years. This study explored the impact of sex and other host factors in pre- and post-vaccination neutralizing antibody (nAb) titers and seroconversion against the H1N1 and H3N2 influenza A viruses (IAVs) among HCWs enrolled into a cross-sectional serosurvey during the annual Johns Hopkins Hospital employee vaccination campaign in the 2017-18 and 2018-19 seasons. The study enrolled 111 participants (male=38, female=73) in 2017-18 and 163 (male=44, female=119) in 2018-19. Serum samples were collected immediately prior to vaccination and approximately 28 days later and nAb titers to vaccine strains determined. An intersectional approach was used to disaggregate the combined effects of sex with age and body mass index (BMI) in the nAb response. Differences between the pre- or post-vaccination geometric mean nAb titers between male and female HCWs were not observed. Male HCWs were 2.86 times more likely to seroconvert compared to female HCWs in 2017-2018, but the same trend was not observed in the following year. When data were disaggregated by age and sex, older female HCWs had higher H1N1 pre- and post-vaccination nAb titers compared to male HCWs in the same age group for both vaccination campaign seasons. In both years, the decline in H3N2 pre-vaccination titers with increasing BMI was greater in female than male HCW. The sex-specific effects of age and BMI on nAb responses to seasonal influenza vaccines require greater consideration.

AP1.2 Introduction

Seasonal influenza epidemics affect 5-15% of the world's population, and the World Health Organization (WHO) attributes 290,000-650,000 annual, global deaths to influenza (117, 277). Healthcare workers (HCWs) are at an increased risk of contracting influenza due to occupational exposure and they can also transmit the virus to patients who have a higher risk of developing severe influenza. The Centers for Disease Control and Prevention (CDC) recommends annual influenza vaccination, with special provisions for HCWs who are directly or indirectly involved in patient care and additional emphasis on the importance of influenza vaccination during the COVID-19 pandemic (278). Healthcare institutions that have mandatory vaccination policies in place have over 90% vaccination rates among HCWs (279), and high rates of vaccination have translated to HCWs receiving many consecutive influenza vaccinations. Previous reports indicate that HCWs with ≥4 previous influenza vaccines have higher pre-vaccination antibody titers compared to first time vaccinees, and the post-vaccination antibody titers are inversely proportional to the pre-vaccination titers (280). Other studies have reported similar findings, where previously vaccinated HCWs were less likely to mount as robust of a response as naïve HCWs receiving the vaccine for the first time due to higher pre-vaccination titers (281-283).

Previous studies illustrate that age and body mass index (BMI) can be determinants of the magnitude of an influenza vaccine response (74, 284-286). Immunosenescence, which refers to the age-associated decline in immune response, has been shown to impact immunity to seasonal influenza vaccines among older adults (74, 284). Older HCWs (age 49-64) are reported to have significantly lower H1N1 pre-vaccination antibody levels compared to younger

HCWs (age 20-48) (287), but with no consideration of the sex of the HCWs. Obesity (i.e., body mass index [BMI]> 30%) also is associated with impaired immune response to the influenza vaccine, which is correlated with a greater decline in the antibody titer to the seasonal influenza vaccine over time (288).

Sex differences in the antibody response to the seasonal influenza vaccine have also been reported, with females generally developing greater antibody responses to seasonal influenza vaccines than males (5, 72, 74, 284, 289, 290). Also, female vaccinees are reported to have greater median pre-vaccination antibody titers than male vaccinees (23), suggesting that females already have elevated antibody responses to influenza prior to receipt of the annual vaccine. Sex differences in the antibody responses to the seasonal influenza vaccine among highly vaccinated HCWs have not been explored, to date.

Females account for 76% of HCWs according the United States Census Bureau, with healthcare occupations projected to increase rapidly in the next four years due to an aging population with greater demand on the healthcare system (291, 292). As more females enter the healthcare workforce, they are more likely to have direct patient contact, which increases risk of exposure to influenza viruses. It is also estimated that more than one tenth of the world population is considered obese, and overweight or obese adults comprise more than two thirds of the US adult population (288). This study explored sex differences in HCWs pre- and post-vaccination neutralizing antibody (nAb) titers and seroconversion against the H1N1 and H3N2 influenza A virus (IAV) vaccine strains after the administration of inactivated influenza vaccine during the 2017-18 and 2018-19 seasons. We also investigated how sex intersects with other

stratifiers, such as age and BMI, to influence the antibody response in HCWs who have received multiple consecutive years of seasonal influenza vaccination.

AP1.3 Methods

AP1.3.1 Study design

This study was a cross-sectional serosurvey, with HCWs recruited from the Johns Hopkins Centers of Excellence for Influenza Research and Surveillance (JHCEIRS) during the annual Johns Hopkins Hospital (JHH) employee influenza vaccination campaign in the 2017-2018 and 2018-2019 influenza seasons. The JHH Institutional Review Board approved the study.

AP1.3.2 Participant eligibility

All HCWs, at least 18 years or older, who visited the vaccination clinic prior to receiving the influenza vaccine during the campaign were eligible to participate. HCWs who were unable to speak or write English, or unable to provide informed consent were excluded from participating in the study.

AP1.3.3 Study procedure

Informed consent was obtained from all HCWs prior to administering the influenza questionnaire, which included demographic information, employment status, influenza exposure, influenza vaccination history in the last five years, and medical history. Prior influenza vaccination information obtained during the interview was verified with JHH Occupational Health records. After completing the questionnaire, 10mL of whole blood was obtained and then the HCWs were vaccinated. HCWs were asked to return for a post-vaccination follow-up visit twenty-eight days later and 10mL of whole blood was obtained.

AP1.3.4 Influenza A virus vaccine strains

In the quadrivalent inactivated influenza vaccines administered at JHH, the 2017-2018 influenza vaccine contained A/Michigan/45/2015 (H1N1) and A/Hong Kong/4801/2014 (H3N2) IAVs. The 2018-2019 influenza vaccine contained A/Michigan/45/2015 (H1N1) and A/Singapore/INFIMH-16-0019/2016 (H3N2). The A/Michigan/45/2015 (H1N1) and A/Hong Kong/4801/2014 (H3N2) vaccine strains were provided by Dr. Doris Bucher at New York Medical College. The A/Singapore/INFIMH-16-0019/2016 (H3N2) vaccine strain was generated using infectious clone technology (293) and was a recombinant virus encoding the HA (GSIAD accession # EPI868856) and NA (GISAID accession # EPI868855) sequences of A/Singapore/INFIMH-16-0019/2016 IVR-186 along with the 6 internal segments of A/Victoria/361/2011. Madin-Darby canine kidney (MDCK) cells were infected with vaccine viruses diluted in infection medium (IM) consisting of Dulbecco modified Eagle medium (Sigma), 0.2% bovine serum albumin (Sigma), 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco), and 2 mM GlutaMAX (Gibco) at 37°C and 5% CO2. Infectious virus titers were determined using a 50% tissue culture infectious dose (TCID50) assay (294).

AP1.3.5 Microneutralization assay

Serum samples were diluted with receptor destroying enzyme (RDE, Denka Seiken) at 1:3 ratio and incubated overnight at 32°C followed by heat inactivation at 56^0C for 35 minutes. Samples were 2-fold serially diluted in IM, mixed with 100 $TCID_{50}$ of each virus, and incubated at room temperature for 1 hour. The virus/serum mixture was transferred in duplicate into the 96-well cell culture plates containing confluent Madin-Darby Canine Kidney (MDCK) cells and incubated at 32°C. After a 24 hour incubation, plates were washed once with 1X PBS, fresh IM

was added, and cells were incubated for 6 days. Plates were fixed with 4% formaldehyde, stained with naphthol blue black solution, and scored as described previously (74).

AP1.3.6 Statistical Analyses

Neutralizing antibody (nAb) titers were log2 transformed and geometric mean titers (GMT) were reported for comparison by sex, age, BMI, and seroconversion. Seropositivity was defined as a ≥40 antibody titer. Seroconversion was defined as at least a four-fold nAb increase between post and pre-vaccination antibody titers. The study enrolled 111 participants (male=38, female=73) in 2017-18 and 163 (male=44, female=119) in 2018-19. Differences between those who seroconverted and those who did not, as well as between male and female HCWs, at each time point for each strain and between pre- and post-vaccination titers were calculated using two-tailed t-tests. Multiple logistic regressions were used to assess the impact of sex, obesity (i.e., BMI > 30%), age, race, comorbid conditions, and pre-vaccination nAb titers on the odds of seroconversion. Unadjusted simple linear regression models were used to determine the effect of age and BMI on pre and post-vaccination antibody titers, in the whole population and separately for males and females. All analyses were performed in Stata 15 (College Station, Texas). A $p<0.05$ was considered statistically significant.

AP1.4 Results

AP1.4.1 Healthcare worker characteristics

The study enrolled 128 participants during the 2017-18 season with 111 (86.7%) HCWs completing both baseline and 28-day post vaccination visits. Of the 111 who completed both visits, 38 (34.2%) were male and 73 (65.8%) were female. During the 2018-19 season, 200

participants were enrolled with 163 HCWs completing both visits (81.5%), and of these 44 (27%) were male and 119 (73%) were female. The HCWs who were lost to follow-up at the 28-day post-vaccination visit were excluded from the analyses. **Table 1** summarizes the demographic characteristics of the study population, and the characteristics of those lost to follow-up are described in **Supplemental Table 1**.

In the 2017-18 season, 73.0% of all HCWs (63.2% of males and 78.1% of females) reported receiving influenza vaccines consecutively in five previous seasons. In the 2018-19 season, 74.2% of all HCWs (61.4% of males and 79.0% of females) reported receiving five previous vaccines. In both study years, the majority of HCW participants were Caucasian females: 62.1% in 2017-18 and 60.8% in 2018-19.

AP1.4.2 HCW who seroconvert have lower pre-vaccination titers

In this study, seroconversion was defined as at least a four-fold increase between pre- and post-vaccination nAb titers, which was used as an indirect indictor of protection after vaccination. Seroconversion, however, is biased when individuals have high pre-vaccination titers, as they may not mount a four-fold rise in titer that is within the detection limits of the assay. Overall, the majority of HCWs did not seroconvert in either season; 61.3% of HCWs in 2017-18 and 72.4% of HCWs in 2018-19 did not seroconvert for H1N1, and 70.3% (2017-2018) and 66.3% (2018-2019) of HCWs did not seroconvert for H3N2 (**Table 2**).

The presence of nAb titers of >1:40 dilution is associated with protection from infection and we classified those individuals as being seroprotected (295). Pre-vaccination, 86.5% and 93.9% of HCWs in the 2017-2018 and 2018-2019 seasons, respectively, were classified as

seroprotected for H1N1 and 96.4% and 92.6% of HCWs were classified as H3N2 seroprotected in each respective study season (**Table 2**). Post-vaccination, 97.3% and 100% of HCWs were classified as seroprotected for H1N1 and 100% and 98.8% were protected for H3N2, in each respective study season.

The pre-vaccination nAb titers to H1N1 were greater among those who did not seroconvert compared to those who did in both seasons (**Figure 1A-B**). The H3N2 pre-vaccination titers were also greater for non-seroconverted HCWs in the 2017-2018 and 2018-2019 seasons, compared to HCWs who seroconverted (**Figure 1C-D)**. For both seasons and both strains, there was a significant increase in nAb titers from pre- to post-vaccination among those who seroconverted. Among those who did not seroconvert, there was only a significant increase in nAb titer against H1N1 in the 2018-19 season.

AP1.4.3 The odds of seroconversion against the H1N1 and H3N2 vaccine viruses in HCWs are affected by pre-vaccination titers

We next examined the impact of sex, BMI, age, race, and the presence of comorbidities on the odds of seroconversion using multiple logistic regression models (**Figure 2**). In the 2017-18 season, sex was a significant determinant of the odds of seroconversion for the H1N1 virus, in which male HCWs were 2.86 times more likely to seroconvert to the H1N1 vaccine strain than female HCWs (**Figure 2A**). This male-bias, however, was not observed in seroconversion against the H3N2 virus in the 2017-18 season nor for seroconversion against either the H1N1 or H3N2 vaccine viruses in the 2018-19 study season (**Figure 2B**). The other host factors of interest did not have a significant impact on the odds of seroconversion.

Unlike the demographic factors, pre-vaccination titers were significant predictors of the odds seroconversion to H1N1 (**Figure 2C)** and H3N2 (**Figure 2D**) for both males and females in the 2017-18 and 2018-19 seasons. In each case, the odds of seroconverting were 0.46 - 0.65 times lower for each one unit increase in pre-vaccination nAb titer.

AP1.4.4 Sex alone is not a determinant of seroconversion H1N1 or H3N2 IAVs

Sex differences have been reported in previous influenza vaccine studies (72, 74, 284); therefore, sex was explored as a possible determinant of pre- and/or post-vaccination titers among HCWs (**Table 2**). In the 2017-18 season, more male HCWs (55.3%) seroconverted to the H1N1 vaccine virus compared to female HCWs (30.1%). In addition, a higher percentage of male HCWs (31.6%) seroconverted to the H3N2 vaccine virus compared to female HCWs (28.8%). In the 2018-19 study season, however, the trend reversed slightly where more female HCWs (29.4%) seroconverted to the H1N1 vaccine virus compared to male HCWs (22.7%), and more female (37.8%) than male (22.7%) HCWs seroconverted to the H3N2 vaccine virus. In both seasons, there were no significant differences between male and female HCW in the pre- or post- geometric mean nAb titer against either the H1N1 or H3N2 vaccine viruses (**Figure 3**). In general, both males and females mounted a response to the vaccine, with post-vaccination titers being significantly higher than pre-vaccination titers.

AP1.4.5 Age intersects with sex to impact nAb titers in HCWs

Previous studies have demonstrated that age is a determinant of both pre- and post-vaccination antibody titers (74, 287). Pre- and post-vaccination nAb titers against the respective seasonal H1N1 and H3N2 vaccine viruses were disaggregated by age and sex to explore the

differential impact of age on vaccine-induced immunity in male and female HCWs. In general, age results in a decline in nAb responses among both male and female HCWs (**Figure 4**). During the 2017-18 season, female HCWs (slope=0.061 for H1N1 and 0.070 for H3N2) had a greater decline in pre vaccination nAb titers with age compared to male HCWs (slope=0.047 for H1N1 and 0.043 for H3N2) (**Figure 4A** and **E**). In post-vaccination nAb titers, male HCWs had a greater decline in H1N1 titers with age (slope=0.056) compared to female HCWs (slope=0.039) (**Figure 4B**), but no difference was observed in the decline of H3N2 titers with age between male and female HCWs (**Figure 4F**). In the 2018-2019 season, male HCWs had a greater decline in both pre- and post-vaccination titers for both H1N1 (slope=0.081 pre and 0.068 post-vaccination) and H3N2 (slope=0.092 pre and 0.070 post-vaccination) compared to female HCWs (slope=0.033 pre and 0.025 post H1N1 vaccination and slope=0.051 pre and 0.045 post H3N2 vaccination) (**Figure 4 C, D, G, H**). Taken together, these data suggest that even among adults below 65 years of age, increasing age is associated with an impaired ability to maintain an antibody response in the year following vaccination and to mount robust responses to the vaccine, and that this effect is modified by sex.

AP1.4.6 BMI intersects with sex to affect H3N2 nAb titers in HCWs

Obesity is known to impair immune response to the influenza vaccine and increase the severity of influenza symptoms (284, 296, 297). For both study seasons, BMI was not an independent determinant of either pre- or post-vaccination nAb titers against the H1N1 vaccine virus (data not shown). Because BMI affected nAb responses to H3N2 vaccine viruses, we further explored the intersection of BMI with sex on pre- and post-vaccination nAb titers against the H3N2 viruses. During each vaccine season, as BMI increased, the decline in pre-

vaccination nAb titers against H3N2 viruses was greater in female HCWs (slope=0.074 in 2017-18 and 0.078 in 2018-19) as compared to male HCWs (slope=0.043 in 2017-18 and 0.052 in 2018-19) (**Figure 5)**. Taken together, these data suggest that BMI has a greater impact on female HCWs' ability to maintain protective antibody levels in the year following immunization.

AP1.5 Discussion

Consecutive years of influenza vaccination does not appear to adversely affect protective influenza antibody titers as >86% of HCWs were seroprotected prior to vaccination and >97% were seroprotected after receiving their annual influenza vaccine. Among HCWs that have received annual influenza vaccinations for at least 5 consecutive seasons, sex alone does not influence pre or post H1N1 or H3N2 vaccination titers or the likelihood of seroconversion. Although we observed that male HCWs were 2.86 times more likely to seroconvert in the 2017-18 season against H1N1 vaccine virus, the same trend was not observed the following year. This is possibly due to the fact that pre-vaccination titers determined the magnitude of the nAb response to the influenza vaccine virus. In the 2017-18 influenza season the male HCWs had lower pre-vaccination nAb titers as compared to female HCWs, possibly increasing the odds of male HCWs seroconverting. In contrast, during the 2018-19 season, male HCWs had slightly higher pre-vaccination nAb titers compared to female HCWs, thereby decreasing the probability of seroconverting among males. It is likely that mandatory repeat vaccinations among HCWs have masked sex differences previously reported in non-HCW populations (72, 74, 284).

The primary predictor of seroconversion was pre-vaccination titers, with the odds of seroconverting decreasing significantly as pre-vaccination titer increased for both males and

females in both seasons. This is consistent with previous reports in highly vaccinated populations (280-283). Age was a significant predictor of pre- and post-vaccination nAb titers against both H1N1 and H3N2 viruses, across both influenza vaccine seasons. When nAb titers were disaggregated by age and sex, we observed that the impact of age on pre- and post-vaccination nAb titers was greater in males than in females in the 2018-19 study seasons, suggesting an age-associated sex difference in nAb titers among HCWs. For most vaccine studies, older age is defined as individuals 65+ years of age. In our study, we showed that declining immunity to QIV occurs among HCWs at a younger age (50-68 years), with the impact being greater for male than female HCWs.

Previous studies have reported that obesity contributes to lower immune responses to seasonal influenza vaccines and increased risk of developing more severe influenza symptoms (284, 296, 297). Higher BMI appears to contribute to a faster decline in the pre-vaccination nAb titers against H3N2, in particular, among HCWs in both study years. Our results suggest that BMI is an important factor associated with the maintenance of nAb against vaccine viruses. Overall, HCWs categorized as overweight or obese had lower pre-vaccination titers compared to normal weight HCWs. The decline in H3N2 pre-vaccination titers with increasing BMI was greater in female compared to male HCWs. The rationale for how BMI would affect neutralization of H3N2 but not H1N1 viruses is not known, but may reflect greater changes in recent years to the H3N2 as compared with the H1N1 components of seasonal influenza vaccines, which likely resulted in the greater seroconversion to H3N2 than H1N1 in HCWs. Taken together, our data supports the hypothesis that that age and BMI may have sex-specific effects on functional antibody responses to influenza A virus vaccine viruses. Increasing age is

associated with a greater decrease in nAb responses overall, whereas increasing BMI is associated with a greater decline in nAb responses in females.

Exploring influenza vaccine-induced immunity through intersectional analyses allows for a deeper understanding of how host factors intersect to impact the quality of the immune response. This study was specifically focused on exploring sex differences in combination with host factors that are well-documented to impact the immune response to the influenza vaccine. Although this study did not find that biological sex alone affected pre- or post-vaccination titers or seroconversion rates in HCWs, sex intersected with age and BMI to explain significant variation in the antibody responses to seasonal influenza vaccines. When vaccine strains remain constant from one year to the next, pre-vaccination nAb titers allowed us to interrogate the duration of the vaccine-induced immune response from the previous year and look at how male and female HCWs may be responding differently to repeat vaccinations, particularly among those above 50 years of age. In addition, as women constitute more than 76% of the healthcare workforce and this is projected to increase within the next four years, it is important to document how sex interacts with other variables to impact vaccine immunogenicity.

This study is not without limitations. The study is an annual observational cross-sectional survey and was not designed specifically to interrogate sex differences in the immune response to the influenza vaccine. The small cohort size did allow for definitive exploration of the effects of sex, age, and BMI as host factors that may affect vaccine efficacy. Further, the lack of racial and ethnic diversity of the enrollees as well as the predominance of females compared with males during the 2017-18 season, especially among older age and normal

weight enrollees must be acknowledged. In addition, due to the mandatory influenza vaccination policy at JHH, the study was not able to recruit unvaccinated HCWs for pre and post-vaccination nAb comparison. Furthermore, there was an H3N2 strain change during the 2018-2019 season; therefore, the 2018-2019 pre-vaccination titers did not necessarily measure H3N2 antibody titers from the previous year. The study also did not continue past the one-month post vaccination; therefore, the durability of the antibody responses was not assessed. The pre-vaccination nAb titers against H1N1 and H3N2 might suggest durability of antibody in the HCW population from previous years. Future studies will incorporate a 6 month follow-up time point to explore nAb durability and confirmation of protection. Finally, this study relied on nAb titers as a measure of immunogenicity, as opposed to hemagglutination inhibition titers, which is more commonly used to measure antibody responses to seasonal influenza vaccines. In conclusion, the impact of host factors, such as sex, is largely masked in the highly vaccinated HCW population. More in-depth intersectional analyses, however, revealed important interactions between sex, age, and BMI in the maintenance of antibody titers in the season following immunization, as well as the magnitude of the response to the seasonal influenza vaccine.

AP1.6 Author contributions

A.P., S.K., K.S-S., and R.R. conceived of the study; H.K., R.M., S.D., J.S., A.P., and S.K. wrote the manuscript; K.F. recruited participants and collected blood samples; H.L. and JWW grew viruses for nAb assays; S.D., ALF, KES, H-S.P., and R.U. processed samples for nAb titers; H.K., R.M., P.S., J.S. and S.D analyzed and graphed data; all authors reviewed the manuscript, provided edits, and approved the final submission.

AP1.7 Acknowledgements

The authors thank the healthcare workers who enrolled and participated in the Johns Hopkins Center for Excellence in Influenza Research and Surveillance study. We are grateful for the efforts of the clinical coordination team JHH who collected samples. We also thank Sharvari Deshpande and Harish Narasimhan for technical assistance with assays.

AP1.8 Tables

Table 1
Characteristics of healthcare workers enrolled during the 2017–18 and 2018–19 influenza vaccine seasons at the Johns Hopkins Hospital.

	2017–2018			2018–2019		
	Male	Female	Total	Male	Female	Total
N (%)	38 (34.2)	73 (65.8)	111	44 (27.0)	119 (73.0)	163
Age - med (IQR)	34 (28–44)	38 (30–52)		30 (27–37)	36 (30–47)	
Age Categories - n (%)						
19–49	33 (86.8)	51 (69.9)	84 (75.7)	38 (86.4)	98 (82.4)	136 (83.4)
50–68	5 (13.2)	22 (30.1)	27 (24.3)	6 (13.6)	21 (17.6)	27 (16.6)
Race - n (%)						
American Indian or Alaskan Native	0 (0.0)	0 (0.0)	0(0.0)	1 (2.3)	0 (0.0)	1 (0.6)
Asian	7 (18.4)	7 (9.6)	0(0.0)	5 (11.4)	8 (6.7)	13 (8.0)
Black or African American	6 (15.8)	13 (17.8)	14 (12.6)	8 (18.2)	28 (23.5)	36 (22.1)
White	21 (55.3)	48 (65.8)	19 (17.1)	28 (63.6)	76 (63.9)	104 (63.8)
Other	3 (7.9)	5 (6.8)	69 (62.2)	2 (4.5)	7 (5.9)	9 (5.5)
Unkown	1 (2.6)	0 (0.0)	8 (7.2)	0 (0.0)	0 (0.0)	0 (0.0)
BMI Categories - n (%)						
Normal	9 (23.7)	31 (42.5)	40 (36.0)	18 (40.9)	55 (46.2)	73 (44.8)
Over weight or obese	29 (76.3)	42 (57.5)	71 (64.0)	25 (56.8)	62 (52.1)	87 (53.4)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	2 (1.7)	3 (1.8)
Vaccination history categories - n (%)						
<5 previous vaccines	14 (36.8)	16 (21.9)	30 (27.0)	12 (27.3)	20 (16.8)	32 (19.6)
5 previous vaccines	24 (63.2)	57 (78.1)	81 (73.0)	27 (61.4)	94 (79.0)	121 (74.2)
Unkown	0 (0.0)	0 (0.0)	0 (0.0)	4 (9.1)	2 (1.7)	6 (3.7)
Missing	(.)	(.)	(.)	1 (2.3)	3 (2.5)	4 (2.5)
Ever smoked - n (%)						
No	(.)	(.)	(.)	42 (95.5)	104 (87.4)	146 (89.6)
Yes	(.)	(.)	(.)	2 (4.5)	14 (11.8)	16 (9.8)
Missing	(.)	(.)	(.)	0 (0.0)	1 (0.8)	1 (0.6)
Underlying conditions						
No	26 (68.4)	31 (42.5)	57 (51.4)	25 (56.8)	64 (53.8)	89 (54.6)
Yes	12 (31.6)	42 (57.5)	54 (48.6)	19 (43.2)	55 (46.2)	74 (45.4)
Patient contact						
No	32 (84.2)	44 (60.3)	76 (68.5)	23 (52.3)	46 (38.7)	69 (42.3)
Yes	5 (13.2)	29 (39.7)	34 (30.6)	21 (47.7)	68 (57.1)	89 (54.6)
Missing	1 (2.6)	0 (0.0)	1 (0.9)	0 (0.0)	5 (4.2)	5 (3.1)

(.) = smoking data not collected in the 2017–18 study season.

Table 2
Pre and post-vaccination geometric mean titer, seroprotection, and seroconversion rates in healthcare workers disaggregated by sex.

	2017–18			2018–19		
	All	Male	Female	All	Male	Female
H1N1						
Pre-vaccination GMT	310 (232–415)	248 (147–419)	349 (245–496)	676 (547–836)	905 (573–1431)	607 (479–770)
Post-vaccination GMT	886 (690–1136)	939 (613–1437)	859 (627–1176)	1418 (1185–1696)	1928 (1332–2791)	1265 (1032–1550)
Pre-vaccination SPR	86.5	84.2	87.7	93.9	95.5	93.3
Post-vaccination SPR	97.3	97.4	97.3	100	100	100
SCR	38.7	55.3	30.1	27.6	22.7	29.4
H3N2						
Pre-vaccination GMT	822 (621–1087)	782 (506–1210)	843 (584–1217)	524 (418–656)	573 (345–953)	506 (394–650)
Post-vaccination GMT	1782 (1409–2255)	1745 (1193–2554)	1802 (1329–2441)	1161 (963–1400)	1043 (682–1596)	1208 (984–1484)
Pre-vaccination SPR	96.4	97.4	95.9	92.6	88.6	94.1
Post-vaccination SPR	100	100	100	98.8	97.7	99.2
SCR	29.7	31.6	28.8	33.7	22.7	37.8

GMT = Geometric mean titer (reported as mean (95% CI)) ; SPR = seroprotection rate ; SCR = Seroconversion rate.
No statistically significant differences.

AP1.9 Figures

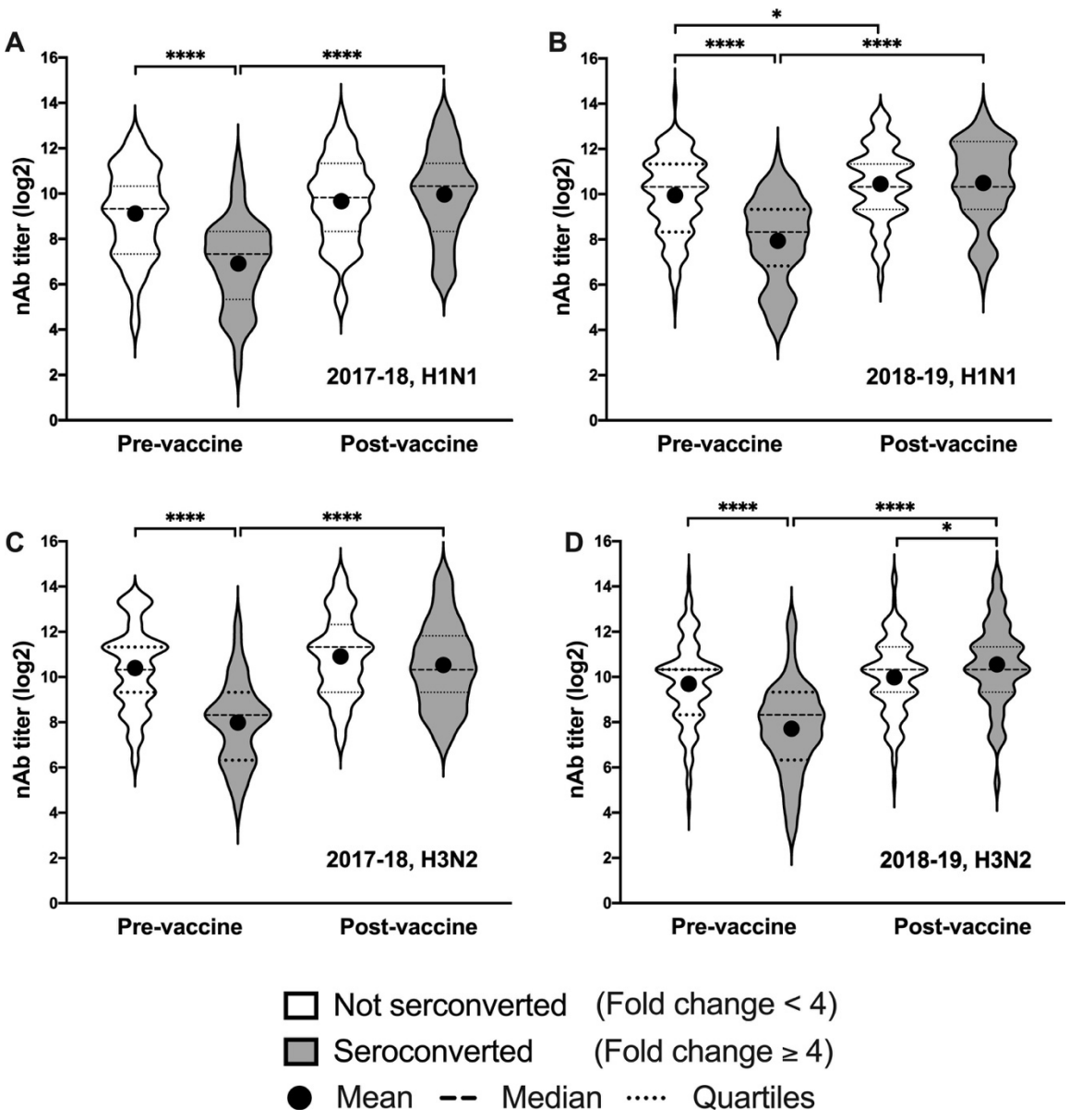


Figure 1: Healthcare workers (HCWs) who did not seroconvert had higher pre-vaccination titers than those who seroconverted. Log_2-transformed neutralizing antibody (nAb) titers pre- and 28 days post-vaccination are shown for HCWs who seroconverted (gray) and those who did not (white) to H1N1 in the 2017-18 season (**A**), H1N1 in the 2018-19 season (**B**), H3N2 in the 2017-18 season (**C**), and H3N2 in the 2018-19 season (**D**). Dashed lines indicate the median titer for each group and dotted lines indicated the 25th and 75th percentiles. Differences between those who seroconverted and those who did not at each time point for each strain and between pre- and post-vaccination titers were calculated using two-tailed t-tests. Asterisks indicate the significance level with * = p-value <0.05 and **** = p-value <0.0001.

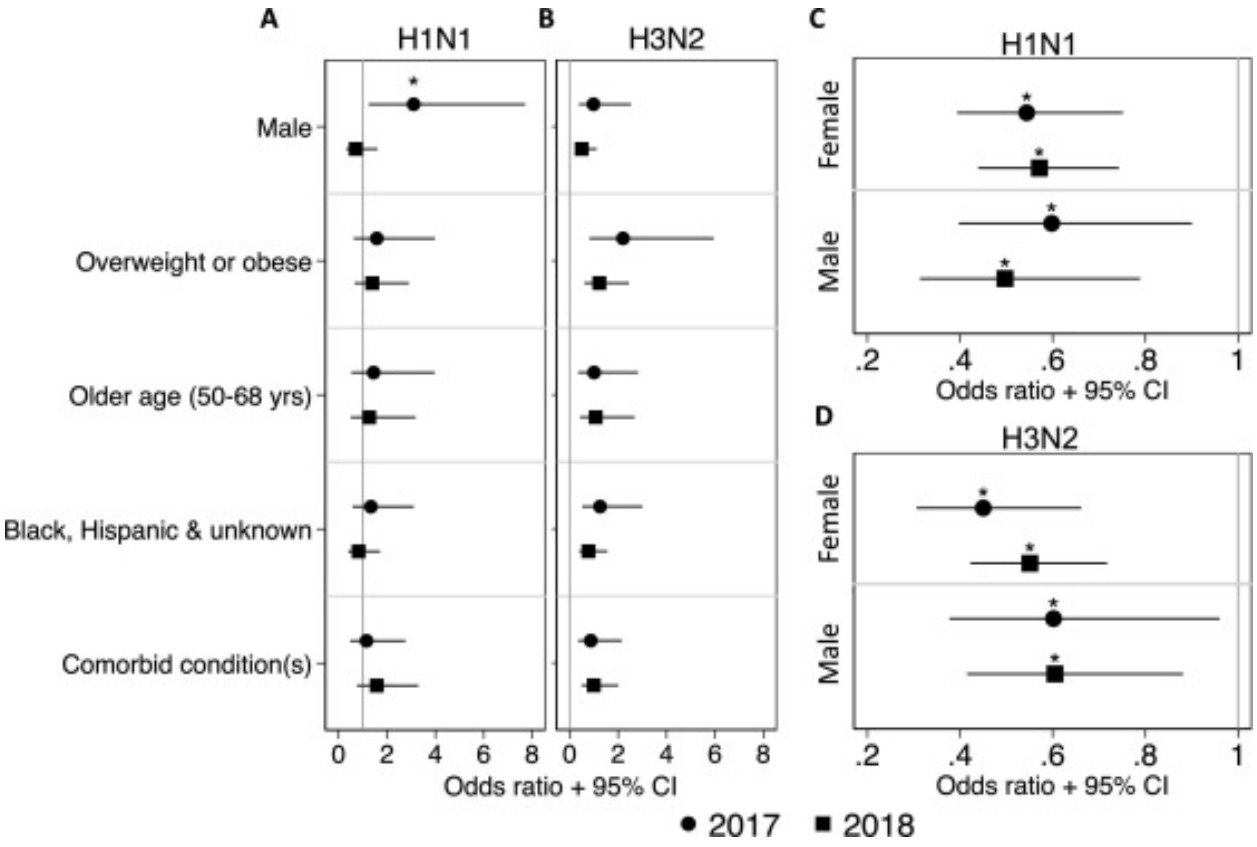


Figure 2: Odds of seroconversion are affected by pre-vaccination titers. Multiple logistic regression models were used to analyze the impact of sex, BMI, race, and the presence of comorbidities on the odds of seroconverting to H1N1 (A) and H3N2 (B) during the 2017-18 and 2018-19 seasons. Point estimates of the log odds ratios with 95% confidence intervals are depicted separately for the 2017-18 (circles) and 2018-19 (squares) seasons. Simple logistic regressions were used to analyze the impact of pre-vaccination titers on the odds of seroconverting to H1N1 (**C**) and H3N2 (**D**) titers for males and females in each season. The odds ratios and 95% confidence are depicted. * = *p*-value < 0.05.

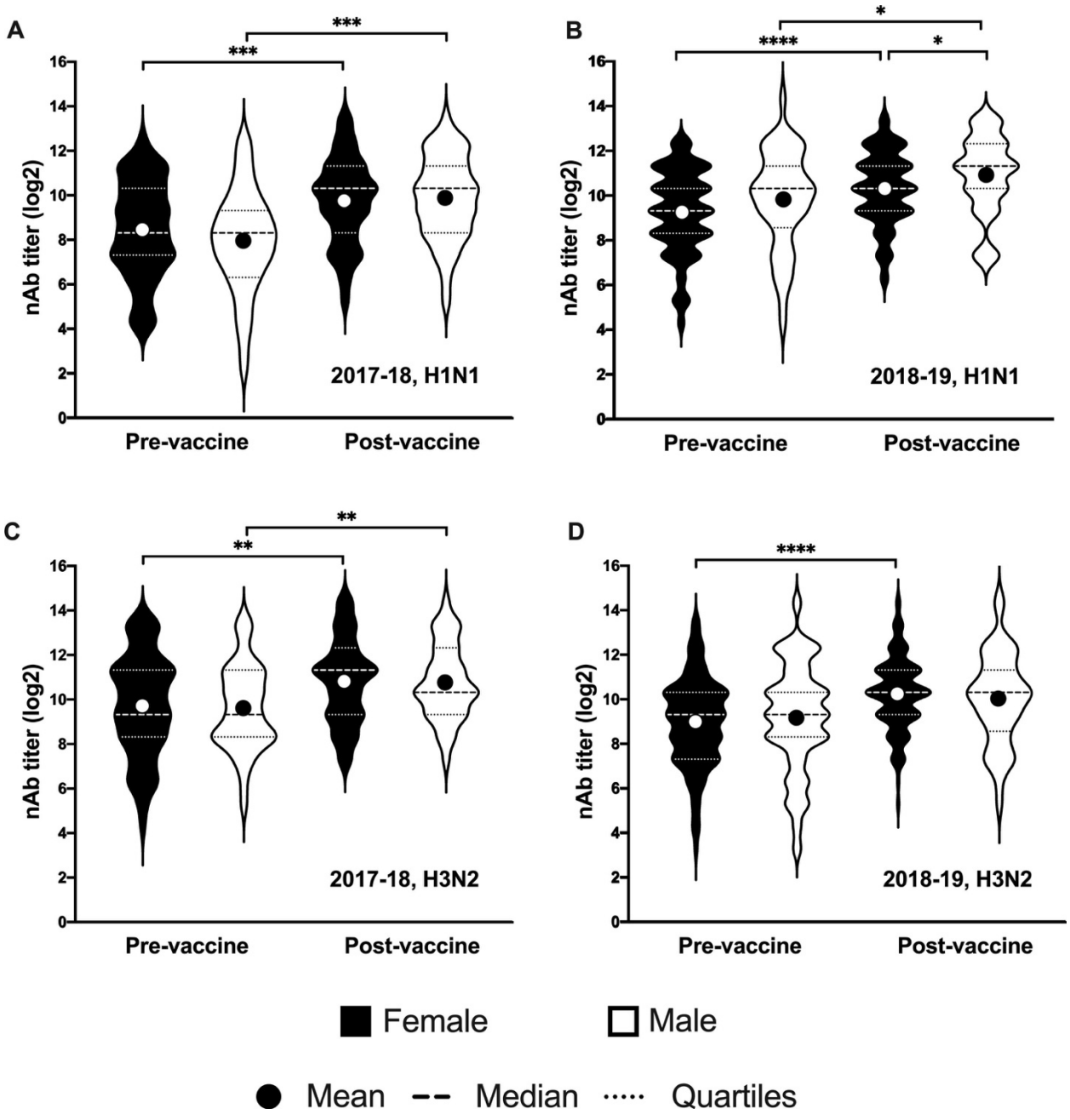


Figure 3: Neutralizing antibody titers increased after receiving an influenza vaccine for both male and female HCWs. Log_2-transformed neutralizing antibody titers pre- and 28 days post-vaccination are shown for female (black) and male (white) HCWs to H1N1 in the 2017-18 season (**A**), H1N1 in the 2018-19 season (**B**), H3N2 in the 2017-18 season (**C**), and H3N2 in the 2018-19 season (**D**). Dashed lines indicate the median titer for each group and dotted lines indicated the 25th and 75th percentiles. Differences between female and male HCWs at each time point for each strain and between pre- and post-vaccination titers were calculated using two-tailed t-tests. Asterisks indicate the significance level with * = p-value <0.05, ** = p-value <0.01, *** = p-value <0.001 and **** = p-value <0.0001.

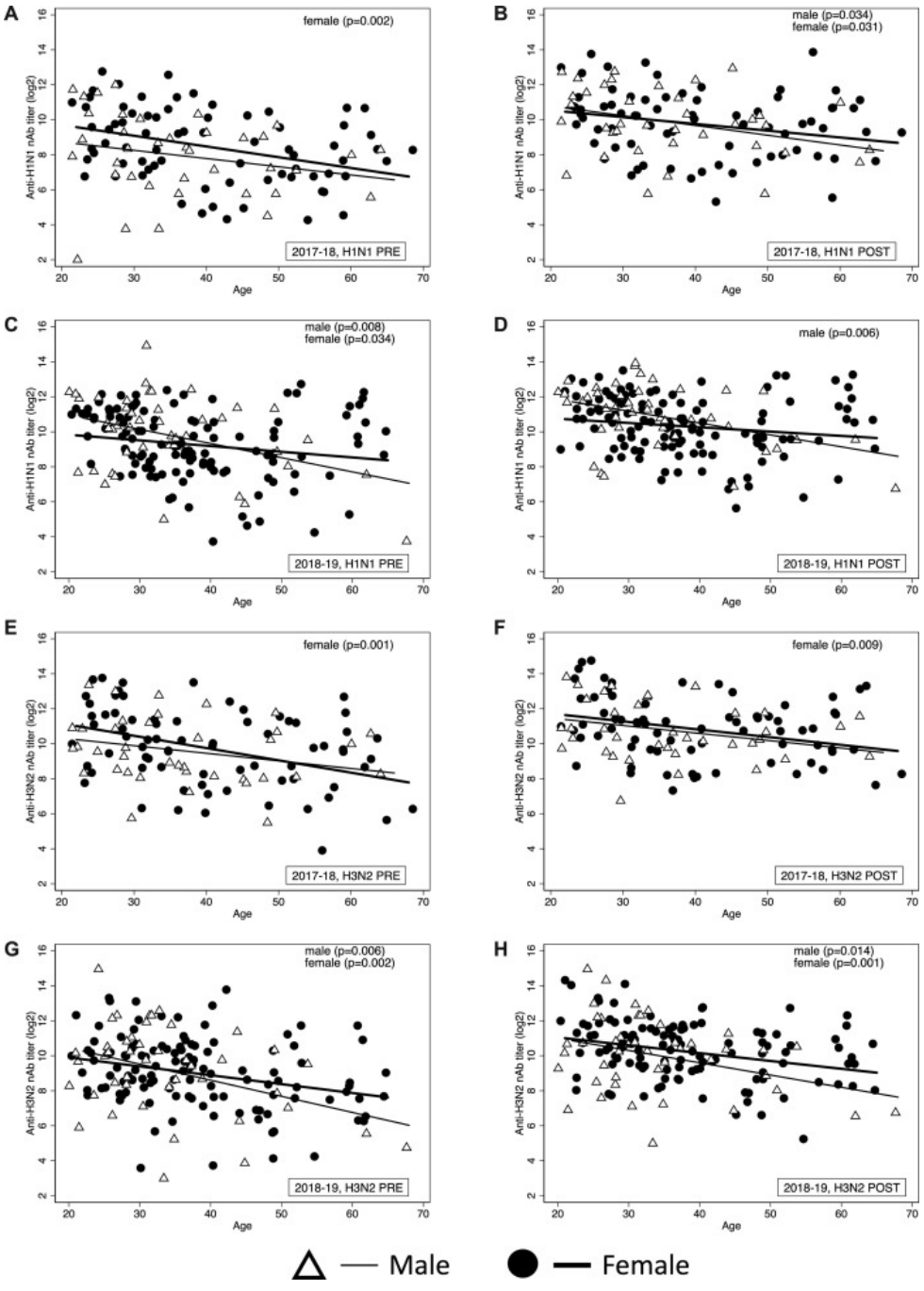


Figure 4: Age intersects with sex to impact neutralizing antibody (nAb) titers in HCWs. Simple linear regression models were used to analyze the effect of age on nAb titers separately for male and female HCWs before vaccination in H1N1 2017-18 season (**A**), 28 days post-vaccination H1N1 in the 2017-18 season (**B**), before vaccination in H1N1 2018-19 season (**C**), 28 days post-vaccination H1N1 in the 2018-19 season (**D**), before vaccination in H3N2 in the 2017-18 season (**E**), and 28 days post-vaccination H3N2 in the 2017-18 season (**F**) before vaccination in H3N2 in the 2018-19 season (**G**), and 28 days post-vaccination H3N2 in the 2018-19 season (**H**).

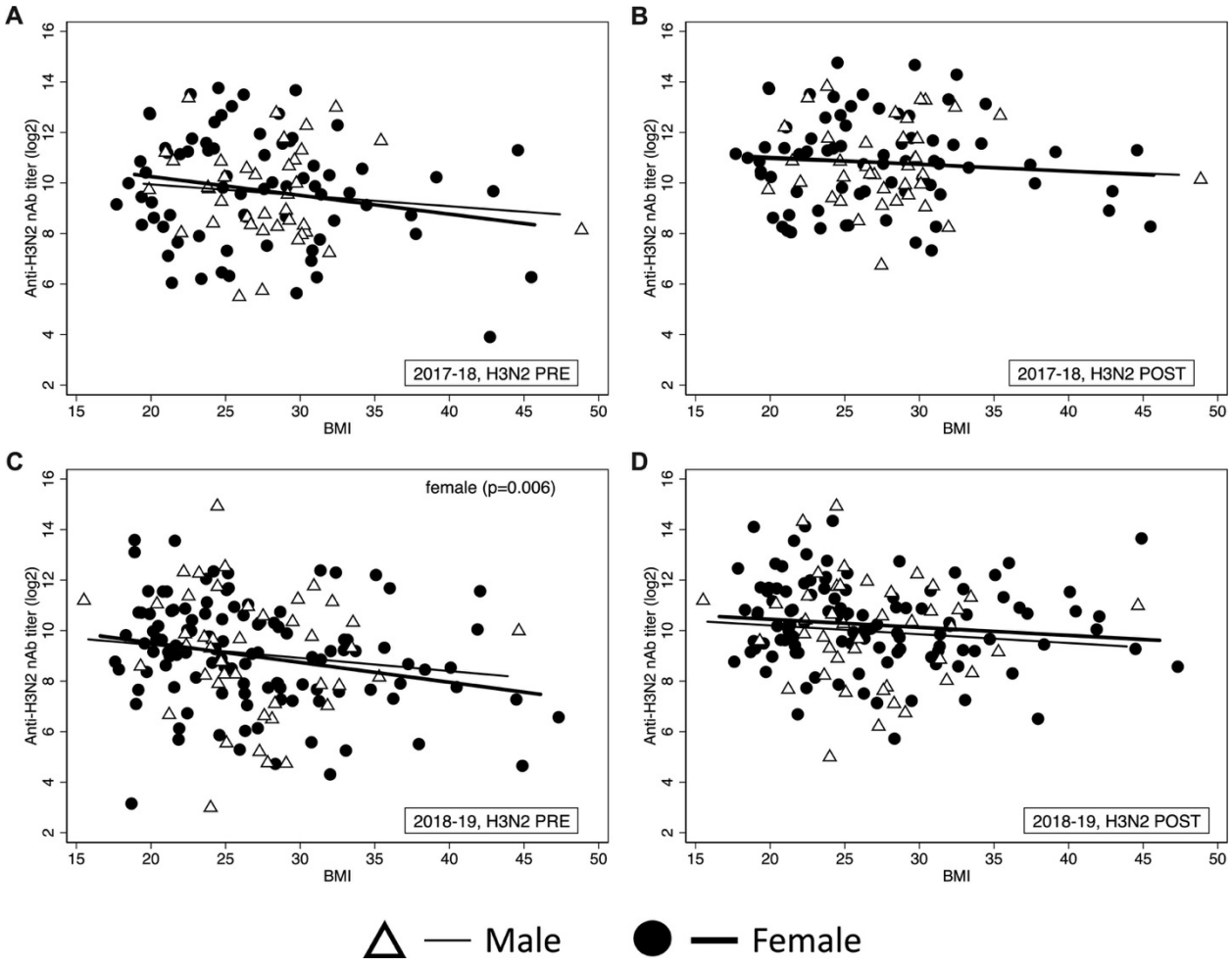


Figure 5: ***Body mass index (BMI) interacts to sex to affect H3N2 nAb titers in HCWs.*** Simple linear regression models were used to analyze the effect of BMI on nAb titers separately for male and female HCWs before vaccination in H3N2 2017-18 season (**A**), 28 days post-vaccination H3N2 in the 2017-18 season (**B**), before vaccination in H3N2 2018-19 season (**C**), 28 days post-vaccination H2N2 in the 2018-19 season (**D**).

APPENDIX 2

ADAPTIVE IMMUNE RESPONSES IN VACCINATED PATIENTS WITH SYMPTOMATIC SARS-COV-2 ALPHA INFECTION

Han-Sol Park*, Janna R. Shapiro*, Ioannis Sitaras*, Bezawit A. Woldemeskel, Caroline C. Garliss, Amanda Dziedzic, Jaiprasath Sachithanandham, Anne Jedlicka, Christopher Caputo, Kimberly E. Rousseau, Manjusha Thakar, San Suwanmanee, Pricila Hauk, Lateef Aliyu, Natalia Majewska, Sushmita Koley, Bela Patel, Patrick Broderick, Giselle Mosnaim, Sonya L. Heath, Emily S. Sydnor, Aarthi Shenoy, Evan M. Bloch, Thomas J. Gniadek, Shmuel Shoham, Arturo Casadevall, Daniel Hanley, Andrea Cox, Oliver Laeyendecker, Michael J. Betenbaugh, Steven M. Cramer, Heba H. Mostafa, Andrew Pekosz, Joel N. Blankson, Sabra L. Klein, Aaron A.R. Tobian, David Sullivan, and Kelly A. Gebo

*Co-first authors

JCI Insights, 2022

AP2.1 Abstract

Benchmarks for protective immunity from infection or severe disease after SARS-CoV-2 vaccination are still being defined. Here we characterized virus neutralizing and ELISA antibody levels, cellular immune responses, and viral variants in 4 separate groups: Healthy controls (HCs) weeks (early) or months (late) following vaccination in comparison to symptomatic SARS-CoV-2 patients after partial or full mRNA vaccination. During the study time, most symptomatic breakthrough infections were caused by the SARS-CoV-2 Alpha variant. In the HCs, neutralizing antibody levels were sustained over time against the vaccine parent virus, but decreased against the Alpha variant, whereas IgG titers and T cell responses against the parent virus and Alpha variant declined over time. Both partially and fully vaccinated patients with symptomatic infections had lower virus neutralizing antibody levels against parent virus than the healthy controls, similar IgG antibody titers and similar virus-specific T cell responses measured by IFN-γ. Compared to healthy controls, neutralization activity against the Alpha variant was lower in the partially vaccinated infected patients and tended to be lower in the fully vaccinated infected patients. In this cohort of breakthrough infections, parent virus neutralization was the superior predictor of breakthrough infections with the Alpha variant of SARS-CoV-2.

Summary: Despite similar antibody titers and T-cell responses to vaccinated noninfected healthy controls, neutralizing capacity was reduced in those with breakthrough infection.

AP2.2 Introduction

The mRNA COVID-19 vaccines are 90% effective at preventing severe disease leading to hospitalization which includes infections with variant SARS-CoV-2 viruses, such as the Alpha and Delta variants (298-300). The United States had 10,262 documented vaccine breakthrough cases from January 1 to April 30, 2021 out of approximately 101 million fully vaccinated individuals (301). During this same period, 706 vaccine breakthrough cases resulted in hospitalization for COVID-19, and of these, 132 were fatal. As of May 1, 2021 the CDC switched to counting only hospitalizations and deaths resulting from vaccine breakthrough cases (302). The total number of people hospitalized in the US after receiving a BNT162b2, mRNA-1273, or Ad26.COV2.S. SARS-CoV-2 vaccine by August 31, 2021 was 10,741 (48% female), with 7,282 being individuals over age 65 (302). Analysis of vaccine breakthroughs from the United Kingdom from December 2020 to July 2021, when the Alpha variant predominated, showed that breakthrough infections in vaccinated people result in fewer COVID-19 symptoms, shorter duration of symptoms, less frequent hospitalizations, and more asymptomatic infections than unvaccinated people (303).

Benchmarks for humoral or cellular immunity that translate to protection against SARS-CoV-2 asymptomatic infection, symptomatic COVID-19, hospitalization, or death are still being defined. The circulation of SARS-CoV-2 variants further complicates the establishment of such benchmarks due to sequence differences compared to the vaccine-seed strains (i.e., parent virus). Many studies show that high antibody titers in the plasma of convalescent or fully vaccinated individuals can adequately neutralize most SARS-CoV-2 variants, suggesting that if individuals develop sufficiently high levels of antiviral antibody, the vaccine can protect against

severe disease (304-306). Less data exist on correlates of protection conferred by cellular immune responses against SARS-CoV-2 in unvaccinated people (307) compared to vaccinated people (308) in the context of antibody responses (309).

During an outpatient trial to evaluate the efficacy of convalescent plasma administered early in infection, we identified vaccinated individuals with confirmed SARS-CoV-2 infection (i.e., breakthrough cases). We conducted a study to compare humoral and cellular responses in infected patients who were at least two weeks post a first dose (partially vaccinated) or second dose (fully vaccinated) of a SARS-CoV-2 mRNA vaccine to healthy fully vaccinated controls. After sequencing the infecting viruses, we compared measures of humoral and cellular immunity to both a parent strain with a spike protein similar to the one used in the mRNA vaccines, and the Alpha variant of SARS-CoV-2, in the infected individuals to healthy vaccinated controls. We report that vaccinated patients with confirmed symptomatic SARS-CoV-2 infections either after the first or second dose had similarly high IgG antibody titers and cell-mediated immune responses, but significantly lower virus neutralizing antibody (nAb) levels compared to healthy vaccinated controls, suggesting that a reduced nAb level is a key factor in greater susceptibility to breakthrough infection.

AP2.3 Results

AP2.3.1 Study population

This study population, described in Table 1, included samples from four separate patient groups: 1) vaccinated uninfected healthy controls sampled 7-14 days post vaccination (early fully vaccinated healthy controls [Early FV-HC]); 2) vaccinated uninfected

healthy controls sampled approximately 95-187 days post vaccination (Late FV-HC); 3) partially vaccinated (PV) patients with symptomatic confirmed SARS-CoV-2 infection (PV-infected [PV-I]); and 4) fully vaccinated (FV) patients with symptomatic confirmed SARS-CoV-2 infection (FV-I). All participants received mRNA-based vaccines (i.e., either BNT162b2 or mRNA-1273). The symptomatic breakthrough patients were identified and evaluated from January 4 to June 4, 2021, before the Delta variant became predominant.

Of the fully vaccinated HC sampled early and late post vaccination, 49% were female. The majority (89%) of the HC had received the BNT162b2 mRNA vaccine. Most of the PV-I patients (n=22) were male (55%) with a median age of 46 years (IQR: 33-55) and had received the BNT162b2 vaccine (72.7%). The mean time from first vaccine dose to presentation with infection (i.e., sample collection) was 20 days (range 14-38). The main exposure was non-work related, with onset of symptoms from 10 known point exposures averaging 2.4 days, and time from symptom onset to blood draw was 4 days (n =13). Cough (81%), fatigue (77%), dyspnea on exertion/shortness of breath (55%), and altered taste or smell (55%) were the predominant symptoms, and a minority (42%) of patients had elevated C-reactive protein.

The majority of the 13 FV-I patients were female (69%), with a median age of 39 years (IQR 33-44). Over three quarters had received the BNT162b2 vaccine. The main exposure sources to SARS-CoV-2 were children or social activities such as travel or dining in a public venue. The median time from second vaccination to confirmed infection was 80 days (range 32-124). The mean time between point exposures and onset of symptoms from 6 known point exposures was 3.6 days and the mean time from symptom onset to screening visit blood draw

was 4 days (N=13). The most common symptoms were fatigue (77%), cough (77%), dyspnea on exertion (69%), and altered taste or smell (54%). The majority (85%) had elevated C-reactive protein. None of the infected patients were immunosuppressed or developed symptoms requiring hospitalization. Most reported being back to their normal healthy state within 2 weeks of symptom onset. Absolute lymphocytes were similar between PV-I (1.74k) and FV-I (1.79k) patients.

AP2.3.2 SARS-CoV-2 Alpha variant caused a majority of infections in vaccinated individuals

In the FV-I, the B.1.1.7 clade (Alpha variant) represented 7 out of 11 sequenced SARS-CoV-2 infections; P.1 (Gamma variant), B.1.526 (Iota variant), B.1.311, and early B.1 lineage (19A Nextstrain) accounted for the remaining 4. In the PV-I, only 8 samples yielded a successful sequence of the infecting viruses, which included: 2 Alpha variants, 2 Gamma variants, and the remaining four consisted of various B.1 lineage viruses. The circulation of variants in the US over time is graphed in Figure 1.

AP2.3.3 Humoral and cellular immune responses to SARS-CoV-2 variants in vaccinated HCs

To assess the kinetics of the humoral immune response post-vaccination, we compared plasma antibodies that bind (measured by indirect IgG ELISA) and neutralize (measured by microneutralization assay) SARS-CoV-2 in the early and late FV-HC groups. Area under the curve (AUC) values were calculated by plotting the OD values (ELISA) or protection from cytopathic effects (microneutralization) against serial dilutions. For SARS-CoV-2 spike (S) and S-receptor binding domain (S-RBD), the specific IgG responses against both the parent strain and Alpha variant were significantly lower in the late FV-HC group compared to the early FV-HC group,

suggesting that responses decrease with time since receipt of the second vaccine dose (Figure 2A-B). The late FV-HC group showed 9%, 23%, 25%, and 24% mean reduction from initial values of IgG responses compared to the early FV-HC group for the parent strain S, Alpha variant S, parent strain S-RBD, and Alpha variant S-RBD, respectively (Figure 2A-B). These reductions were all statistically significant, and the reduction in anti-S-RBD IgG was greater in magnitude than the reduction in anti-S IgG for both the parent and Alpha variant viruses. Furthermore, in early FV-HC, the IgG responses to the parent S and S-RBD were lower than the IgG responses to the Alpha variant S and S-RBD by 3% ($p=0.0004$) and 7% ($p=0.0225$), respectively. The late FV-HC group showed 12% lower ($p<0.0001$) IgG response to the Alpha variant S compared to the parent S, whereas the IgG response to the Alpha variant S-RBD was 8% higher ($p=0.0030$) than the response to parent S-RBD (Figure 2A-B).

Microneutralization assays demonstrated a significant difference in nAb activity (as expressed by AUC) against the Alpha variant between the early FV-HC group and the late FV-HC group, with a 23% decrease in the late FV-HC group compared to early FV-HCs (Figure 2C). The difference in nAb AUC against the parent virus between early and late FV-HCs, however, was negligible, suggesting that the antibody response against the Alpha variant significantly waned over 4 months in contrast to minimal waning of the nAb response to the parent virus. This decreasing trend with time was confirmed when assessing antibody responses relative to the days post second vaccination in the late FV-HC group (Supplemental figure 1C and F). In parallel to the AUC values, geometric mean titers (GMT) are summarized for each group in Supplemental Table 1, and follow similar patterns as the AUC values.

Humoral and cellular immune responses are known to interact closely to provide protection against viral infection. We performed interferon (IFN)-γ enzyme-linked immunosorbent spot (ELISpot) assays on peripheral blood mononuclear cells (PBMCs) obtained from FV-HCs to quantify the frequency of virus-specific T cells (Figure 2D). Due to low sample availability, cellular assays were not completed for all participants. Exact sample sizes are detailed in Supplemental Table 2. As expected, treatment with SARS-CoV-2 S peptide pool stimulated a significant T cell response at both early and late time points in HCs. The early FV-HC group elicited stronger T cell response, with a median of 197 spot-forming units (SFU) per million cells against SARS-CoV-2 parent strain spike peptide pool as previously reported (308), compared with less than 110 SFU per million cells in the late FV-HC group (Figure 2D). As with the antibody data, this decreasing trend with time was confirmed when assessing T cell responses relative to the days post second vaccination in the late FV-HC group (Supplemental Figure 1G).

AP2.3.4 Humoral and cellular immune responses to SARS-CoV-2 in infected, but vaccinated individuals.

We compared antibody responses to SARS-CoV-2 in partially (PV-I) and fully (FV-I) vaccinated and infected patients with responses in the late FV-HC group using ELISA and microneutralization assays. The samples in the late FV-HC group were collected in a similar time frame relative to the second vaccine dose in the FV-I group (Supplemental Figure 1). Among the infected patients, there was no significant difference in IgG responses to SARS-CoV-2 S or S-RBD between PV-I and FV-I patients to either the parent or Alpha variant viruses (Figures 3A-B and 3D-E). The IgG responses to the parent strain and Alpha variant S and S-RBD tended to be

higher in the infected groups than in the late FV-HC group, and was statistically significantly different for the fully vaccinated group for the parent virus S-RBD ($p = 0.0365$; Figure 3D). The anti-S IgG AUC values were higher for the parent than Alpha variant virus (i.e., trending below the line of agreement) among PV-I, FV-I, and HC groups (Figure 3C). In contrast, anti-S-RBD IgG AUC values were similar against the parent and Alpha variant (i.e., primarily on the line of agreement) (Figure 3F). Finally, anti-nucleocapsid IgG responses were not significantly different between HCs and either PV-I or FV-I patients (Supplemental Figure 2).

While IgG responses to SARS-CoV-2 antigens (parent and Alpha variant) were lower in late FV-HC group compared to vaccinated and infected participants (Figures 3A-F), the functional nAb response to the parent virus was significantly lower in the PV-I and FV-I group compared to that in late FV-HC group (mean reduction of 73% and 43%, respectively; Figure 3G). Similarly, the nAb response to the Alpha variant was significantly lower in the PV-I group than in the late FV-HC group, but this difference was not significant for the FV-I group. nAb responses to the Alpha variant in the PV-I and FV-I groups were 63% ($p<0.0001$) and 16% (ns) lower compared to those of late FV-HC group, respectively (Figure 3H). Furthermore, the functional nAb responses against both the parent virus and the Alpha variant were significantly lower in the PV-I group compared to that in the FV-I group (52% and 58% lower in the PV-I group for Parent and Alpha, respectively) (Figures 3G-H), supporting that full vaccination is important in eliciting nAb responses to SARS-CoV-2 variants of concern. Additional analysis showed a correlation between nAb responses against the parent virus and the Alpha variant (Figure 3I). Furthermore, patterns in AUC values were replicated on the GMT scale (Supplemental Table 1).

To determine if cell-mediated immunity was reduced in patients with vaccinated SARS-CoV-2 infections, T cell IFN-γ responses to a SARS-CoV-2 S peptide pool were evaluated. Like the HC group, cells from infected patients (i.e., PV-I and FV-I) treated with the peptide pool mounted a significantly greater IFN-γ response than untreated cells (Figure 3J). Further, the IFN-γ response was similar among the PV-I, FV-I, and the late-FV-HC groups (Figure 3J). To determine if there was a reduction in cell-mediated immune responses to the Alpha variant in the vaccinated patients, we compared T cell responses to the parent and Alpha strain. There was no difference in T cell IFN-γ responses among either PV-I or FV-I to parent or Alpha spike peptide pools (Figure 3K).

AP2.3.5 Humoral and cell-mediated immune parameters are not associated in either healthy controls or vaccinated, but infected patients

Within the HCs, PV-I and FV-I patients, levels of binding IgG against either S or S-RBD and nAb to the parent strain all strongly correlated with each other (correlation coefficients (R) ranged from 0.55 to 0.94), indicating high levels of agreement between the three measures of humoral immunity (Figure 4A-D). However, antibody responses were poorly correlated with measures of T cell-mediated immunity to the parent strain (R values ranging from -0.31 to 0.40; Figures 4A-D). When assessing the correlation between cellular and humoral responses to the Alpha variant, similar trends were observed as for the parent strain (Figure 5).

AP2.4 Discussion

Symptomatic SARS-CoV-2 infections in vaccinated individuals may be due to low antibody levels, low cellular responses, mismatches between cellular and humoral responses to the parent strain and the variants, or high exposure to infectious cases. We have quantified the

magnitude of antibody and cellular responses to parent strain and Alpha variant in four separate groups: partially vaccinated individuals infected with SARS-CoV-2, fully vaccinated individuals infected with SARS-CoV-2, as well as uninfected healthy controls sampled early and late after vaccination.

This study demonstrates several important findings about infections in this vaccinated cohort. The antibody responses to the spike and S-RBD antigens were comparable at similar timepoints among fully vaccinated healthy controls and in those who developed breakthrough infections. Regardless of whether fully or partially vaccinated, however, SARS-CoV-2 infection was associated with lower levels of neutralizing antibodies to the parent strain. These data are consistent with a recent study reporting an association between lower titers of neutralizing antibodies and breakthrough infections (306) and two studies identifying nAb levels as potential correlates of protection (310, 311). Our study complements and extends those findings by showing the presence of robust spike peptide-specific T cell responses in infected vaccinated individuals suggesting that lower neutralization titers are specifically associated with infections in vaccinated individuals, regardless of T cell responses. Our study also further reinforces the impact of the second vaccine dose in boosting nAb responses.

In addition to contributing to a better understanding of infections in vaccinated individuals, this study provides insight into vaccine-induced immune response by evaluating early and late healthy control groups. First, measures of both cellular and humoral immunity decreased with time since the second dose. Measures of the cellular response, however, did not correlate well with antibody responses. Second, these data demonstrate that IgG titers

against the Alpha variant S and S-RBD were higher than those to the parent strain in the early healthy control group. Interestingly, IgG levels to the Alpha variant spike were markedly lower than those to the parent strain in the late HC group. These findings suggest that while mRNA vaccines initially stimulate robust humoral responses to variant viruses, these responses may be short-lived (312-314). Finally, microneutralization assay results indicated that the nAb response against the parent virus was maintained through 6 months in late healthy control groups although binding IgG response decreased, an observation also seen in a study of vaccinated health care workers followed for six months (313). While the nAb responses to parent virus and the Alpha variant were comparable in the early FV-HC group, the level of nAb against Alpha variant was significantly lower in the late FV-HC group. This decrease in nAb response to the Alpha variant over time again suggests waning of vaccine-induced immunity to variants of concern.

The magnitude of the antiviral antibody response and the duration of detectable neutralizing antibodies was assessed, with neutralizing antibodies being detectable longer in convalescent (108 days) than vaccinated (65 days) individuals (311, 315). The nAb titers necessary for protection against infection were much higher than needed for protection from severe disease (310, 311). One important factor that has not been carefully addressed to date is the level of mucosal antibodies present after vaccination, as these antibodies are critical for protection from infection in the upper respiratory tract (316). The presence of anti-SARS-CoV-2 IgG in the nasal tract of infected individuals is inversely correlated to the presence of infectious virus (317, 318) emphasizing the importance of mucosal antibody response for rapid neutralization of SARS-CoV-2.

Samples from infected individuals were collected in the first 8 days after symptom onset. The decrease in nAb titers against the Alpha variant relative to the parent virus observed in the FV-HC group was not observed in the infected groups. Among patients in the FV-I group, infection represented the third exposure to SARS-CoV-2, and a rapid memory response likely contributed to the elevated measures of humoral and cellular immunity reported here. Consistent with this hypothesis, emerging data on the Omicron variant suggests that multiple exposures (i.e., either three doses of mRNA vaccines or a combination of infection and two vaccine doses) are needed to develop broad immunity to SARS-CoV-2 variants of concern (319). Since the Alpha variant is antigenically distinct from the parent virus, a boosting effect against the parent virus may not be as strong as that against the Alpha variant, thus, a difference in parent virus neutralizing antibody levels remained

There are several limitations to this study. The study had a small sample size, and consistent with having received the vaccination, none of the infected participants were hospitalized, suggesting mild disease. Also, the timing of sample collection in this study allowed for evaluation of breakthrough infections with the Alpha variant, but not the Delta or Omicron variants, which may have different pathology. In addition, all HCs in the study had received two doses of vaccine, while infected cases were either fully or partially vaccinated, meaning that the control group for the PV-I is imperfect. While the timeframe of this nonlongitudinal, convenience sample collection relative to vaccination overlapped for the FV-I and late FV-HC groups, the late FV-HC group was sampled slightly longer after vaccination, on average, than the FV-I group. Because of this, responses may have waned to a greater degree in the FV-HC than the FV-I, thus attenuating differences between the two groups. Finally, low availability of

PBMC from HC groups did not allow testing of T cell responses against the Alpha variant, such that data comparing T cell responses to the parent and Alpha strains in infected participants must be interpreted with caution.

Overall, the study demonstrated humoral and cellular responses decreased with time from vaccination date, potentially increasing the likelihood of infections. It is critically important to understand the magnitude and duration of the protective immune response induced by vaccination and boosting to determine how best to end the COVID-19 pandemic.

AP2.5 Methods

AP2.5.1 Study participants, blood sample processing, and storage

From an outpatient trial recruiting symptomatic newly infected patients, which did not exclude vaccinated individuals, we identified 13 fully vaccinated (more than 14 days post second vaccination) patients who developed symptomatic breakthrough SARS-CoV-2 infection (FV-I) and 22 partially vaccinated (more than 14 days after first vaccination) patients who developed symptomatic SARS-CoV-2 infection (PV-I). We then compared these patients to 22 fully vaccinated non-infected health care workers (i.e., healthy control (HC)) evaluated 1-2 weeks post vaccine (Early FV-HC) and 15 fully vaccinated uninfected health care workers evaluated at 5 months post vaccine (Late FV-HC). All study participants had received either BNT162b2 (Pfizer) or mRNA-1273 (Moderna) mRNA vaccines. For those with SARS-CoV-2 infection, clinical symptoms information, nasal swabs, and serum samples were obtained as close to onset of symptoms as possible. Demographic and clinical data was self-reported by the research participants.

The study called Convalescent Plasma to Limit SARS-CoV-2 Associated Complications (CSSC-004) was a phase 2 double blinded randomized control trial with either high titer SARS-COV-2 convalescent plasma or placebo control plasma. This study was designed as a separate protocol under Johns Hopkins University Investigational New Drug application (19725) and filed as NCT04373460 at clinical trials.gov. Johns Hopkins was the central IRB (IRB00247590).

AP2.5.2 SARS-CoV-2 genome sequencing

Automated nucleic acid extraction was performed as described previously (317, 318) using the chemagic 360 (PerkinElmer) following the manufacturer's protocol. Whole genome sequencing and analysis were performed as previously described (320).

For a subset of samples, 25ng of RNA, previously extracted using Qiagen's Viral RNA mini kit, was processed following the Illumina RNA Prep with Enrichment (L) Tagmentation protocol with Illumina Respiratory Virus Oligo Panel for one plex enrichment. Libraries were sequenced on the Illumina MiSeq (2x76 bp) or iSeq (2x151 bp) platform. FASTQ files were analyzed in Illumina's BaseSpace using the DRAGEN Pathogen Detection application to generate consensus files. The pangolin web-based application, Phylogenetic Assignment of named Global Outbreak LINeages (PANGOLIN) (https://pangolin.cog-uk.io/) was used to identify the SARS-CoV2 lineages from these consensus sequences. Nextclade (https://clades.nextstrain.org/) was used for clade assignment, sequence quality check and phylogenetic tree construction.

AP2.5.3 Expression and purification of parent strain and Alpha variant S- and S-RBD Plasmid preparation

The plasmids expressing recombinant S and S-RBD for the vaccine strain of SARS-CoV-2 have been described previously (321) The sequence from the SARS-CoV-2 Alpha variant hCoV19/USA/MD-HP01101/2021 (EPI_ISL_825013) was used to engineer S and S-RBD expression plasmids. The Alpha variant S gene was synthesized in its entirety (GeneScript) before cloning into the pCAGGS vector. Site directed mutagenesis was used to introduce a N501Y substitution into the plasmid expressing the S-RBD from the vaccine strain. The plasmids were extracted using GigaPrep kits (Thermo Fisher Scientific) and eluted in molecular biology grade water.

Protein purification by immobilized metal affinity chromatography (IMAC) and gravity flow was adapted from previous methods (321). After washing with PBS (Thermo Fisher Scientific), nickel nitrilotriacetic acid (Ni-NTA) agarose (QIAGEN) was added to the culture supernatant, followed by overnight incubation for 12–16 hours at 4°C on a rotator. For every 150 mL of culture supernatant, 2.5 mL Ni-NTA agarose was added. Five-milliliter gravity-flow polypropylene columns (QIAGEN) were equilibrated with PBS. One polypropylene column was used for every 150 mL culture supernatant. The supernatant-agarose mixture was then loaded onto the column to retain the agarose beads, with recombinant proteins bound to the beads. Each column was then washed, first with 1× culture supernatant volume of PBS and then with 25 mL of 20 mM imidazole (Millipore MilliporeSig- ma) in PBS wash buffer to remove host cell proteins. Recombinant proteins were then eluted from each column in 3 fractions with 5 mL of 250 mM imidazole in PBS elution buffer per fraction, giving a total of 15 mL eluate per column.

The eluate was subsequently dialyzed several times against PBS using Amicon Ultra Centrifugal Filters (Millipore MilliporeSigma) at 5000g for 20 minutes at 10°C to remove the imidazole and concentrate the eluate. Filters with a 10 kDa molecular weight cutoff were used for RBD eluate, whereas filters with a 50 kDa molecular weight cutoff were used for full-length S protein eluate. The final concentration of the recombinant S and S-RBD proteins was measured by bicinchoninic acid (BCA) assay (Thermo Fisher Scientific), and purity was assessed on 10% SDS-PAGE gels (Bio-Rad) followed by Coomassie blue staining. After sufficient destaining in water over-night, clear single bands were visible for S- and S-RBD proteins at their respective molecular sizes.

For the scale-up purification, preparative IMAC chromatography was performed using an Äkta Explorer 100 (Amersham Biosciences, Uppsala, Sweden) controlled by Unicorn 5.31 software. HisTrap excel (1mL) prepacked columns (Cytiva) were used for generating the purified S and S-RBD proteins. For the S-RBD purification process, the equilibration step was performed with PBS buffer for 5 column volumes (CV) at 1 mL/min, followed by loading of 40 CV of the harvest material at 1 mL/min. A wash step with 20 mM imidazole in PBS was performed for 20 CV at 1 mL/min, immediately followed by the step gradient elution of the bound proteins using 15 CV of 500 mM imidazole in PBS at 1 mL/min. 1 mL fractions were collected during this step and stored for further purity analysis. The column was then re-equilibrated with PBS, regenerated using 0.5 M NaOH for 10 CV at 1 mL/min and finally stored at 20% v/v ethanol. For the S protein purification process, a similar setup was used with slight modifications in the purification protocol. The flow rate during the loading step was reduced to 0.5 mL/min to increase the residence time during loading, thereby increasing the yield of the target protein.

The buffer compositions were optimized to enhance the purity in the elution step. This included an addition of 0.4 M NaCl in the equilibration, wash and elution buffers to help mitigate the electrostatic interactions of contaminants with the tagged protein or resin. The imidazole concentration in the wash buffer was also increased to 30 mM to help remove the tagged contaminants from the elution fractions. SDS-PAGE analysis (Any kDa gel, Bio-Rad) was performed followed by silver staining to analyze the purity of these fractions. The pure fractions were then pooled and buffer-exchanged against PBS (as described above) to generate ~ 10x concentrated S and RBD protein solutions.

AP2.5.4 Viruses and cells

Vero-E6-TMPRSS2 cells (181) were cultured in complete media (CM) consisting of DMEM containing 10% FBS (Gibco, Thermo Fisher Scientific), 1 mM glutamine (Invitrogen, Thermo Fisher Scientific), 1 mM sodium pyruvate (Invitrogen, Thermo Fisher Scientific), 100 U/mL penicillin (Invitrogen, Thermo Fisher Scientific), and 100 µg/mL streptomycin (Invitrogen, Thermo Fisher Scientific). Cells were incubated in a 5% CO2 humidified incubator at 37°C.

The SARS–CoV-2/USA-WA1/2020 virus was obtained from BEI Resources. The Alpha variant of SARS-CoV-2 (hCoV19/USA/MD-HP01101/2021, EPI_ISL_825013) was isolated on Vero-TMPRSS2 cells plated in 24-well dishes and grown to 75% confluence. The CM was removed and replaced with 150 µL of infection medium (IM), which is identical to CM but with the fetal bovine serum reduced to 2.5%, and 150 ul of the viral transport media containing a swab from a patient confirmed to be SARS-CoV-2 positive was added to the culture. The cultures were incubated at 37°C for 2 hours, the inoculum was aspirated and replaced with 0.5

mL of IM and the cells cultured at 37°C for up to 5 days. When a cytopathic effect was visible in most of the cells, the IM was harvested and stored at −70°C. The presence of SARS-CoV-2 was verified by extracting RNA from the harvested supernatant using the Qiagen Viral RNA extraction kit (Qiagen), and viral RNA detected using quantitative RT-PCR (184). The consensus sequence of the virus isolate did not differ from the sequence derived from the clinical specimen. The infectious virus titer was determined on Vero-TMPRSS2 cells using a 50% tissue culture infectious dose (TCID50) assay as previously described for SARS-CoV-2 (185, 186). Serial 10-fold dilutions of the virus stock were made in infection media (IM) (identical to CM except the FBS was reduced to 2.5%), and then 100 µL of each dilution was added to the cells in a 96-well plate in sextuplicate. The cells were incubated at 37°C for 4 days, visualized by staining with naphthol blue-black, and scored visually for cytopathic effect. A Reed and Muench calculation was used to determine the TCID50 per mL (187).

AP2.5.5 Indirect Enzyme-linked immunoassays (ELISAs)

The ELISA protocol was adapted from a protocol published by the Florian Krammer laboratory (321). Ninety-six well plates (Immulon 4HBX, Thermo Fisher Scientific) were coated with either full-length S protein or S-RBD of the parent strain or Alpha variant at a volume of 50 µL of 2 µg/mL diluted antigen in filtered, sterile 1× PBS (Thermo Fisher Scientific) at 4°C overnight. Coating buffer was removed, and the plates were washed 3 times with 300 µL 1× PBS plus 0.1% Tween-20 (PBST) wash buffer (Thermo Fisher Scientific) and then blocked with 200 µL PBST with 3% nonfat milk (milk powder, American Bio) by volume for 1 hour at room temperature. All plasma samples were heat-inactivated at 56°C on a heating block for 1 hour before use. Negative control samples were prepared at 1:10 dilutions in PBST in 1%

nonfat milk and plated at a final dilution of 1:100. A monoclonal antibody against the SARS–CoV-2 S protein was used as a positive control (1:5000 dilution, catalog #40150- D001, Sino Biological). Plasma samples were prepared in 3-fold serial dilutions starting at 1:20 in PBST in 1% nonfat milk. Blocking solution was removed, and 100 µL diluted plasma was added in duplicate to the plates and incubated at room temperature for 2 hours. Plates were washed 3 times with PBST wash buffer, and 50 µL secondary antibody was added to the plates and incubated at room temperature for 1 hour. Anti–human secondary antibody, Fc-specific total IgG HRP (1:5000 dilution, catalog A18823, Invitrogen, Thermo Fisher Scientific), was prepared in PBST plus 1% nonfat milk. Plates were washed, and all residual liquid was removed before addition of 100 µL SIGMAFAST OPD (o-phenylenediamine dihydrochloride) solution (MilliporeSigma) to each well, followed by incubation in darkness at room temperature for 10 minutes. To stop the reaction, 50 µL 3M HCl (Thermo Fisher Scientific) was added to each well. The OD of each plate was read at 490 nm (OD490) on a SpectraMax i3 ELISA Plate Reader (BioTek Instruments). A cutoff value for each plate was calculated by summing the average of the OD values of the negative controls and 3 times the standard deviations of the OD values of the negative controls. This cut-off value was subtracted from all sample OD values, and negative values set to zero. Background-subtracted OD values were then plotted against the dilution factor to calculate the AUC. For all ELISA data, a titer of 1:180 was determined as a cut-off for positivity using pre-pandemic and convalescent samples. On the AUC scale, this cut-off was established by taking the average AUC values of samples with a titer of 1:180.

AP2.5.6 Microneutralization assay

Plasma nAbs were determined as described for SARS-CoV-2 (188). Two- fold dilutions of plasma (starting at a 1:20 dilution) were made in infection media (IM). Infectious virus was added to the plasma dilutions at a final concentration of 1×10^4 $TCID_{50}$/mL (100 $TCID_{50}$ per 100 µL). The samples were incubated for 1 hour at room temperature, and then 100 µL of each dilution was added to 1 well of a 96-well plate of VeroE6-TMPRSS2 cells in hexaplicate. The cells were incubated for 6 hours at 37°C, 5% CO_2. The inocula were removed, fresh IM was added, and the cells were incubated at 37° C, 5% CO_2 for 2 days. The cells were fixed by the addition of 100 µL of 4% formaldehyde per well, incubated for at least 4 hours at room temperature, and then stained with Napthol Blue Black (MilliporeSigma). The nAb titer was calculated as the highest serum dilution that eliminated the cytopathic effect in 50% of the wells (NT50) and the area under the curve (AUC) was calculated using Graphpad Prism.

AP2.5.7 T cell interferon response to SARS-CoV-2 spike peptide

Peptides and ELISPOT assays. Peptides for the spike protein of SARS-CoV-2 were obtained from BEI Resources and reconstituted with DMSO at a concentration of 10 mg/mL. The SARS-CoV-2 peptides are 12, 13, or 17 mer, with 10 amino acid overlaps. The spike protein peptide pool consisted of 181 peptides. The peptides were combined into 1 pool for each viral protein at 10 µg/mL. The Alpha variant spike peptides (15mers with 11 amino acid overlap) were purchased from JPT Peptide Technologies (Berlin, Germany) and used at a concentration of 1ug/ml. For comparison, parent virus spike peptides from the same company were used at the same concentration. Stimulation with anti-CD3 antibody (Mabtech, 1 µg/mL) was used as a positive control for each study participant.

IFN-γ ELISPOT assays were performed as previously described (322). Briefly, ELISPOT Pro and ELISPOT Plus kits with precoated plates were purchased from Mabtech. The wells were plated with unfractionated PBMCs at 250,000 cells/well, and the cells were cultured for 20 hours with the SARS-CoV-2 peptide pool. The plates were then processed according to the manufacturer's protocol and read by a blinded independent investigator using an automated reading system. Four replicates per pool were run, and the mean of replicates was plotted. The replicate farthest from the median was not used. If 2 values were equally distant from the median, then the higher value was discarded. Spot forming units (SFU) per million PBMCs were calculated by multiplying SFU generated by the automated plate reader by 4 (i.e., SFU/250,000 cells were multiplied to yield the standard SFU/million). A response was counted as positive only if treatment induced at least a 3-fold increase and the SFU exceeded 20 spots/million.

AP2.5.8 Statistical analyses

AUC values were $\log_{10}$ transformed to achieve a normal distribution. Immune read-outs between groups and viruses were compared by two-tailed t-tests, paired t-tests, or one-way ANOVAs with Tukey's correction for multiple comparisons where appropriate. A p value of less than 0.05 was considered statistically significant. Correlations between IgG antibody, microneutralization assay and ELISpot assays were assessed using Pearson's correlation coefficients (R). Percent changes were calculated as 100*[(initial value – final value)/initial value] using $\log_{10}$-tranformed values. Analyses were performed using GraphPad Prism 8 (GraphPad Software) and Stata 15 (StataCorp).

AP2.6 Author contributions

K.G., D.S., J.B., S.K., A.P., O.L., A.C., and A.T conceived and designed the study; D.S., E.B., A.C., and A.T. wrote the IRB protocol; K.G., B.P.,P.B., G.M., S.H., E.S., A.S., E.B., D. H. and D.S. recruited participants; D.S. coordinated collection of all samples; M.T. cataloged and organized samples and assisted with blinded data organization; H-S.P., J.S., B.W., C.G., K.R., A.J.,I.S., H.M., A.D. S.S. and C.C. carried out all experiments; P.H., L.A.,N.M. SK., S.M.C. and M.J.B. produced and purified recombinant SARS-CoV-2 proteins; J.S. performed statistical analyses; H-S.P., J.S., S.K., A.T.,J. B, A.P, D.S., and K.G. wrote the manuscript with substantial input from all co-authors. For co–first authorship, HSP is named first, for antibody level determinations and data analysis, JS for coordinating data analysis and IS for virus nAb determinations and data analysis.

AP2.7 Tables

Table 1. Participant demographic information

SARS-CoV-2 infection	Early FV-HC[a]	Late FV-HC[b]	PV-I[c]	FV-I[d]
N	22	15	22	13
Sample collection - mean (range)				
Days post dose 1			20 (14 - 38)	103 (54 - 145)
Days post dose 2	9 (7 - 14)	142 (95-187)		80 (32 - 124)
Sex - n (%)				
Male	13 (59.0)	6 (40.0)	12 (54.5)	4 (30.8)
Female	9 (41.0)	9 (60.0)	10 (45.5)	9 (69.2)
Age - n (%)				
21-30	7 (31.8)	12 (80.0)	2 (9.1)	3 (23.1)
31-40	4 (18.2)	2 (13.3)	7 (31.8)	4 (30.8)
41-50	6 (27.3)	1 (6.7)	5 (22.7)	5 (38.5)
51-60	5 (22.7)	0 (0)	6 (27.3)	1 (7.7)
61-70	0 (0)	0 (0)	2 (9.1)	0 (0)
Vaccine type				
Moderna	1 (4.5)	3 (20.0)	6 (27.3)	3 (23.1)
Pfizer	21 (95.5)	12 (80.0)	16 (72.7)	10 (76.9)

[a] Fully vaccinated healthy controls sampled early after receipt of the second vaccine dose.

[b] Fully vaccinated healthy controls sampled late after receipt of the second vaccine dose.

[c] Partially vaccinated (>14 days after first vaccination) patients who developed symptomatic breakthrough SARS-CoV-2 infection

[d] Fully vaccinated (>14 days after second vaccination) patients who developed symptomatic breakthrough SARS-CoV-2 infection

Supplement Table 1. Geometric mean titers

GMT (95% CI)	Early FV-HC	Late FV-HC	PV-I	FV-I
Parent S IgG	35820 (20992 - 61122)	10109 (6509 - 15702)	27906 (15394 - 50586)	20444 (9327 - 44812)
Parent S-RBD IgG	43740 (25439 - 75208)	2705 (1721 - 4252)	12551 (6717 - 23453)	14580 (6188 - 34354)
Alpha S IgG	35820 (25915 - 49511)	3901 (1807 - 8422)	7618 (4202 - 13809)	6262 (2888 - 13579)
Alpha S-RBD IgG	102226 (70886 - 147423)	5229 (2790 - 9802)	10279 (5303 - 19925)	18787 (7303 - 48330)
Parent nAb	181 (121 - 273)	202 (132 - 309)	18 (12 - 26)	38 (20 - 71)
Alpha nAb	206 (140 - 303)	76 (51 - 114)	21 (13 - 34)	47 (24 - 93)

Supplemental Table 2. Samples numbers used in assays.

Group	Early FV-HC	Late FV-HC	PV-I	FV-I
N	22	15	22	13
Data sources - n (%)				
ELISA	22 (100)	15 (100)	22 (100.0)	13 (100.0)
Neutralizing	22 (100)	15 (100)	22 (100.0)	13 (100.0)
ELISpot (vaccine virus)	15 (68)	13 (87)	11 (50.0)	8 (61.5)
Complete data	15 (68)	13 (87)	11 (50)	8 (61.5)

AP2.7 Figures

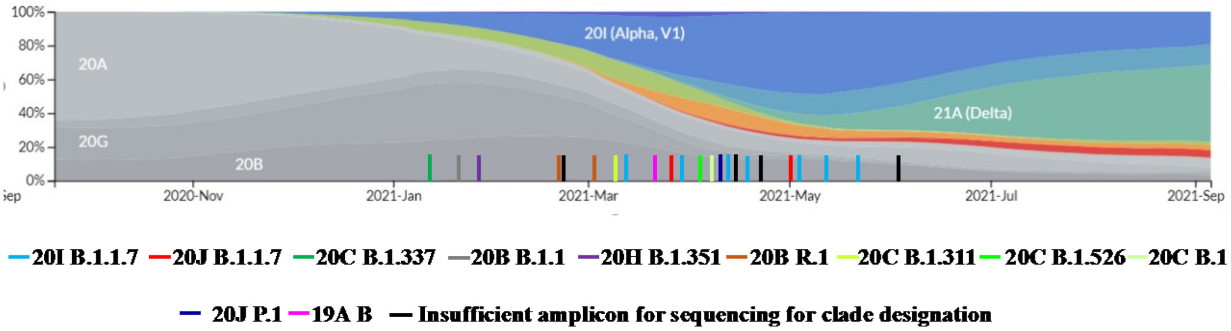


Figure 1 The incidence of majority breakthrough infections caused by SARS-CoV-2 variants. The frequency of SARS CoV2 circulating lineages in the USA between Sep 2020 and Sep 2021. Each bar with specific color indicates the time when the breakthrough infections occurred. Data were retrieved from Nextstrain Genomic epidemiology of novel coronavirus-North America-focused subsampling, further filtered data set by country- USA on 9/27/2021.

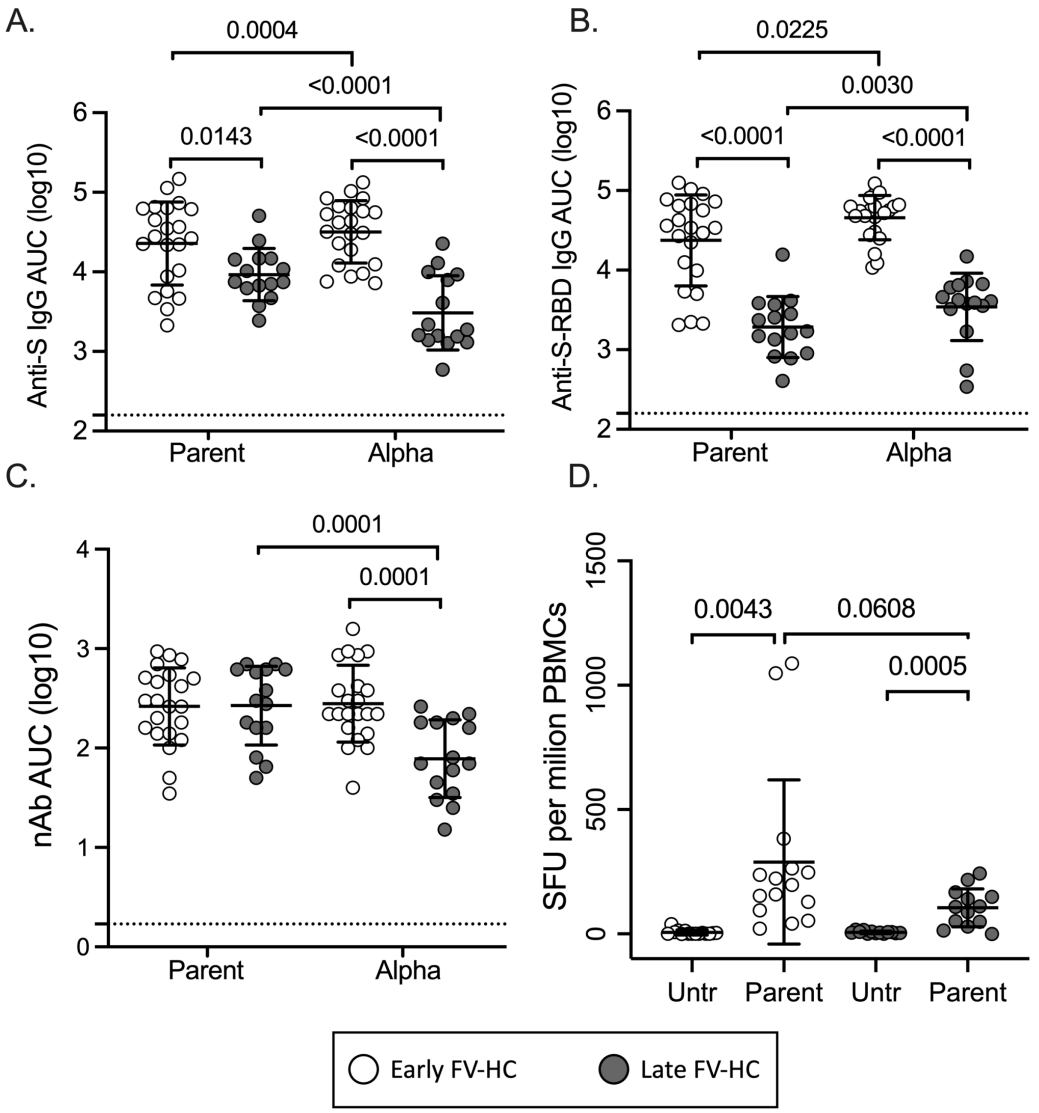


Figure 2. Measures of vaccine-induced humoral and cellular immunity decrease with time in HCs, with the exception of nAb to the parent strain. Plasma and PBMC samples were collected from fully vaccinated HCs, with no history of testing positive for COVID-19, either 7-14 days (early, n = 22) or 95-187 days (late, n = 15) after the second dose. Indirect ELISAs were used to measure IgG against S (**A**) and the S-RBD (**B**) from either the parent strain or Alpha variant viruses and are graphed as area under the curve (AUC) values. Microneutralization assays were also performed against the parent virus and Alpha variant, and AUC values are shown (**C**). An IFN-γ ELISPOT was used to measure the spot-forming units (SFU) per million PBMCs in response to SARS-Cov-2 spike parent strain peptide pools (**D**). The dashed lines indicate the limit of detection (**A-C**). Two-tailed, unpaired t-tests were used to compare between groups, and paired two-tailed t-tests were used to compare outcomes on the same individuals. P-values below 0.05 are shown, but since four comparisons were made in each panel, the Bonferroni correction for multiple comparisons suggests that only values below 0.0125 (i.e., 0.05/4) be considered statistically significant.

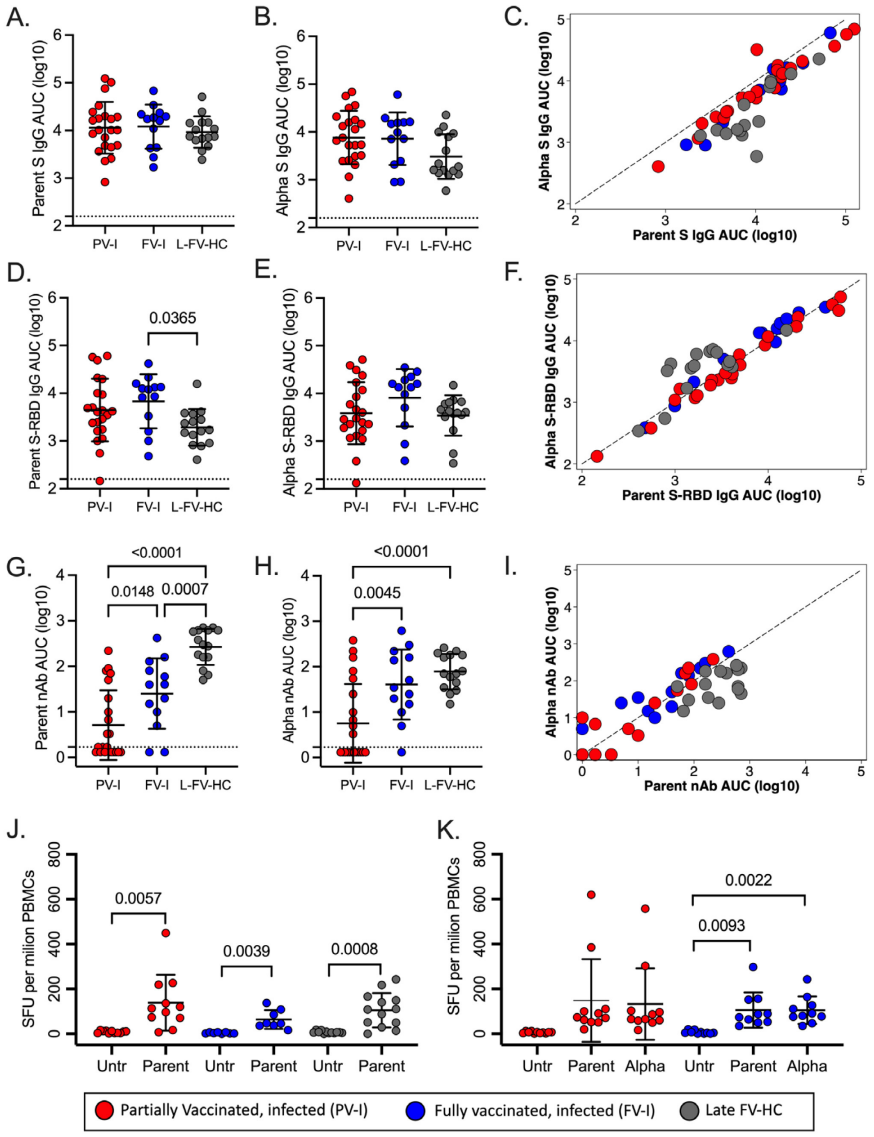


Figure 3. Antibody responses are greater in FV-I as compared to FV-HCs. Plasma samples were collected from confirmed breakthrough infections that occurred either after receipt of the first vaccine dose (red circles, PV-I, n = 22) or after receipt of the second dose (blue circles, FV-I, n = 13). For comparison, plasma samples from fully vaccinated HCs were collected (grey circles, Late FV-HC, n = 15). Log_{10}-transformed AUC values for anti-S IgG (indirect ELISA; **A-C**), anti-S-RBD IgG (indirect ELISA; **D-F**) and neutralizing antibodies (microneutralization assay; **G-I**) are shown for the three study groups for the parent virus (**A, D** and **G**), the Alpha variant (**B, E,** and **H**), and as the correlation between the parent and Alpha variants (**C, F,** and **I**). Dashed lines indicate the limit of detction (**A-B, D-E, G-H**) or the line of agreement (**C,F, I**). One-way ANOVA with Tukey's correction for multiple comparisons were used to compare groups for antibody data, and paired two-tailed t-tests (**J**) or repeated-measures ANOVA with Tukey's correction for multiple comparisons (**K**) were used to analyze ELISpot data. All p-values below 0.05 are shown and were considered statically significant.

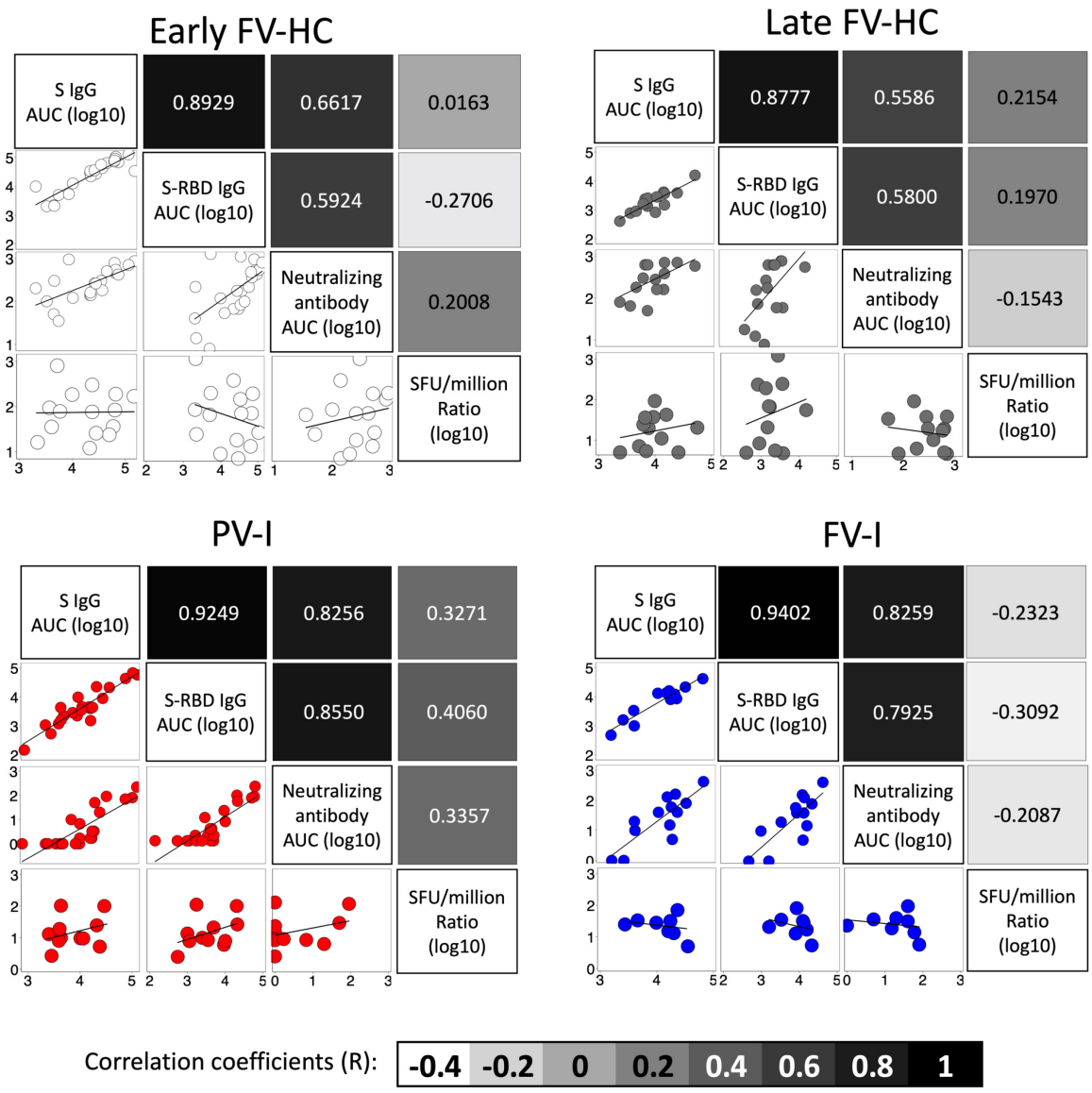


Figure 4. Antibody responses correlate well with each other but correlate poorly with measures of T cell-mediated immunity. The correlation between various measures of humoral and cell-mediated immunity were assessed seperately for HC sampled 7-14 days post-vaccination (**A**, Early FV-HC), HC sampled 95-187 days post-vaccination (**B**, Late FV-HC), indiviuals with confirmed SARS-CoV-2 after receipt of the first dose of a vaccine (**C**, PV-I), and indiviuals with confirmed SARS-CoV-2 after receipt of the second dose of a vaccine (**D**, FV-I). Scatter plots and trendlines are shown in the lower half of matrices, and correlation coefficients (R), color coded by the strength of the correlation, are shown in the upper half of the matrices. For cell-based measures, data shown is the ratio spot-formuing units (SFU) per smillion for treated to untreated cells, transformed on a $\log_{10}$ scale.

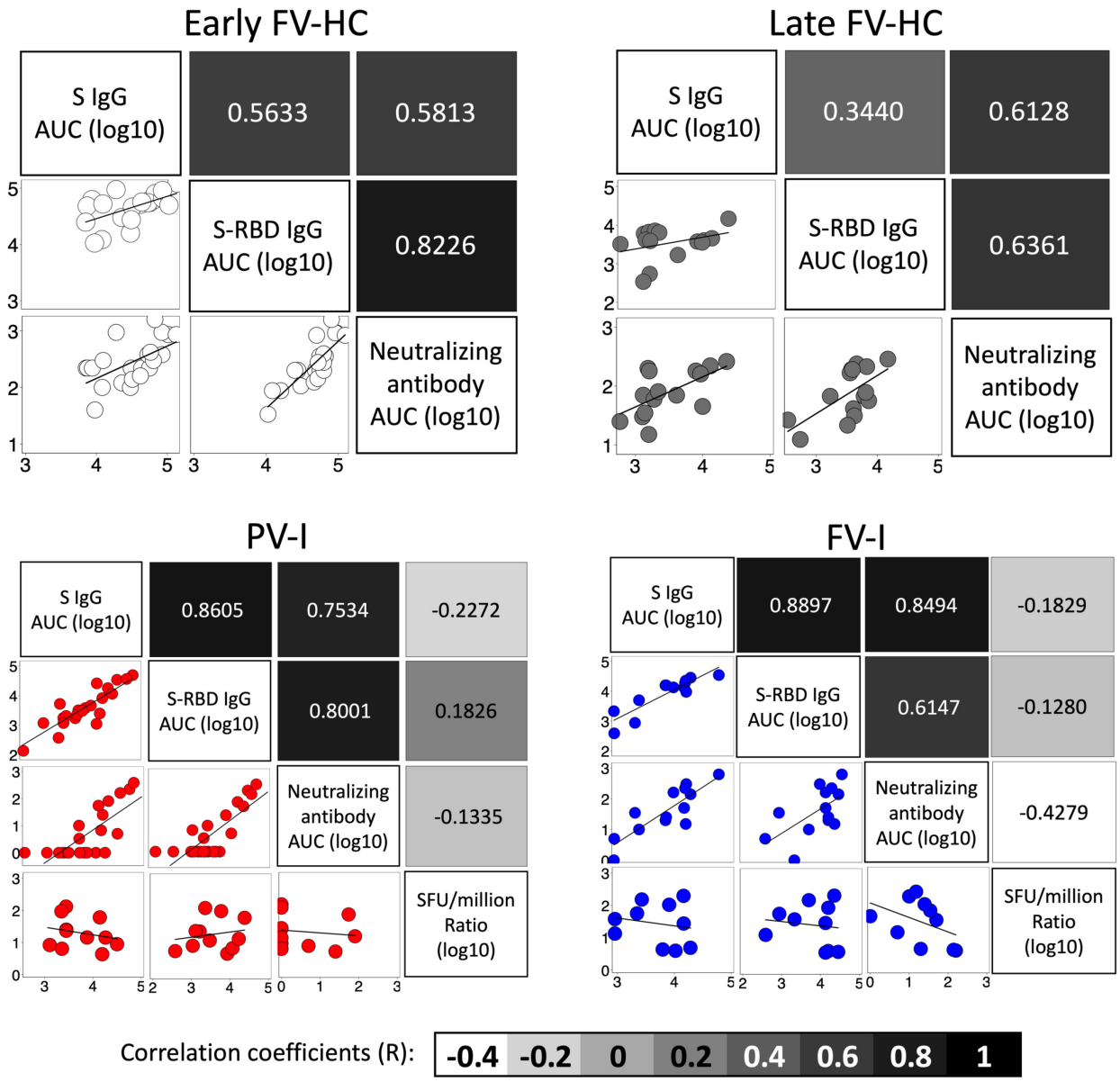
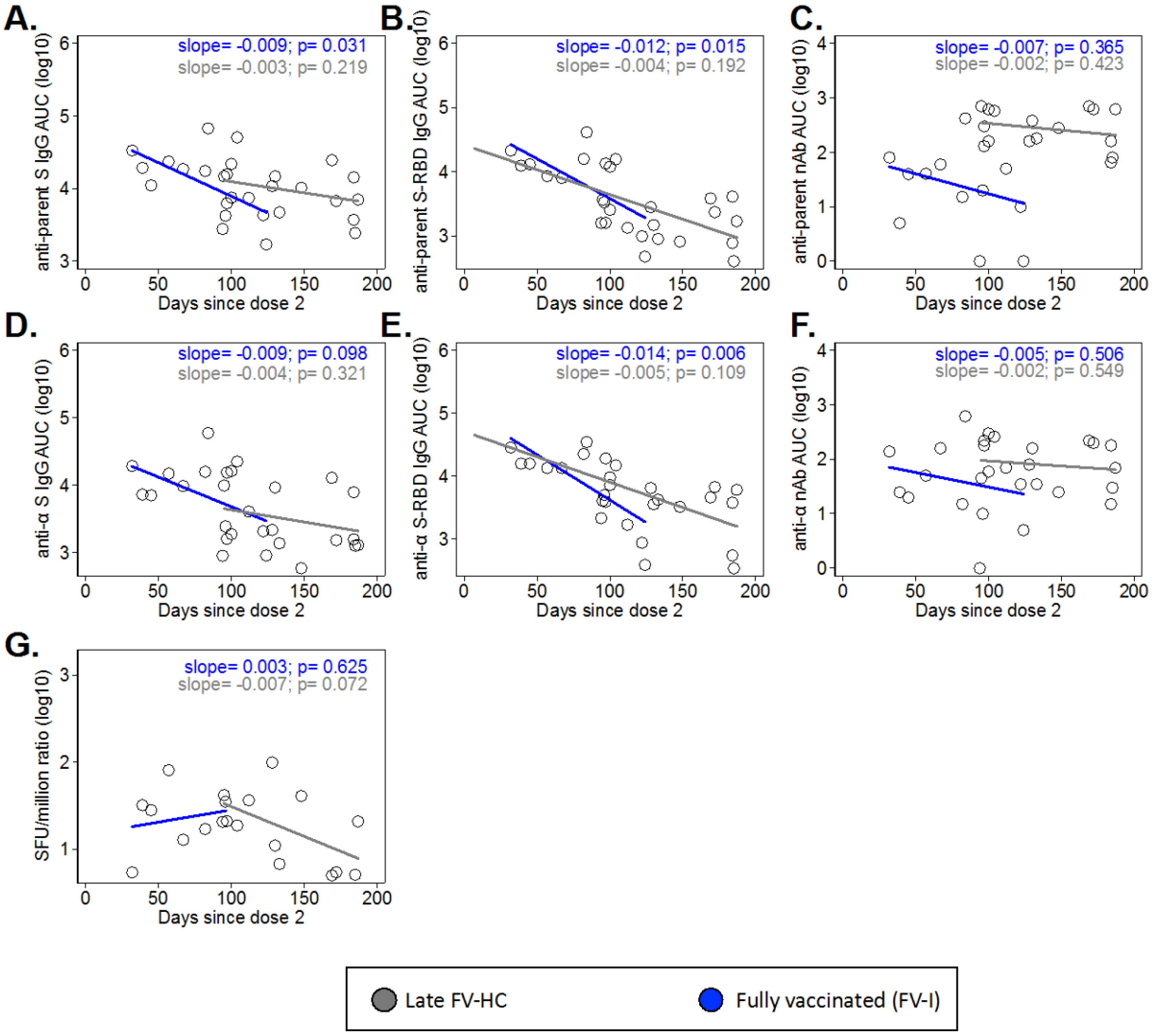
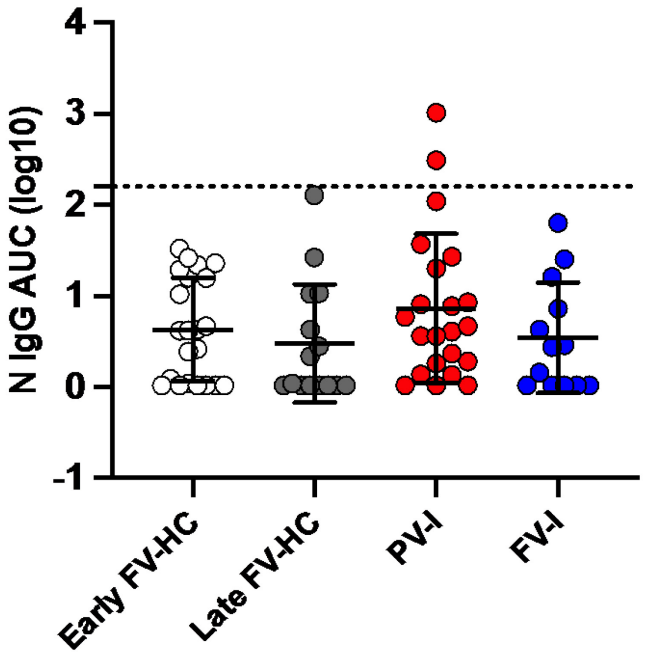


Figure 5. Antibody responses to the alpha variant correlated well with each other but correlate poorly with measures of T cell-mediated immunity. The correlation between various measures of humoral and cell-mediated immunity to the alpha variant were assessed seperately early FV-HC (**A**), late FV-HC (**B**), PV-Is (**C**), and FV-Is (**D**). Scatter plots and trendlines are shown in the lower half of matrices, and correlation coefficients (R), color coded by the strength of the correlation, are shown in the upper half of the matrices. For cell-based measures, data shown is the ratio of spot-formuing units (SFU) per smillion for treated to untreated cells, transformed on a $\log_{10}$ scale.



Supplemental figure 1. Measures of humoral and cellular immunity tend to decline with time after vaccination, regardless of infection status. Humoral immune responses are shown as the parent strain anti-S-IgG AUC (A), anti-S-RBD-IgG AUC (B), and neutralizing antibody (nAb) AUC (C) versus days since receipt of the second dose of a SARS-CoV-2 mRNA vaccine for individuals with breakthrough infection (n = 13) and healthy controls without evidence of breakthrough infection (n = 15). Humoral immune responses to the alpha variant are shown as anti-S-IgG AUC (D), anti-S-RBD-IgG AUC (E), and neutralizing antibody (nAb) AUC (F). Similarly, measures of cellular immunity are shown as a ratio of SFU/million (G)) in treated and untreated cells versus days since receipt of the second dose of a SARS-CoV-2 mRNA vaccine. Slopes and associated p-values were derived from simple linear regressions for each outcome for both groups.



Supplement figure 2. Antibody response to SARS-CoV-2 virus nucleocapsid (N) in vaccinated people. Indirect ELISAs were used to measure IgG against the nucleocapsid protein and results graphed as area under the curve (AUC) values. Data is shown for healthy controls (HCs), with no evidence of SARS-CoV-2 infection 7-14 days (white circles, early FV-HC, n =22) or 95-187 days (gray circles, Late FV-HC, n = 15) after the second dose. Red and blue circles indicate samples obtained from participants who had received either 1 (PV-I, n = 22) or 2 (FV-I, n= 13) vaccine doses, and who had confirmed SARS-CoV-2 infection. The dashed line indicates the limit of detction. A one-way ANOVA with Tukey's correction for multiple comparions was used to compare groups, and, p-values below 0.05 were considered statically significant.

APPENDIX 3

STOP 'CONTROLLING' FOR SEX AND GENDER IN GLOBAL HEALTH RESEARCH

Janna R. Shapiro, Sabra L. Klein, and Rosemary Morgan

BMJ Global Health, 2021

AP3.1 Summary box

- Sex and gender are often 'controlled' for in global health research, which forces the relationship between the predictor and outcome of interest to be the same across sex (i.e. males, females and intersex) or gender (i.e. men, women and gender minorities).
- There are many examples where controlling for sex, gender, or both led to incorrect findings that were detrimental to equitably improving global health.
- Instead of controlling for sex or gender, we urge researchers to consider sex and gender as variables of importance that can explain, rather than confound, their research.

AP3.2 Commentary

If you read any global health publication – whether it be about injury prevention, non-communicable diseases, or vaccines – you are likely to find a footnote in a table or a sentence in the statistical methods section indicating that the results were 'controlled' for sex or gender. Although the terms sex and gender are often used interchangeably in the literature, the distinction between them is important. Sex, or the biological differences between males and females, is based on the sex chromosome complement, reproductive tissues, and sex steroid concentrations. In contrast, gender is based on behaviors, occupations, and activities defined by social or cultural norms, and can refer to differences between men, women, and gender minorities. There is ample evidence that both sex and gender contribute meaningfully to global health outcomes. In this article, we explore what it means when we 'control' for sex or gender, and whether this practice can have unintended outcomes.

Statistically, we seek to uncover how predictors influence a health outcome. In global health, predictors can be demographic (e.g., age or race), medical (e.g., type or presence of treatment) or intervention-based (e.g., access to intervention or not). In many cases, a third type of variable, known as a "confounder", must also be taken into account. In the statistical

literature, there are many technical definitions of confounding.(323) For our purposes, a confounding variable is a risk factor for the outcome that is also associated with the predictor, such that the observed relationship between predictor and outcome is confused by the presence of the confounder (Figure 1A).(324) For example, in studying the relationship between age and the likelihood of getting a COVID-19 vaccine, sex or gender could be considered confounders if, in your study population, women were older and more likely to get the vaccine than men. In this case, sex, gender, or both might make it difficult to understand the causal relationship between age and the likelihood of vaccination.

Controlling for sex or gender means treating these variables as confounding factors, rather than variables of importance to the research question. Technically, this usually means that a term was included in a regression model to account for the fact that sex, gender, or both might influence the predictor and the outcome, and possibly confuse the relationship under investigation. While this allows for sex or gender differences in the outcome at baseline, it also forces this difference to be the same at all levels of the predictor (note the parallel lines in Figure 1A). For example, if we return to our example of how age (predictor) impacts the likelihood of getting the COVID-19 vaccine (outcome), controlling for sex or gender forces the difference between men and women to be the same at all ages. This approach assumes that the change in the likelihood of getting vaccinated with age is the same for men and women.

In reality, there are countless examples that demonstrate that the true relationships between our predictors and outcomes of interest do, in fact, differ by both sex and gender. We argue that relationships such as the ones depicted in Figure 1B, where the sex/gender

difference changes across levels of the predictor, are often more accurate than the one depicted in Figure 1A. There is considerable danger in ignoring such sex and gender differences by controlling for them statistically. For example, re-analysis of a randomized-controlled trial comparing different anti-retroviral therapy regimens for HIV management found higher rates of efficacy, adverse events, and treatment discontinuation in women compared to men.(325) Evidence suggests that this may be due to sex differences in drug metabolism, leading to higher drug exposure in females[2], yet the original analysis of this data completely ignored the role of sex as a biological variable by controlling for sex in statistical analyses.(326, 327) In this case, and in many others, assuming the outcome of the drug regimen was the same in men and women was not only incorrect, but detrimental to the health of women who were likely over-dosed, more likely to suffer side effects, and to discontinue treatment than men.

Although the tendency to 'control' for sex or gender is based in statistics, it is also pervasive in public health interventions and messaging, which often ignore sex as a biological variable or are blind to gender inequalities. For example, the link between gender norms and tobacco use has been thoroughly exploited by the tobacco industry. Tobacco advertising highlights associations between smoking and masculinity, or alternately, promotes smoking as a symbol of independence and sexuality to target women.(328) Public health interventions for tobacco control, however, such as increasing prices and taxation of tobacco products, often do not consider the gendered aspect of this issue.(328) The absence of gender-responsive tobacco control measures may explain why more countries saw significant decreases in the prevalence of smoking in men than in women between 2005 and 2015.(329) Once again, treating sex and

gender as confounding variables to be controlled for (or ignored), instead of meaningful sources of variation in the population, is detrimental to equitably improving global health.

Instead of controlling for sex and gender – be it statistically or in the application of an intervention - we urge those working in global health to consider sex and gender as variables of importance that can explain, rather than confuse, their research. A first, and necessary, step is to disaggregate data to interrogate how sex and gender intersect with each other or with the predictors and outcomes under investigation.(329) Disaggregation of data is a trigger for sex- and gender-responsive research that allows for understanding how the true relationship between a predictor and outcome differs between males and females or between men, women, and gender minorities. This avoids the pitfalls and unintended consequences of ignoring sex as a biological variable and gender as a social variable, and adds richness and depth to the field of global health, which undoubtedly benefits the populations we serve.

AP3.3 Figure

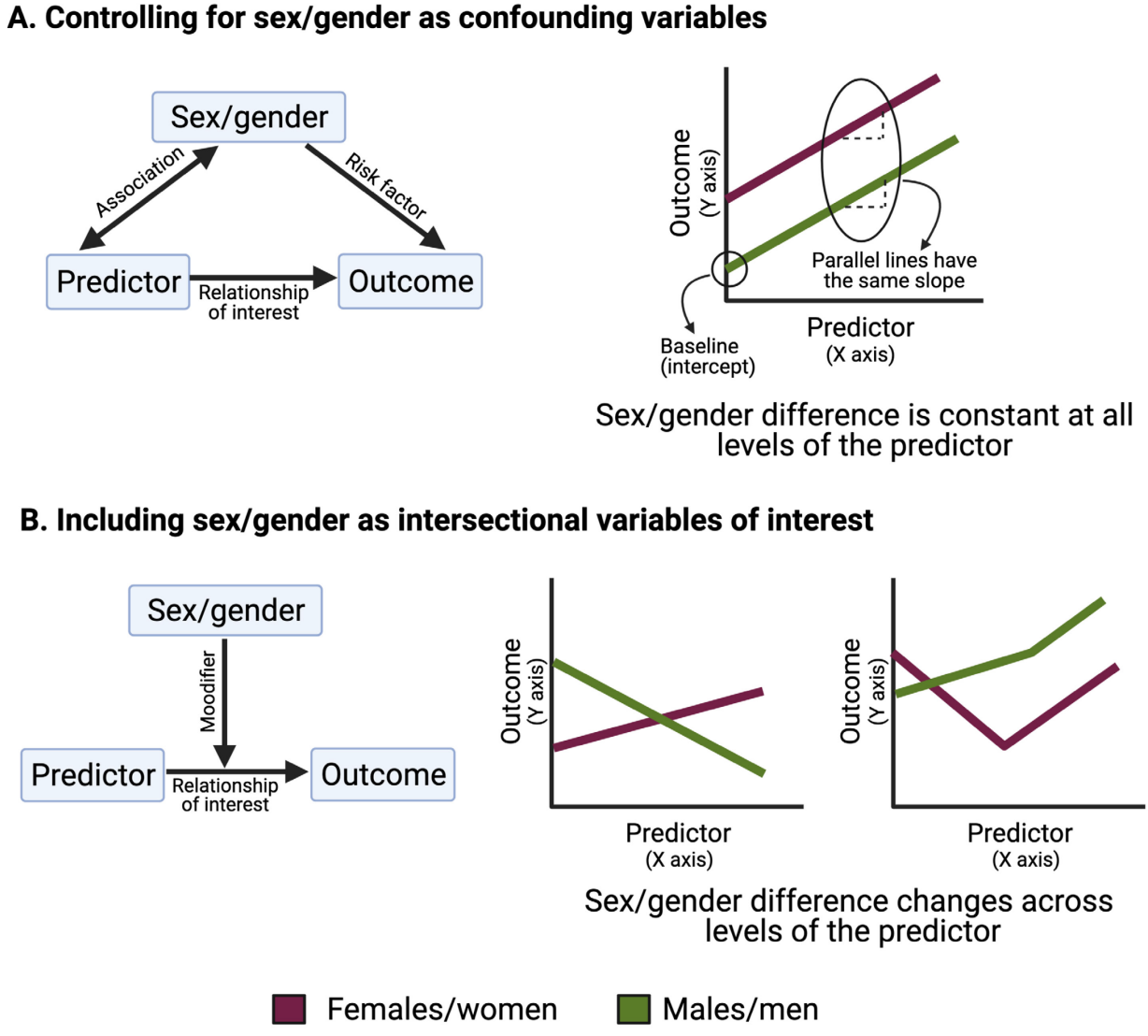


Figure 1. Controlling for sex and gender as confounding variables compared with including sex and gender as intersectional variables of interest.

APPENDIX 4

COVID-19: USE INTERSECTIONAL ANALYSES TO CLOSE GAPS IN OUTCOMES AND VACCINATION

Janna R. Shapiro, Sabra L. Klein, and Rosemary Morgan

Nature, 2021

AP4.1 Correspondence

We call for an intersectional approach to COVID-19 research and vaccination programmes to better serve people. Socially, gender, race, ethnicity, disability, class and geography are key mediators of exposure to SARS-CoV-2, access to care and the impact of lockdowns. Biologically, age, male sex, obesity and co-morbidities are important risk factors for severe disease and mortality. More investigation is needed on how these factors interact to affect health and vaccination.

For example, mild to moderate adverse events following messenger RNA COVID-19 vaccines (such as fatigue and pain) are more likely to be reported by women than men (CDC COVID Response Team, Food and Drug Administration (330). Meanwhile, fewer women, younger adults and Black individuals intend to get a COVID-19 vaccine (331). Clearly, intersectionality is key in studying and communicating the risks and benefits of vaccination.

Despite interest in how the pandemic differentially affects people, biomedical and social scientists have siloed variables to focus on one group or risk factor. Instead, we need models that evaluate, for example, how the impact of age on COVID-19 outcomes differs by sex, race, gender, co-morbidities or frailty. Such approaches have borne fruit in flu vaccine development

REFERENCES

1. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol. 2008;8(9):737-44.
2. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16(10):626-38.
3. Crooke SN, Ovsyannikova IG, Poland GA, Kennedy RB. Immunosenescence: A systems-level overview of immune cell biology and strategies for improving vaccine responses. Exp Gerontol. 2019;124:110632.
4. Flanagan KL, Fink AL, Plebanski M, Klein SL. Sex and Gender Differences in the Outcomes of Vaccination over the Life Course. Annu Rev Cell Dev Bi. 2017;33(1):577-99.
5. Fink AL, Klein SL. Sex and Gender Impact Immune Responses to Vaccines Among the Elderly. Physiology (Bethesda). 2015;30(6):408-16.
6. Scully EP, Schumock G, Fu M, Massaccesi G, Muschelli J, Betz J, et al., editors. Sex and gender differences in testing, hospital admission, clinical presentation, and drivers of severe outcomes from COVID-19. Open Forum Infect Dis; 2021: Oxford University Press US.
7. Giurgea LT, Cervantes-Medina A, Walters K-A, Scherler K, Han A, Czajkowski LM, et al. Sex Differences in Influenza: The Challenge Study Experience. J Infect Dis. 2021;Corrected proof.
8. Gnanasekaran G, Biedenbender R, Davidson HE, Gravenstein S. Vaccinations for the Older Adult. Clin Geriatr Med. 2016;32(3):609-25.
9. Piroth L, Cottenet J, Mariet A-S, Bonniaud P, Blot M, Tubert-Bitter P, et al. Comparison of the characteristics, morbidity, and mortality of COVID-19 and seasonal influenza: a nationwide, population-based retrospective cohort study. The Lancet Respiratory Medicine. 2021;9(3):251-9.
10. Hamborsky J, Kroger A, Wolfe S. Epidemiology and prevention of vaccine-preventable diseases: US Department of Health & Human Services, Centers for Disease Control and Prevention 2015.
11. Wong KC, Luscombe GM, Hawke C. Influenza infections in Australia 2009–2015: is there a combined effect of age and sex on susceptibility to virus subtypes? BMC Infectious Diseases. 2019;19(1):42.
12. Wang C-S, Wang S-T, Chou P. Efficacy and cost-effectiveness of influenza vaccination of the elderly in a densely populated and unvaccinated community. Vaccine. 2002;20(19-20):2494-9.
13. Wang X-L, Yang L, Chan K-H, Chan K-P, Cao P-H, Lau EH-Y, et al. Age and Sex Differences in Rates of Influenza-Associated Hospitalizations in Hong Kong. Am J Epidemiol. 2015;182(4):335-44.
14. Crighton E, Elliott S, Moineddin R, Kanaroglou P, Upshur R. An exploratory spatial analysis of pneumonia and influenza hospitalizations in Ontario by age and gender. Epidemiology & Infection. 2007;135(2):253-61.

15. Azziz-Baumgartner E, Cabrera AM, Cheng PY, Garcia E, Kusznierz G, Calli R, et al. Incidence of influenza-associated mortality and hospitalizations in Argentina during 2002–2009. Influenza and other respiratory viruses. 2013;7(5):710-7.
16. Kang S-J, Jung SI. Age-related morbidity and mortality among patients with COVID-19. Infection & chemotherapy. 2020;52(2):154.
17. O'Driscoll M, Dos Santos GR, Wang L, Cummings DA, Azman AS, Paireau J, et al. Age-specific mortality and immunity patterns of SARS-CoV-2. Nature. 2021;590(7844):140-5.
18. Salje H, Kiem CT, Lefrancq N, Courtejoie N, Bosetti P, Paireau J, et al. Estimating the burden of SARS-CoV-2 in France. Science. 2020;369(6500):208-11.
19. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. JAMA. 2020;323(20):2052-9.
20. Meng Y, Wu P, Lu W, Liu K, Ma K, Huang L, et al. Sex-specific clinical characteristics and prognosis of coronavirus disease-19 infection in Wuhan, China: A retrospective study of 168 severe patients. PLoS pathogens. 2020;16(4):e1008520.
21. Bauer P, Brugger J, Koenig F, Posch M. An international comparison of age and sex dependency of COVID-19 Deaths in 2020-a descriptive analysis. medRxiv. 2021.
22. Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering how biological sex impacts immune responses and COVID-19 outcomes. Nat Rev Immunol. 2020:1-6.
23. Voigt EA, Ovsyannikova IG, Kennedy RB, Grill DE, Goergen KM, Schaid DJ, et al. Sex differences in older adults' immune responses to seasonal influenza vaccination. Front Immunol. 2019;10:180.
24. Cook IF, Barr I, Hartel G, Pond D, Hampson AW. Reactogenicity and immunogenicity of an inactivated influenza vaccine administered by intramuscular or subcutaneous injection in elderly adults. Vaccine. 2006;24(13):2395-402.
25. Moehling KK, Zhai B, Schwarzmann WE, Chandran UR, Ortiz M, Nowalk MP, et al. The impact of physical frailty on the response to inactivated influenza vaccine in older adults. Aging. 2020;12.
26. Loeb N, Andrew MK, Loeb M, George KA, Haynes L, McElhaney JE, et al. Frailty is associated with increased hemagglutination-inhibition titres in a 4-year randomized trial comparing standard and high dose influenza vaccination. Open Forum Infect Dis. 2020;7(5):ofaa148.
27. Falsey Ann R, Treanor John J, Tornieporth N, Capellan J, Gorse Geoffrey J. Randomized, Double-Blind Controlled Phase 3 Trial Comparing the Immunogenicity of High-Dose and Standard-Dose Influenza Vaccine in Adults 65 Years of Age and Older. J Infect Dis. 2009;200(2):172-80.
28. Talaat KR, Greenberg ME, Lai MH, Hartel GF, Wichems CH, Rockman S, et al. A single dose of unadjuvanted novel 2009 H1N1 vaccine is immunogenic and well tolerated in young and elderly adults. J Infect Dis. 2010;202(9):1327-37.
29. Gubbels Bupp MR, Potluri T, Fink AL, Klein SL. The Confluence of Sex Hormones and Aging on Immunity. Front Immunol. 2018;9:1269.

30. Tadount F, Doyon-Plourde P, Quach C. Is there a difference in the immune response, efficacy, effectiveness and safety of seasonal influenza vaccine in males and females?–A systematic review. Vaccine. 2019;38(3):444-59.
31. Chambers C, Skowronski DM, Rose C, Serres GD, Winter A-L, Dickinson JA, et al., editors. Should sex be considered an effect modifier in the evaluation of influenza vaccine effectiveness? Open Forum Infect Dis; 2018: Oxford University Press US.
32. Govaert TM, Dinant GJ, Aretz K, Masurel N, Sprenger MJ, Knottnerus JA. Adverse reactions to influenza vaccine in elderly people: randomised double blind placebo controlled trial. Bmj. 1993;307(6910):988-90.
33. Donalisio MR, Ramalheira RM, Cordeiro R. Adverse reactions to influenza vaccine in the elderly, Campinas District, SP, 2000. Revista da Sociedade Brasileira de Medicina Tropical. 2015.
34. Keitel WA, Atmar RL, Cate TR, Petersen NJ, Greenberg SB, Ruben F, et al. Safety of high doses of influenza vaccine and effect on antibody responses in elderly persons. Arch Intern Med. 2006;166(10):1121-7.
35. Couch RB, Winokur P, Brady R, Belshe R, Chen WH, Cate TR, et al. Safety and immunogenicity of a high dosage trivalent influenza vaccine among elderly subjects. Vaccine. 2007;25(44):7656-63.
36. Cook IF. Sex differences in injection site reactions with human vaccines. Hum Vaccines. 2009;5(7):441-9.
37. Beyer W, Palache A, Kerstens R, Masurel N. Gender differences in local and systemic reactions to inactivated influenza vaccine, established by a meta-analysis of fourteen independent studies. European Journal of Clinical Microbiology and Infectious Diseases. 1996;15(1):65-70.
38. Honkanen PO, Keistinen T, Kivelä S-L. Reactions Following Administration of Influenza Vaccine Alone or With Pneumococcal Vaccine to the Elderly. Arch Intern Med. 1996;156(2):205.
39. Abe KT, Hu Q, Mozafarihashjin M, Samson R, Manguiat K, Robinson A, et al. Neutralizing antibody responses to SARS-CoV-2 variants in vaccinated Ontario long-term care home residents and workers. medRxiv. 2021:2021.08.06.21261721.
40. Brockman MA, Mwimanzi F, Lapointe HR, Sang Y, Agafitei O, Cheung P, et al. Reduced magnitude and durability of humoral immune responses to COVID-19 mRNA vaccines among older adults. J Infect Dis. 2021:jiab592.
41. Kontopoulou K, Nakas CT, Ainatzoglou A, Ifantidou A, Ntotsi P, Katsioulis C, et al. Immunogenicity of the BNT162b2 mRNA Covid-19 vaccine in elderly people over 85 years of age in Greece: the GREVAXIMO study. Aging Clin Exp Res. 2021:1-5.
42. Ríos SS, Romero MM, Zamora EBC, Sahuquillo MTT, Rizos LR, Sánchez-Jurado PM, et al. Immunogenicity of the BNT162b2 vaccine in frail or disabled nursing home residents: COVID-A study. J Am Geriatr Soc. 2021;69(6):1441-7.
43. Causa R, Almagro-Nievas D, Rivera-Izquierdo M, Benítez-Muñoz N, López-Hernández B, García-García F, et al. Antibody Response 3 Months after 2 Doses of BNT162b2 mRNA COVID-19 Vaccine in Residents of Long-Term Care Facilities. Gerontology. 2021:1-7.

44. Canaday DH, Carias L, Oyebanji O, Keresztesy D, Wilk D, Payne M, et al. Reduced BNT162b2 mRNA vaccine response in SARS-CoV-2-naive nursing home residents. medRxiv. 2021.
45. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. New England Journal of Medicine. 2020.
46. Falsey AR, Sobieszczyk ME, Hirsch I, Sproule S, Robb ML, Corey L, et al. Phase 3 safety and efficacy of AZD1222 (ChAdOx1 nCoV-19) COVID-19 vaccine. New England Journal of Medicine. 2021;385(25):2348-60.
47. Sadoff J, Gray G, Vandebosch A, Cárdenas V, Shukarev G, Grinsztejn B, et al. Safety and efficacy of single-dose Ad26. COV2. S vaccine against Covid-19. New England Journal of Medicine. 2021;384(23):2187-201.
48. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. New England Journal of Medicine. 2020;383(27):2603-15.
49. Gomes D, Beyerlein A, Katz K, Hoelscher G, Nennstiel U, Liebl B, et al. Is the BNT162b2 COVID-19 vaccine effective in elderly populations? Results from population data from Bavaria, Germany. Plos One. 2021;16(11):e0259370.
50. Hollinghurst J, North L, Perry M, Akbari A, Gravenor MB, Lyons RA, et al. COVID-19 infection risk amongst 14,104 vaccinated care home residents: a national observational longitudinal cohort study in Wales, UK, December 2020–March 2021. Age Ageing. 2021.
51. Antonelli M, Penfold RS, Merino J, Sudre CH, Molteni E, Berry S, et al. Risk factors and disease profile of post-vaccination SARS-CoV-2 infection in UK users of the COVID Symptom Study app: a prospective, community-based, nested, case-control study. Lancet Infect Dis. 2021.
52. Havers FP, Pham H, Taylor CA, Whitaker M, Patel K, Anglin O, et al. COVID-19-associated hospitalizations among vaccinated and unvaccinated adults≥ 18 years–COVID-NET, 13 states, January 1–July 24, 2021. medRxiv. 2021.
53. Choi YY, Kim M-K, Kwon HC, Kim GH. Safety Monitoring after the BNT162b2 COVID-19 Vaccine among Adults Aged 75 Years or Older. J Korean Med Sci. 2021;36(45):0.
54. Hoffmann MA, Wieler HJ, Enders P, Buchholz H-G, Plachter B. Age- and Sex-Graded Data Evaluation of Vaccination Reactions after Initial Injection of the BNT162b2 mRNA Vaccine in a Local Vaccination Center in Germany. Nato Adv Sci Inst Se. 2021;9(8):911.
55. Xiong X, Yuan J, Li M, Jiang B, Lu ZK. Age and Gender Disparities in Adverse Events Following COVID-19 Vaccination: Real-World Evidence Based on Big Data for Risk Management. Frontiers Medicine. 2021;8:700014.
56. Somiya M, Mine S, Yasukawa K, Ikeda S. Sex differences in the incidence of anaphylaxis to LNP-mRNA COVID-19 vaccines. Vaccine. 2021;39(25):3313-4.
57. Shimabukuro TT, Cole M, Su JR. Reports of anaphylaxis after receipt of mRNA COVID-19 vaccines in the US—December 14, 2020-January 18, 2021. Jama. 2021;325(11):1101-2.
58. Blumenthal KG, Robinson LB, Camargo CA, Shenoy ES, Banerji A, Landman AB, et al. Acute allergic reactions to mRNA COVID-19 vaccines. Jama. 2021;325(15):1562-5.

59. Lai C-C, Ko W-C, Chen C-J, Chen P-Y, Huang Y-C, Lee P-I, et al. COVID-19 vaccines and thrombosis with thrombocytopenia syndrome. Expert Review of Vaccines. 2021;20(8):1027-35.
60. Klein NP, Lewis N, Goddard K, Fireman B, Zerbo O, Hanson KE, et al. Surveillance for Adverse Events After COVID-19 mRNA Vaccination. JAMA. 2021;326(14):1390-9.
61. Bulati M, Caruso C, Colonna-Romano G. From lymphopoiesis to plasma cells differentiation, the age-related modifications of B cell compartment are influenced by "inflamm-ageing". Ageing Res Rev. 2017;36:125-36.
62. Pinti M, Appay V, Campisi J, Frasca D, Fülöp T, Sauce D, et al. Aging of the immune system: Focus on inflammation and vaccination. Eur J Immunol. 2016;46(10):2286-301.
63. Giglio T, Imro MA, Filaci G, Scudeletti M, Puppo F, De Cecco L, et al. Immune cell circulating subsets are affected by gonadal function. Life Sciences. 1994;54(18):1305-12.
64. Kumru S, Godekmerdan A, Yılmaz B. Immune effects of surgical menopause and estrogen replacement therapy in peri-menopausal women. Journal of Reproductive Immunology. 2004;63(1):31-8.
65. Kamada M, Irahara M, Maegawa M, Yasui T, Yamano S, Yamada M, et al. B cell subsets in postmenopausal women and the effect of hormone replacement therapy. Maturitas. 2001;37(3):173-9.
66. Maggio M, Basaria S, Ble A, Lauretani F, Bandinelli S, Ceda GP, et al. Correlation between Testosterone and the Inflammatory Marker Soluble Interleukin-6 Receptor in Older Men. The Journal of Clinical Endocrinology & Metabolism. 2006;91(1):345-7.
67. Márquez EJ, Chung C-h, Marches R, Rossi RJ, Nehar-Belaid D, Eroglu A, et al. Sexual-dimorphism in human immune system aging. Nature communications. 2020;11(1):1-17.
68. Marttila S, Jylhävä J, Nevalainen T, Nykter M, Jylhä M, Hervonen A, et al. Transcriptional Analysis Reveals Gender-Specific Changes in the Aging of the Human Immune System. Plos One. 2013;8(6):e66229.
69. Hirokawa K, Utsuyama M, Hayashi Y, Kitagawa M, Makinodan T, Fulop T. Slower immune system aging in women versus men in the Japanese population. Immun Ageing. 2013;10(1):19.
70. Wikby A, Månsson IA, Johansson B, Strindhall J, Nilsson SE. The immune risk profile is associated with age and gender: findings from three Swedish population studies of individuals 20–100 years of age. Biogerontology. 2008;9(5):299-308.
71. Goetzl EJ, Huang MC, Kon J, Patel K, Schwartz JB, Fast K, et al. Gender specificity of altered human immune cytokine profiles in aging. The FASEB Journal. 2010;24(9):3580-9.
72. Furman D, Hejblum BP, Simon N, Jojic V, Dekker CL, Thiébaut R, et al. Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. Proc Natl Acad Sci U S A. 2014;111(2):869-74.
73. Shapiro JR, Li H, Morgan R, Chen Y, Kuo H, Ning X, et al. Sex-specific effects of aging on humoral immune responses to repeated influenza vaccination in older adults. Npj Vaccines. 2021;6(1):147.
74. Potluri T, Fink AL, Sylvia KE, Dhakal S, Vermillion MS, Steeg Lv, et al. Age-associated changes in the impact of sex steroids on influenza vaccine responses in males and females. Npj Vaccines. 2019;4(1):29.

75. Bates TA, Leier HC, Lyski ZL, Goodman JR, Curlin ME, Messer WB, et al. Age-Dependent Neutralization of SARS-CoV-2 and P.1 Variant by Vaccine Immune Serum Samples. JAMA. 2021;326(9).
76. Jabal KA, Ben-Amram H, Beiruti K, Batheesh Y, Sussan C, Zarka S, et al. Impact of age, ethnicity, sex and prior infection status on immunogenicity following a single dose of the BNT162b2 mRNA COVID-19 vaccine: real-world evidence from healthcare workers, Israel, December 2020 to January 2021. Eurosurveillance. 2021;26(6):2100096.
77. Collier DA, Ferreira IATM, Kotagiri P, Datir RP, Lim EY, Touizer E, et al. Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. Nature. 2021;596(7872):417-22.
78. Bajema KL, Dahl RM, Prill MM, Meites E, Rodriguez-Barradas MC, Marconi VC, et al. Effectiveness of COVID-19 mRNA Vaccines Against COVID-19–Associated Hospitalization—Five Veterans Affairs Medical Centers, United States, February 1–August 6, 2021. Morbidity and Mortality Weekly Report. 2021;70(37):1294.
79. Robles-Fontan MM, Nieves EG, Cardona-Gerena I, Irizarry RA. Time-varying effectiveness of the mRNA-1273, BNT162b2 and Ad26. COV2. S vaccines against SARS-CoV-2 infections and COVID-19 hospitalizations and deaths: an analysis based on observational data from Puerto Rico. medRxiv. 2021.
80. Cerqueira-Silva T, Oliveira VdA, Boaventura VS, Pescarini JM, Júnior JB, Machado TM, et al. Influence of age on the effectiveness and duration of protection of Vaxzevria and CoronaVac vaccines: A population-based study. Lancet Regional Heal Am. 2022;6:100154-.
81. Haas EJ, Angulo FJ, McLaughlin JM, Anis E, Singer SR, Khan F, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. Lancet. 2021;397(10287):1819-29.
82. Chemaitelly H, Tang P, Hasan MR, AlMukdad S, Yassine HM, Benslimane FM, et al. Waning of BNT162b2 vaccine protection against SARS-CoV-2 infection in Qatar. New England Journal of Medicine. 2021;385(24):e83.
83. Aldridge RW, Yavlinsky A, Nguyen V, Eyre MT, Shrotri M, Navaratnam AMD, et al. Waning of SARS-CoV-2 antibodies targeting the Spike protein in individuals post second dose of ChAdOx1 and BNT162b2 COVID-19 vaccines and risk of breakthrough infections: analysis of the Virus Watch community cohort. medRxiv. 2021:2021.11.05.21265968.
84. Lustig Y, Sapir E, Regev-Yochay G, Cohen C, Fluss R, Olmer L, et al. BNT162b2 COVID-19 vaccine and correlates of humoral immune responses and dynamics: a prospective, single-centre, longitudinal cohort study in health-care workers. The Lancet Respiratory Medicine. 2021.
85. Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. New England Journal of Medicine. 2021;385(24):e84.
86. Agrawal U, Katikireddi SV, McCowan C, Mulholland RH, Azcoaga-Lorenzo A, Amele S, et al. COVID-19 hospital admissions and deaths after BNT162b2 and ChAdOx1 nCoV-19 vaccinations in 2· 57 million people in Scotland (EAVE II): a prospective cohort study. The Lancet Respiratory Medicine. 2021;9(12):1439 - 49.

87. Nordström P, Ballin M, Nordström A. Effectiveness of Covid-19 Vaccination Against Risk of Symptomatic Infection, Hospitalization, and Death Up to 9 Months: A Swedish Total-Population Cohort Study. Preprints with THE LANCET. 2021.
88. Liu C, Lee J, Ta C, Soroush A, Rogers JR, Kim JH, et al. A Retrospective Analysis of COVID-19 mRNA Vaccine Breakthrough Infections – Risk Factors and Vaccine Effectiveness. medRxiv. 2021:2021.10.05.21264583.
89. Chen X, Mao G, Leng SX. Frailty syndrome: an overview. Clinical interventions in aging. 2014;9:433.
90. Chen Y, Liu S, Leng SX. Chronic low-grade inflammatory phenotype (CLIP) and senescent immune dysregulation. Clinical therapeutics. 2019;41(3):400-9.
91. Gordon EH, Hubbard RE. Differences in frailty in older men and women. Medical Journal of Australia. 2020;212(4):183-8.
92. Park C, Ko FC. The Science of Frailty: Sex Differences. Clin Geriatr Med. 2021;37(4):625-38.
93. Gordon EH, Peel NM, Samanta M, Theou O, Howlett SE, Hubbard RE. Sex differences in frailty: A systematic review and meta-analysis. Exp Gerontol. 2016;89:30-40.
94. Nevalainen T, Autio A, Kummola L, Salomaa T, Junttila I, Jylhä M, et al. CD27- IgD- B cell memory subset associates with inflammation and frailty in elderly individuals but only in males. Immunity & ageing : I & A. 2019;16(1):19.
95. Gale CR, Baylis D, Cooper C, Sayer AA. Inflammatory markers and incident frailty in men and women: the English Longitudinal Study of Ageing. Age. 2013;35(6):2493-501.
96. Moehling KK, Nowalk MP, Lin CJ, Bertolet M, Ross TM, Carter CE, et al. The effect of frailty on HAI response to influenza vaccine among community-dwelling adults ≥ 50 years of age. Hum Vacc Immunother. 2018;14(2):361-7.
97. Narang V, Lu Y, Tan C, Camous XFN, Nyunt SZ, Carre C, et al. Influenza Vaccine-Induced Antibody Responses Are Not Impaired by Frailty in the Community-Dwelling Elderly With Natural Influenza Exposure. Front Immunol. 2018;9:2465.
98. DiazGranados CA, Dunning AJ, Robertson CA, Talbot HK, Landolfi V, Greenberg DP. Efficacy and immunogenicity of high-dose influenza vaccine in older adults by age, comorbidities, and frailty. Vaccine. 2015;33(36):4565-71.
99. Bauer JM, Castro AD, Bosco N, Romagny C, Diekmann R, Benyacoub J, et al. Influenza vaccine response in community-dwelling German prefrail and frail individuals. Immun Ageing. 2017;14(1):17.
100. Epps PV, Tumpey T, Pearce MB, Golding H, Higgins P, Hornick T, et al. Preexisting Immunity, Not Frailty Phenotype, Predicts Influenza Postvaccination Titers among Older Veterans. Clin Vaccine Immunol. 2017;24(3):e00498-16.
101. Yao X, Hamilton RG, Weng N-p, Xue Q-L, Bream JH, Li H, et al. Frailty is associated with impairment of vaccine-induced antibody response and increase in post-vaccination influenza infection in community-dwelling older adults. Vaccine. 2011;29(31):5015-21.
102. Andrew MK, Shinde V, Ye L, Hatchette T, Haguinet F, Santos GD, et al. The Importance of Frailty in the Assessment of Influenza Vaccine Effectiveness Against Influenza-Related Hospitalization in Elderly People. J Infect Dis. 2017;216(4):405-14.

103. Talbot HK, Nian H, Chen Q, Zhu Y, Edwards KM, Griffin MR. Evaluating the case-positive, control test-negative study design for influenza vaccine effectiveness for the frailty bias. Vaccine. 2016;34(15):1806-9.
104. Seiffert P, Konka A, Kasperczyk J, Kawa J, Lejawa M, Maślanka-Seiffert B, et al. Immunogenicity of the BNT162b2 mRNA COVID-19 vaccine in older residents of a long-term care facility: relation with age, frailty and prior infection status. Biogerontology. 2021.
105. Demaret J, Corroyer-Simovic B, Alidjinou EK, Goffard A, Trauet J, Miczek S, et al. Impaired Functional T-Cell Response to SARS-CoV-2 After Two Doses of BNT162b2 mRNA Vaccine in Older People. Front Immunol. 2021;12(4639).
106. Kwetkat A, Heppner HJ. Comorbidities in the elderly and their possible influence on vaccine response. Vaccines for Older Adults: Current Practices and Future Opportunities. 2020;43:73-85.
107. Zimmerman RK, Lauderdale DS, Tan SM, Wagener DK. Prevalence of high-risk indications for influenza vaccine varies by age, race, and income. Vaccine. 2010;28(39):6470-7.
108. Clark A, Jit M, Warren-Gash C, Guthrie B, Wang HH, Mercer SW, et al. Global, regional, and national estimates of the population at increased risk of severe COVID-19 due to underlying health conditions in 2020: a modelling study. The Lancet Global Health. 2020;8(8):e1003-e17.
109. Boikos C, Imran M, Nguyen VH, Ducruet T, Sylvester GC, Mansi JA. Effectiveness of the Adjuvanted Influenza Vaccine in Older Adults at High Risk of Influenza Complications. Nato Adv Sci Inst Se. 2021;9(8):862.
110. Butt AA, Omer SB, Yan P, Shaikh OS, Mayr FB. SARS-CoV-2 vaccine effectiveness in a high-risk national population in a real-world setting. Annals of Internal Medicine. 2021;174(10):1404-8.
111. Lewis NM, Naioti EA, Self WH, Ginde AA, Douin DJ, Talbot HK, et al. Effectiveness of mRNA vaccines in preventing COVID-19 hospitalization by age and burden of chronic medical conditions among immunocompetent US adults, March–August 2021. J Infect Dis. 2021:jiab619.
112. Shapiro JR, Klein SL, Morgan R. Stop 'controlling' for sex and gender in global health research. Bmj Global Heal. 2021;6(4):e005714.
113. Columb M, Atkinson M. Statistical analysis: sample size and power estimations. Bja Education. 2016;16(5):159-61.
114. Klein SL, Schiebinger L, Stefanick ML, Cahill L, Danska J, De Vries GJ, et al. Opinion: sex inclusion in basic research drives discovery. Proc National Acad Sci. 2015;112(17):5257-8.
115. United Nations Department of Economic and Social Affairs Population Division. World Population Prospects 2019: Highlights. 2019.
116. Macias AE, McElhaney JE, Chaves SS, Nealon J, Nunes MC, Samson SI, et al. The disease burden of influenza beyond respiratory illness. Vaccine. 2020.
117. Iuliano AD, Roguski KM, Chang HH, Muscatello DJ, Palekar R, Tempia S, et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. Lancet. 2018;391(10127):1285-300.

118. Global Burden of Disease Influenza Collaborators, Troeger CE, Blacker BF, Khalil IA, Zimsen SRM, Albertson SB, et al. Mortality, morbidity, and hospitalisations due to influenza lower respiratory tract infections, 2017: an analysis for the Global Burden of Disease Study 2017. Lancet Respir Medicine. 2018;7(1):69-89.
119. Grohskopf LA, Alyanak E, Broder KR, Blanton LH, Fry AM, Jernigan DB, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices—United States, 2020–21 influenza season. MMWR Recommendations and Reports. 2020;69(8):1.
120. DiazGranados CA, Dunning AJ, Kimmel M, Kirby D, Treanor J, Collins A, et al. Efficacy of High-Dose versus Standard-Dose Influenza Vaccine in Older Adults. New England Journal of Medicine. 2014;371(7):635-45.
121. Centers for Disease Control and Prevention. Flu Vaccination Coverage, United States, 2019–20 Influenza Season 2020 [Available from: https://www.cdc.gov/flu/fluvaxview/coverage-1920estimates.htm.
122. Weinberger B. Vaccines for the elderly: current use and future challenges. Immunity & ageing : I & A. 2018;15(1):3.
123. Demicheli V, Jefferson T, Ferroni E, Rivetti A, Di Pietrantonj C. Vaccines for preventing influenza in healthy adults. Cochrane database of systematic reviews. 2018(2).
124. Nichols MK, Andrew MK, Hatchette TF, Ambrose A, Boivin G, Bowie W, et al. Influenza vaccine effectiveness to prevent influenza-related hospitalizations and serious outcomes in Canadian adults over the 2011/12 through 2013/14 influenza seasons: A pooled analysis from the Canadian Immunization Research Network (CIRN) Serious Outcomes Surveillance (SOS Network). Vaccine. 2018;36(16):2166-75.
125. Rondy M, El Omeiri N, Thompson MG, Levêque A, Moren A, Sullivan SG. Effectiveness of influenza vaccines in preventing severe influenza illness among adults: a systematic review and meta-analysis of test-negative design case-control studies. Journal of Infection. 2017;75(5):381-94.
126. Kwong JC, Chung H, Jung JK, Buchan SA, Campigotto A, Campitelli MA, et al. The impact of repeated vaccination using 10-year vaccination history on protection against influenza in older adults: a test-negative design study across the 2010/11 to 2015/16 influenza seasons in Ontario, Canada. Eurosurveillance. 2020;25(1):1900245.
127. McLean HQ, Thompson MG, Sundaram ME, Meece JK, McClure DL, Friedrich TC, et al. Impact of Repeated Vaccination on Vaccine Effectiveness Against Influenza A(H3N2) and B During 8 Seasons. Clin Infect Dis. 2014;59(10):1375-85.
128. Sung M-H, Shen Y, Handel A, Bahl J, Ross TM. Longitudinal Assessment of Immune Responses to Repeated Annual Influenza Vaccination in a Human Cohort of Adults and Teenagers. Front Immunol. 2021;12:472.
129. Belongia EA, Skowronski DM, McLean HQ, Chambers C, Sundaram ME, Serres GD. Repeated annual influenza vaccination and vaccine effectiveness: review of evidence. Expert Review of Vaccines. 2017;16(7):723-36.
130. Bartoszko JJ, McNamara IF, Aras OAZ, Hylton DA, Zhang YB, Malhotra D, et al. Does consecutive influenza vaccination reduce protection against influenza: A systematic review and meta-analysis. Vaccine. 2018;36(24):3434-44.

131. Cheng AC, Macartney KK, Waterer GW, Kotsimbos T, Kelly PM, Blyth CC, et al. Repeated Vaccination Does Not Appear to Impact Upon Influenza Vaccine Effectiveness Against Hospitalization With Confirmed Influenza. Clin Infect Dis. 2017;64(11):1564-72.
132. Casado I, Domínguez Á, Toledo D, Chamorro J, Astray J, Egurrola M, et al. Repeated influenza vaccination for preventing severe and fatal influenza infection in older adults: a multicentre case–control study. Canadian Medical Association Journal. 2018;190(1):E3-E12.
133. Örtqvist Å, Brytting M, Leval A, Hergens M-P. Impact of repeated influenza vaccinations in persons over 65 years of age: A large population-based cohort study of severe influenza over six consecutive seasons, 2011/12–2016/17. Vaccine. 2018;36(37):5556-64.
134. Jang H, Ross TM. Preexisting influenza specific immunity and vaccine effectiveness. Expert Review of Vaccines. 2019;18(10):1-9.
135. Henry C, Palm A-KE, Krammer F, Wilson PC. From Original Antigenic Sin to the Universal Influenza Virus Vaccine. Trends Immunol. 2018;39(1):70-9.
136. Linderman SL, Ellebedy AH, Davis C, Eberhardt CS, Antia R, Ahmed R, et al. Influenza Immunization in the Context of Preexisting Immunity. Csh Perspect Med. 2020:a040964.
137. Guthmiller JJ, Utset HA, Wilson PC. B Cell Responses against Influenza Viruses: Short-Lived Humoral Immunity against a Life-Long Threat. Viruses. 2021;13(6):965.
138. Guthmiller JJ, Wilson PC. Harnessing immune history to combat influenza viruses. Current Opinion in Immunology. 2018;53:187-95.
139. Ellebedy AH. Immunizing the Immune: Can We Overcome Influenza's Most Formidable Challenge? Nato Adv Sci Inst Se. 2018;6(4):68.
140. Ciarambino T, Para O, Giordano M. Immune system and COVID-19 by sex differences and age. Women's Health. 2021;17:17455065211022262.
141. Meester I, Manilla-Muñoz E, León-Cachón RB, Paniagua-Frausto GA, Carrión-Alvarez D, Ruiz-Rodríguez CO, et al. SeXY chromosomes and the immune system: reflections after a comparative study. Biol Sex Differ. 2020;11(1):1-13.
142. Chen Y, Klein SL, Garibaldi BT, Li H, Wu C, Osevala NM, et al. Aging in COVID-19: Vulnerability, immunity and intervention. Ageing Res Rev. 2020:101205.
143. Frasca D, Ferracci F, Diaz A, Romero M, Lechner S, Blomberg BB. Obesity decreases B cell responses in young and elderly individuals. Obesity. 2016;24(3):615-25.
144. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in Older Adults: Evidence for a Phenotype. Journals Gerontology Ser Biological Sci Medical Sci. 2001;56(3):M146-M57.
145. Ward BJ, Pillet S, Charland N, Trepanier S, Couillard J, Landry N. The establishment of surrogates and correlates of protection: Useful tools for the licensure of effective influenza vaccines? Hum Vacc Immunother. 2018;14(3):647-56.
146. Al-Khayatt R, Jennings R, Potter C. Interpretation of responses and protective levels of antibody against attenuated influenza A viruses using single radial haemolysis. Epidemiology & Infection. 1984;93(2):301-12.
147. Hobson D, Curry R, Beare A, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. Epidemiology & Infection. 1972;70(4):767-77.

148. Reber A, Katz J. Immunological assessment of influenza vaccines and immune correlates of protection. Expert Review of Vaccines. 2013;12(5):519-36.
149. Klein SL, Jedlicka A, Pekosz A. The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis. 2010;10(5):338-49.
150. Kuo H, Shapiro JR, Dhakal S, Morgan R, Fink AL, Lui H, et al. Sex-specific effects of age and body mass index on antibody responses to seasonal influenza vaccines in healthcare workers. Vaccine. 2021.
151. Weinberger B, Herndler-Brandstetter D, Schwanninger A, Weiskopf D, Grubeck-Loebenstein B. Biology of Immune Responses to Vaccines in Elderly Persons. Clin Infect Dis. 2008;46(7):1078-84.
152. Centers for Disease Control and Prevention. Influenza Historic Timeline 2019 [Available from: https://www.cdc.gov/flu/pandemic-resources/pandemic-timeline-1930-and-beyond.htm.
153. Bitzegeio J, Majowicz S, Matysiak-Klose D, Sagebiel D, Werber D. Estimating age-specific vaccine effectiveness using data from a large measles outbreak in Berlin, Germany, 2014/15: evidence for waning immunity. Eurosurveillance. 2019;24(17):1800529.
154. Crooke SN, Riggenbach MM, Ovsyannikova IG, Warner ND, Chen M-H, Hao L, et al. Durability of humoral immune responses to rubella following MMR vaccination. Vaccine. 2020;38(51):8185-93.
155. Savage RD, Bell CA, Righolt CH, Wilkinson K, Schwartz KL, Chen C, et al. A multisite study of pertussis vaccine effectiveness by time since last vaccine dose from three Canadian provinces: A Canadian Immunization Research Network study. Vaccine. 2021;39(20):2772-9.
156. Posuwan N, Wanlapakorn N, Sa-Nguanmoo P, Wasitthankasem R, Vichaiwattana P, Klinfueng S, et al. The success of a universal hepatitis B immunization program as part of Thailand's EPI after 22 years' implementation. Plos One. 2016;11(3):e0150499.
157. Miller MS, Gardner TJ, Krammer F, Aguado LC, Tortorella D, Basler CF, et al. Neutralizing Antibodies Against Previously Encountered Influenza Virus Strains Increase over Time: A Longitudinal Analysis.
158. Liu M, Zhao X, Hua S, Du X, Peng Y, Li X, et al. Antigenic patterns and evolution of the human influenza A (H1N1) virus. Scientific reports. 2015;5(1):1-8.
159. Vijaykrishna D, Holmes EC, Joseph U, Fourment M, Su YC, Halpin R, et al. The contrasting phylodynamics of human influenza B viruses. Elife. 2015;4:e05055.
160. Caini S, Kusznierz G, Garate VV, Wangchuk S, Thapa B, Júnior FJdP, et al. The epidemiological signature of influenza B virus and its B/Victoria and B/Yamagata lineages in the 21st century. Plos One. 2019;14(9):e0222381.
161. World Health Organization. WHO recommendations on the composition of influenza virus vaccines 2021 [Available from: https://www.who.int/influenza/vaccines/virus/recommendations/en/.
162. Auladell M, Jia X, Hensen L, Chua B, Fox A, Nguyen THO, et al. Recalling the Future: Immunological Memory Toward Unpredictable Influenza Viruses. Front Immunol. 2019;10:1400.

163. Hannoun C. The evolving history of influenza viruses and influenza vaccines. Expert review of vaccines. 2013;12(9):1085-94.
164. Dunning AJ, DiazGranados CA, Voloshen T, Hu B, Landolfi VA, Talbot HK. Correlates of Protection against Influenza in the Elderly: Results from an Influenza Vaccine Efficacy Trial. Clin Vaccine Immunol. 2016;23(3):228-35.
165. Roy M, Sherrard L, Dubé È, Gilbert NL. Determinants of non-vaccination against seasonal influenza. Health reports. 2018;29(10):12-22.
166. Shapiro JR, Klein SL, Morgan R. COVID-19: use intersectional analyses to close gaps in outcomes and vaccination. Nature. 2021;591(7849):202.
167. Greenberg DP, Robertson CA, Noss MJ, Blatter MM, Biedenbender R, Decker MD. Safety and immunogenicity of a quadrivalent inactivated influenza vaccine compared to licensed trivalent inactivated influenza vaccines in adults. Vaccine. 2013;31(5):770-6.
168. Schafer JL, Graham JW. Missing data: our view of the state of the art. Psychological methods. 2002;7(2):147.
169. Newson RB. Sensible parameters for univariate and multivariate splines. The Stata Journal. 2012;12(3):479-504.
170. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine. New England Journal of Medicine. 2020.
171. Canaday DH, Carias L, Oyebanji OA, Keresztesy D, Wilk D, Payne M, et al. Reduced BNT162b2 Messenger RNA Vaccine Response in Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)–Naive Nursing Home Residents. Clin Infect Dis. 2021;73(11):2112-5.
172. Bauer P, Brugger J, König F, Posch M. An international comparison of age and sex dependency of COVID-19 deaths in 2020: a descriptive analysis. Scientific reports. 2021;11(1):1-11.
173. Bischof E, Wolfe J, Klein SL. Clinical trials for COVID-19 should include sex as a variable. The Journal of Clinical Investigation. 2020;130(7).
174. Shapiro JR, Morgan R, Leng SX, Klein SL. Roadmap for sex-responsive influenza and COVID-19 vaccine research in older adults. Frontiers in Aging. 2022;in press.
175. Zhong D, Xiao S, Debes AK, Egbert ER, Caturegli P, Colantuoni E, et al. Durability of Antibody Levels After Vaccination With mRNA SARS-CoV-2 Vaccine in Individuals With or Without Prior Infection. JAMA. 2021.
176. Klein SL, Pekosz A, Park H-S, Ursin RL, Shapiro JR, Benner SE, et al. Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population. The Journal of Clinical Investigation. 2020.
177. NCI Serological Sciences Network for COVID-19 (SeroNet): National Cancer Institute; [cited 2022. Available from: https://www.cancer.gov/research/key-initiatives/covid-19/coronavirus-research-initiatives/serological-sciences-network.
178. Karaba AH, Zhu X, Liang T, Wang KH, Rittenhouse AG, Akinde O, et al. A Third Dose of SARS-CoV-2 Vaccine Increases Neutralizing Antibodies Against Variants of Concern in Solid Organ Transplant Recipients. American Journal of Transplantation. 2021.

179. Park H-S, Shapiro JR, Sitaras I, Woldemeskel BA, Garliss C, Dziedzic A, et al. Adaptive immune responses in vaccinated patients with symptomatic SARS-CoV-2 Alpha infection. JCI Insight. 2022.
180. Stadlbauer D, Amanat F, Chromikova V, Jiang K, Strohmeier S, Arunkumar GA, et al. SARS-CoV-2 seroconversion in humans: a detailed protocol for a serological assay, antigen production, and test setup. Current protocols in microbiology. 2020;57(1):e100.
181. Matsuyama S, Nao N, Shirato K, Kawase M, Saito S, Takayama I, et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proc Natl Acad Sci U S A. 2020;117(13):7001-3.
182. Luo CH, Morris CP, Sachithanandham J, Amadi A, Gaston DC, Li M, et al. Infection with the SARS-CoV-2 Delta Variant is Associated with Higher Recovery of Infectious Virus Compared to the Alpha Variant in both Unvaccinated and Vaccinated Individuals. Clin Infect Dis. 2021.
183. Fall A, Eldesouki RE, Sachithanandham J, Morris CP, Norton JM, Gaston DC, et al. The displacement of the SARS-CoV-2 variant Delta with Omicron: An investigation of hospital admissions and upper respiratory viral loads. EBioMedicine. 2022;79:104008.
184. Waggoner JJ, Stittleburg V, Pond R, Saklawi Y, Sahoo MK, Babiker A, et al. Triplex real-time RT-PCR for severe acute respiratory syndrome coronavirus 2. Emerg Infect Dis. 2020;26(7):1633.
185. Schaecher SR, Touchette E, Schriewer J, Buller RM, Pekosz A. Severe acute respiratory syndrome coronavirus gene 7 products contribute to virus-induced apoptosis. J Virol. 2007;81(20):11054-68.
186. Schaecher SR, Mackenzie JM, Pekosz A. The ORF7b protein of severe acute respiratory syndrome coronavirus (SARS-CoV) is expressed in virus-infected cells and incorporated into SARS-CoV particles. J Virol. 2007;81(2):718-31.
187. Reed L, Meunch H. A simple method of estimating 50 percent endpoints. Am J Epidemiol. 1938;27(3):493-7.
188. Schaecher SR, Stabenow J, Oberle C, Schriewer J, Buller RM, Sagartz JE, et al. An immunosuppressed Syrian golden hamster model for SARS-CoV infection. Virology. 2008;380(2):312-21.
189. Grobben M, Straten Kvd, Brouwer PJM, Brinkkemper M, Maisonnasse P, Dereuddre-Bosquet N, et al. Cross-reactive antibodies after SARS-CoV-2 infection and vaccination. eLife. 2021;10:e70330.
190. Anderson EM, Eiloa T, Goodwin E, Bolton MJ, Gouma S, Goel RR, et al. SARS-CoV-2 infections elicit higher levels of original antigenic sin antibodies compared to SARS-CoV-2 mRNA vaccinations. medRxiv. 2021.
191. Wang J, Tong Y, Li D, Li J, Li Y. The Impact of Age Difference on the Efficacy and Safety of COVID-19 Vaccines: A Systematic Review and Meta-Analysis. Front Immunol. 2021;12.
192. Mwimanzi F, Lapointe H, Cheung PK, Sang Y, Yaseen F, Umviligihozo G, et al. Older Adults Mount Less Durable Humoral Responses to a Two-dose COVID-19 mRNA Vaccine Regimen, but Strong Initial Responses to a Third Dose. medRxiv. 2022.
193. Goldblatt D, Fiore-Gartland A, Johnson M, Hunt A, Bengt C, Zavadska D, et al. Towards a population-based threshold of protection for COVID-19 vaccines. Vaccine. 2021.

194. Carreño JM, Alshammary H, Tcheou J, Singh G, Raskin A, Kawabata H, et al. Activity of convalescent and vaccine serum against SARS-CoV-2 Omicron. Nature. 2021.
195. Bartsch YC, Tong X, Kang J, Avendaño MJ, Serrano EF, García-Salum T, et al. Omicron variant Spike-specific antibody binding and Fc activity is preserved in recipients of mRNA or inactivated COVID-19 vaccines. Sci Transl Med. 2022:eabn9243.
196. Chan CEZ, Seah SGK, Chye DH, Massey S, Torres M, Lim APC, et al. The Fc-mediated effector functions of a potent SARS-CoV-2 neutralizing antibody, SC31, isolated from an early convalescent COVID-19 patient, are essential for the optimal therapeutic efficacy of the antibody. Plos One. 2021;16(6):e0253487.
197. Gorman MJ, Patel N, Guebre-Xabier M, Zhu AL, Atyeo C, Pullen KM, et al. Fab and Fc contribute to maximal protection against SARS-CoV-2 following NVX-CoV2373 subunit vaccine with Matrix-M vaccination. Cell Reports Medicine. 2021;2(9).
198. Vorland CJ. Statistics: Sex difference analyses under scrutiny. Elife. 2021;10:e74135.
199. Shattuck-Heidorn H, Danielsen AC, Gompers A, Bruch JD, Zhao H, Boulicault M, et al. A finding of sex similarities rather than differences in COVID-19 outcomes. Nature. 2021;597(7877):E7-E9.
200. Hsieh C-L, Goldsmith JA, Schaub JM, DiVenere AM, Kuo H-C, Javanmardi K, et al. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. Science. 2020;369(6510):1501-5.
201. MacDonald NE, The Sage Working Group on Vaccine Hesitancy. Vaccine hesitancy: Definition, scope and determinants. Vaccine. 2015;33(34):4161-4.
202. Salmon DA, Dudley MZ, Glanz JM, Omer SB. Vaccine Hesitancy Causes, Consequences, and a Call to Action. American Journal of Preventive Medicine. 2015;49(6):S391-S8.
203. Dubé E, Laberge C, Guay M, Bramadat P, Roy R, Bettinger JA. Vaccine hesitancy. Hum Vacc Immunother. 2013;9(8):1763-73.
204. Brewer NT, Chapman GB, Rothman AJ, Leask J, Kempe A. Increasing vaccination: putting psychological science into action. Psychological Science in the Public Interest. 2017;18(3):149-207.
205. Reny TT. Masculine Norms and Infectious Disease: The Case of COVID-19. Polit Gender. 2020;16(4):1-8.
206. Courtenay WH. Constructions of masculinity and their influence on men's well-being: a theory of gender and health. Soc Sci Med. 2000;50(10):1385-401.
207. Gustafson PE. Gender Differences in Risk Perception: Theoretical and Methodological Perspectives. Risk Analysis. 1998;18(6):805-11.
208. Alsharawy A, Spoon R, Smith A, Ball S. Gender Differences in Fear and Risk Perception During the COVID-19 Pandemic. Front Psychol. 2021;12:689467.
209. Witteman HO, Chipenda Dansokho S, Exe N, Dupuis A, Provencher T, Zikmund-Fisher BJ. Risk communication, values clarification, and vaccination decisions. Risk Analysis. 2015;35(10):1801-19.
210. Morgan R, Klein SL. The intersection of sex and gender in the treatment of influenza. Curr Opin Virol. 2019;35(mBio 8 2017):35-41.
211. Kapilashrami A, Hankivsky O. Intersectionality and why it matters to global health. Lancet. 2018;391(10140):2589-91.

212. Quinn S, Jamison A, Musa D, Hilyard K, Freimuth V. Exploring the Continuum of Vaccine Hesitancy Between African American and White Adults: Results of a Qualitative Study. Plos Curr. 2016;8:ecurrents.outbreaks.3e4a5ea39d8620494e2a2c874a3c4201.
213. Jamison AM, Quinn SC, Freimuth VS. "You don't trust a government vaccine": Narratives of institutional trust and influenza vaccination among African American and white adults. Soc Sci Med. 2019;221:87-94.
214. Bhanu C, Gopal DP, Walters K, Chaudhry UAR. Vaccination uptake amongst older adults from minority ethnic backgrounds: A systematic review. PLoS Medicine. 2021;18(11):e1003826.
215. Harris LM, Chin NP, Fiscella K, Humiston S. Barrier to pneumococcal and influenza vaccinations in Black elderly communities: mistrust. J Natl Med Assoc. 2006;98(10):1678-84.
216. Quinn SC, Hilyard KM, Jamison AM, An J, Hancock GR, Musa D, et al. The influence of social norms on flu vaccination among African American and White adults. Health Educ Res. 2017;32(6):473-86.
217. Freimuth VS, Jamison A, Hancock G, Musa D, Hilyard K, Quinn SC. The Role of Risk Perception in Flu Vaccine Behavior among African-American and White Adults in the United States. Risk Analysis. 2017;37(11):2150-63.
218. Freimuth VS, Jamison AM, An J, Hancock GR, Quinn SC. Determinants of trust in the flu vaccine for African Americans and Whites. Soc Sci Medicine 1982. 2017;193:70-9.
219. Quinn SC, Jamison A, Freimuth VS, An J, Hancock GR, Musa D. Exploring racial influences on flu vaccine attitudes and behavior: Results of a national survey of White and African American adults. Vaccine. 2017;35(8):1167-74.
220. Fast HE, Zell E, Murthy BP, Murthy N, Meng L, Scharf LG, et al. Booster and Additional Primary Dose COVID-19 Vaccinations Among Adults Aged ≥65 Years — United States, August 13, 2021–November 19, 2021. Morbidity and Mortality Weekly Report. 2021;70(50):1735-9.
221. International Vaccine Access Center and Morgan State University. Evaluation of Baltimore City Flu Vaccination Initiative, 2020. Baltimore, MD; 2021.
222. Kreps S, Kriner D. Factors influencing Covid-19 vaccine acceptance across subgroups in the United States: Evidence from a conjoint experiment. Vaccine. 2021;39(24):3250-8.
223. QuickFacts Baltimore City, Maryland (County): United States Census Bureau, ; 2021 [Available from: https://www.census.gov/quickfacts/fact/table/baltimorecitymaryland/INC110219.
224. United Sates Census Bureau. American Community Survey Data 2019 [Available from: https://www.census.gov/programs-surveys/acs/data.html.
225. Maryland Department of Health. Coronavirus Disease 2019 (COVID-19) Outbreak - Vaccinations in Maryland 2022 [updated 2/21/22. Available from: https://coronavirus.maryland.gov/#Vaccine.
226. Maul A, Reddy K, Joshi M. Vaccine equity index shows reduction in Maryland COVID-19 vaccination disparity in less than two months. NEJM Catalyst Innovations in Care Delivery. 2021;2(2).

227. Cardona S, Felipe N, Fischer K, Sehgal NJ, Schwartz BE. Vaccination disparity: quantifying racial inequity in COVID-19 vaccine administration in Maryland. Journal of Urban Health. 2021;98(4):464-8.
228. Lee KH, Marx M. Baltimore City: County-level comparisons of COVID-19 cases and deaths. 2021.
229. Centers for Disease Control and Prevention. COVIDVavView - COVID-19 Vaccination Coverage and Vaccine Confidence Among Adults 2022 [Available from: https://www.cdc.gov/vaccines/imz-managers/coverage/covidvaxview/interactive/adults.html.
230. Ritchie J, Lewis J. Qualitative research practice: A guide for social science students and researchers. London: sage; 2003.
231. Gale NK, Heath G, Cameron E, Rashid S, Redwood S. Using the framework method for the analysis of qualitative data in multi-disciplinary health research. Bmc Med Res Methodol. 2013;13(1):1-8.
232. Heise L, Greene ME, Opper N, Stavropoulou M, Harper C, Nascimento M, et al. Gender inequality and restrictive gender norms: framing the challenges to health. Lancet. 2019;393(10189):2440-54.
233. Corbie-Smith G. Vaccine Hesitancy Is a Scapegoat for Structural Racism. Jama Heal Forum. 2021;2(3):e210434.
234. Bajaj SS, Stanford FC. Beyond Tuskegee — Vaccine Distrust and Everyday Racism. New England Journal of Medicine. 2021;384(5):e12.
235. Centers for Disease Control. Demographic trends of people receiving COVID-19 vaccinations in the United States. 2021.
236. Finucane ML, Slovic P, Mertz CK, Flynn J, Satterfield TA. Gender, race, and perceived risk: The 'white male' effect. Heal Risk Soc. 2000;2(2):159-72.
237. Green MA, Evans CR, Subramanian SV. Can intersectionality theory enrich population health research? Soc Sci Med. 2017;178:214-6.
238. Larson E, George A, Morgan R, Poteat T. 10 Best resources on… intersectionality with an emphasis on low- and middle-income countries. Health Policy and Planning. 2016;31(8):964-9.
239. Agénor M, Pérez AE, Peitzmeier SM, Potter J, Borrero S. Human Papillomavirus Vaccination Initiation Among Sexual Orientation Identity and Racial/Ethnic Subgroups of Black and White U.S. Women and Girls: An Intersectional Analysis. J Women's Heal. 2018;27(11):1349-58.
240. Feletto M, Sharkey A. The influence of gender on immunisation: using an ecological framework to examine intersecting inequities and pathways to change. Bmj Global Heal. 2019;4(5):e001711.
241. Tan L. Adult vaccination: Now is the time to realize an unfulfilled potential. Hum Vacc Immunother. 2015;11(9):2158-66.
242. World Health Organization. Immunization agenda 2030. Geneva, Switzerland2018.
243. Okwo-Bele J-M, Cherian T. The expanded programme on immunization: a lasting legacy of smallpox eradication. Vaccine. 2011;29:D74-D9.

244. Doherty TM, Giudice GD, Maggi S. Adult vaccination as part of a healthy lifestyle: moving from medical intervention to health promotion. Ann Med. 2019;51(2):128-40.
245. Coll PP, Costello VW, Kuchel GA, Bartley J, McElhaney JE. The Prevention of Infections in Older Adults: Vaccination. J Am Geriatr Soc. 2020;68(1):207-14.
246. Privor-Dumm LA, Poland GA, Barratt J, Durrheim DN, Knoll MD, Vasudevan P, et al. A global agenda for older adult immunization in the COVID-19 era: a roadmap for action. Vaccine. 2020.
247. Jaoude JA, Kouzy R, Lin TA, Alam MBE, Subbiah V, Taniguchi CM, et al. Exclusion of Older Adults in COVID-19 Clinical Trials. Mayo Clin Proc. 2020;95(10):2293-4.
248. Ridda I, MacIntyre CR, Lindley RI, Tan TC. Difficulties in recruiting older people in clinical trials: An examination of barriers and solutions. Vaccine. 2010;28(4):901-6.
249. Witham MD, George J. Clinical trial design for older people—time for a rethink. Qjm Int J Medicine. 2014;107(1):15-6.
250. Rochon PA, Mason R, Gurwitz JH. Increasing the visibility of older women in clinical research. Lancet. 2020;395(10236):1530-2.
251. Liljas AE, Walters K, Jovicic A, Iliffe S, Manthorpe J, Goodman C, et al. Strategies to improve engagement of 'hard to reach'older people in research on health promotion: a systematic review. BMC public health. 2017;17(1):1-12.
252. Novick G. Is there a bias against telephone interviews in qualitative research? Research in nursing & health. 2008;31(4):391-8.
253. Menon S, Sonderegger P, Totapally S. Five questions to consider when conducting COVID-19 phone research. Bmj Global Heal. 2021;6(Suppl 5):e004917.
254. Azad A, Sernbo E, Svärd V, Holmlund L, Björk Brämberg E. Conducting in-depth interviews via mobile phone with persons with common mental disorders and multimorbidity: the challenges and advantages as experienced by participants and researchers. Int J Environ Res Pu. 2021;18(22):11828.
255. Chang AY, Skirbekk VF, Tyrovolas S, Kassebaum NJ, Dieleman JL. Measuring population ageing: an analysis of the Global Burden of Disease Study 2017. Lancet Public Heal. 2019;4(3):e159-e67.
256. Jazwinski SM, Kim S. Examination of the Dimensions of Biological Age. Frontiers Genetics. 2019;10:263.
257. Ribeiro AR, Howlett SE, Fernandes A. Frailty - A promising concept to evaluate disease vulnerability. Mech Ageing Dev. 2020;187:111217.
258. Bandeen-Roche K, Seplaki CL, Huang J, Buta B, Kalyani RR, Varadhan R, et al. Frailty in Older Adults: A Nationally Representative Profile in the United States. Journals Gerontology Ser Biological Sci Medical Sci. 2015;70(11):1427-34.
259. Dent E, Martin FC, Bergman H, Woo J, Romero-Ortuno R, Walston JD. Management of frailty: opportunities, challenges, and future directions. Lancet Lond Engl. 2019;394(10206):1376-86.
260. Leng SX, Margolick JB. Aging, sex, inflammation, frailty, and CMV and HIV infections. Cell Immunol. 2020;348:104024.
261. Yao X, Li H, Leng SX. Inflammation and Immune System Alterations in Frailty. Clin Geriatr Med. 2011;27(1):79-87.

262. Poland GA, Ovsyannikova IG, Kennedy RB. Personalized vaccinology: A review. Vaccine. 2017;36(36):5350-7.
263. Klein SL, Poland GA. Personalized vaccinology: One size and dose might not fit both sexes. Vaccine. 2013;31(23):2599-600.
264. Yakerson A. Women in clinical trials: a review of policy development and health equity in the Canadian context. Int J Equity Health. 2019;18(1):1-8.
265. Vidaver RM, Lafleur B, Tong C, Bradshaw R, Marts SA. Women subjects in NIH-funded clinical research literature: lack of progress in both representation and analysis by sex. J Womens Health Gend Based Med. 2000;9(5):495-504.
266. Arnegard ME, Whitten LA, Hunter C, Clayton JA. Sex as a Biological Variable: A 5-Year Progress Report and Call to Action. J Women's Heal. 2020;29(6):858-64.
267. Shapiro JR, Morgan R, Leng SX, Klein SL. Roadmap for Sex-Responsive Influenza and COVID-19 Vaccine Research in Older Adults. Frontiers in Aging. 2022;3.
268. Morgan R, George A, Ssali S, Hawkins K, Molyneux S, Theobald S. How to do (or not to do)… gender analysis in health systems research. Health policy and planning. 2016;31(8):1069-78.
269. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol. 2021;21(2):83-100.
270. Schotsaert M, Saelens X, Leroux-Roels G. Influenza vaccines: T-cell responses deserve more attention. Expert Review of Vaccines. 2012;11(8):949-62.
271. Korenkov D, Isakova-Sivak I, Rudenko L. Basics of CD8 T-cell immune responses after influenza infection and vaccination with inactivated or live attenuated influenza vaccine. Expert Review of Vaccines. 2018;17(11):977-87.
272. Altenburg AF, Rimmelzwaan GF, de Vries RD. Virus-specific T cells as correlate of (cross-) protective immunity against influenza. Vaccine. 2015;33(4):500-6.
273. Sureshchandra S, Lewis SA, Doratt BM, Jankeel A, Ibraim IC, Messaoudi I. Single-cell profiling of T and B cell repertoires following SARS-CoV-2 mRNA vaccine. JCI insight. 2021;6(24).
274. Oberhardt V, Luxenburger H, Kemming J, Schulien I, Ciminski K, Giese S, et al. Rapid and stable mobilization of CD8+ T cells by SARS-CoV-2 mRNA vaccine. Nature. 2021;597(7875):268-73.
275. Lozano-Ojalvo D, Camara C, Lopez-Granados E, Nozal P, del Pino-Molina L, Bravo-Gallego LY, et al. Differential effects of the second SARS-CoV-2 mRNA vaccine dose on T cell immunity in naive and COVID-19 recovered individuals. Cell reports. 2021;36(8):109570.
276. Naradikian MS, Hao Y, Cancro MP. Age-associated B cells: key mediators of both protective and autoreactive humoral responses. Immunol Rev. 2015;269(1):118-29.
277. Dini G, Toletone A, Sticchi L, Orsi A, Bragazzi NL, Durando P. Influenza vaccination in healthcare workers: A comprehensive critical appraisal of the literature. Hum Vacc Immunother. 2018;14(3):772-89.
278. Centers for Disease Control and Prevention National Center for Immunization and Respiratory Diseases (NCIRD). Influenza Vaccination Information for Health Care Workers 2020 [Available from: https://www.cdc.gov/flu/professionals/healthcareworkers.htm.

279. Wang TL, Jing L, Bocchini JA. Mandatory influenza vaccination for all healthcare personnel: a review on justification, implementation and effectiveness. Current Opinion in Pediatrics. 2017;29(5):606-15.
280. Huang K-YA, Chang S-C, Huang Y-C, Chiu C-H, Lin T-Y. Antibody responses to trivalent inactivated influenza vaccine in health care personnel previously vaccinated and vaccinated for the first time. Scientific reports. 2017;7(1):1-10.
281. Sacadura-Leite E, Sousa-Uva A, Rebelo-de-Andrade H. Antibody response to the influenza vaccine in healthcare workers. Vaccine. 2012;30(2):436-41.
282. Tete SM, Jul-Larsen Å, Rostami S, Lunde THF, Søland H, Krammer F, et al. Impact of pre-existing immunity on the induction of functional cross-reactive anti-hemagglutinin stalk antibodies following vaccination with an AS03 adjuvanted pandemic H1N1 vaccine. Vaccine. 2018;36(16):2213-9.
283. Leung VK, Carolan LA, Worth LJ, Harper SA, Peck H, Tilmanis D, et al. Influenza vaccination responses: Evaluating impact of repeat vaccination among health care workers. Vaccine. 2017;35(19):2558-68.
284. Engler RJ, Nelson MR, Klote MM, VanRaden MJ, Huang C-Y, Cox NJ, et al. Half-vs full-dose trivalent inactivated influenza vaccine (2004-2005): age, dose, and sex effects on immune responses. Arch Intern Med. 2008;168(22):2405-14.
285. Frasca D, Diaz A, Romero M, Landin AM, Phillips M, Lechner SC, et al. Intrinsic defects in B cell response to seasonal influenza vaccination in elderly humans. Vaccine. 2010;28(51):8077-84.
286. Frasca D, Diaz A, Romero M, Blomberg BB. The generation of memory B cells is maintained, but the antibody response is not, in the elderly after repeated influenza immunizations. Vaccine. 2016;34(25):2834-40.
287. Strengell M, Ikonen N, Ziegler T, Kantele A, Anttila VJ, Julkunen I. Antibody responses against influenza A (H1N1) pdm09 virus after sequential vaccination with pandemic and seasonal influenza vaccines in Finnish healthcare professionals. Influenza and other respiratory viruses. 2013;7(3):431-8.
288. Sheridan PA, Paich HA, Handy J, Karlsson EA, Hudgens MG, Sammon AB, et al. Obesity is associated with impaired immune response to influenza vaccination in humans. Int J Obesity. 2011;36(8):1072-7.
289. Klein SL, Pekosz A. Sex-based Biology and the Rational Design of Influenza Vaccination Strategies. Journal of Infectious Diseases. 2014;209(suppl 3):S114-S9.
290. Ursin RL, Liu H, Powell HR, Westerbeck J, Shaw-Saliba K, Sylvia KE, et al. Differential antibody recognition of H3N2 vaccine and seasonal influenza virus strains based on age, vaccine status, and sex in the 2017-18 season. J Infect Dis. 2020.
291. Day JC, Christnacht C. Your Health Care Is in Women's Hands: United States Census Bureau; 2019 [Available from: https://www.census.gov/library/stories/2019/08/your-health-care-in-womens-hands.html.
292. U.S. Bureau of Labor and Statistics. Occupational Outlook Handbook - Healthcare Occupations 2019 [Available from: https://www.bls.gov/ooh/healthcare/home.htm.

293. Powell H, Pekosz A. Neuraminidase antigenic drift of H3N2 clade 3c. 2a viruses alters virus replication, enzymatic activity and inhibitory antibody binding. PLoS pathogens. 2020;16(6):e1008411.
294. Wohlgemuth N, Lane AP, Pekosz A. Influenza A Virus M2 Protein Apical Targeting Is Required for Efficient Virus Replication. Journal of Virology. 2018;92(22):e01425-18.
295. Module 23: Influenza Vaccines. Geneva: World Health Organization; 2017.
296. Louie JK, Acosta M, Winter K, Jean C, Gavali S, Schechter R, et al. Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. Jama. 2009;302(17):1896-902.
297. Neidich SD, Green WD, Rebeles J, Karlsson EA, Schultz-Cherry S, Noah TL, et al. Increased risk of influenza among vaccinated adults who are obese. Int J Obes (Lond). 2017;41(9):1324-30.
298. Thompson MG, Stenehjem E, Grannis S, Ball SW, Naleway AL, Ong TC, et al. Effectiveness of Covid-19 Vaccines in Ambulatory and Inpatient Care Settings. N Engl J Med. 2021.
299. Thomas SJ, Moreira ED, Jr., Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine through 6 Months. N Engl J Med. 2021.
300. Lopez Bernal J, Andrews N, Gower C, Gallagher E, Simmons R, Thelwall S, et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. N Engl J Med. 2021;385(7):585-94.
301. Team CC-VBCI. COVID-19 Vaccine Breakthrough Infections Reported to CDC - United States, January 1-April 30, 2021. MMWR Morb Mortal Wkly Rep. 2021;70(21):792-3.
302. CDC. COVID-19 Vaccine Breakthrough Case Investigation and Reporting 2021 [updated September 1, 2021. Available from: https://www.cdc.gov/vaccines/covid-19/health-departments/breakthrough-cases.html.
303. Antonelli M, Penfold RS, Merino J, Sudre CH, Molteni E, Berry S, et al. Risk factors and disease profile of post-vaccination SARS-CoV-2 infection in UK users of the COVID Symptom Study app: a prospective, community-based, nested, case-control study. Lancet Infect Dis. 2021.
304. Planas D, Veyer D, Baidaliuk A, Staropoli I, Guivel-Benhassine F, Rajah MM, et al. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature. 2021;596(7871):276-80.
305. Bates TA, Leier HC, Lyski ZL, McBride SK, Coulter FJ, Weinstein JB, et al. Neutralization of SARS-CoV-2 variants by convalescent and BNT162b2 vaccinated serum. Nat Commun. 2021;12(1):5135.
306. Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, et al. Covid-19 Breakthrough Infections in Vaccinated Health Care Workers. N Engl J Med. 2021.
307. Strafella C, Caputo V, Guerrera G, Termine A, Fabrizio C, Cascella R, et al. Case Report: Sars-CoV-2 Infection in a Vaccinated Individual: Evaluation of the Immunological Profile and Virus Transmission Risk. Front Immunol. 2021;12:708820.
308. Woldemeskel BA, Garliss CC, Blankson JN. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. J Clin Invest. 2021;131(10).

309. Geers D, Shamier MC, Bogers S, den Hartog G, Gommers L, Nieuwkoop NN, et al. SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. Sci Immunol. 2021;6(59).
310. Earle KA, Ambrosino DM, Fiore-Gartland A, Goldblatt D, Gilbert PB, Siber GR, et al. Evidence for antibody as a protective correlate for COVID-19 vaccines. Vaccine. 2021;39(32):4423-8.
311. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med. 2021;27(7):1205-11.
312. Suthar MS, Arunachalam PS, Hu M, Reis N, Trisal M, Raeber O, et al. Durability of immune responses to the BNT162b2 mRNA vaccine. bioRxiv. 2021:2021.09.30.462488.
313. Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months. N Engl J Med. 2021.
314. Chemaitelly H, Tang P, Hasan MR, AlMukdad S, Yassine HM, Benslimane FM, et al. Waning of BNT162b2 Vaccine Protection against SARS-CoV-2 Infection in Qatar. N Engl J Med. 2021.
315. Gaebler C, Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Tokuyama M, et al. Evolution of antibody immunity to SARS-CoV-2. Nature. 2021;591(7851):639-44.
316. Lavelle EC, Ward RW. Mucosal vaccines - fortifying the frontiers. Nat Rev Immunol. 2021.
317. Mostafa HH, Luo CH, Morris CP, Li M, Swanson NJ, Amadi A, et al. SARS-CoV-2 Infections in mRNA Vaccinated Individuals are Biased for Viruses Encoding Spike E484K and Associated with Reduced Infectious Virus Loads that Correlate with Respiratory Antiviral IgG levels. medRxiv. 2021.
318. Luo CH, Morris CP, Sachithanandham J, Amadi A, Gaston D, Li M, et al. Infection with the SARS-CoV-2 Delta Variant is Associated with Higher Infectious Virus Loads Compared to the Alpha Variant in both Unvaccinated and Vaccinated Individuals. medRxiv. 2021.
319. Roessler A, Riepler L, Bante D, von Laer D, Kimpel J. SARS-CoV-2 B.1.1.529 variant (Omicron) evades neutralization by sera from vaccinated and convalescent individuals. medRxiv. 2021:2021.12.08.21267491.
320. Morris CP, Luo CH, Amadi A, Schwartz M, Gallagher N, Ray SC, et al. An Update on SARS-CoV-2 Diversity in the United States National Capital Region: Evolution of Novel and Variants of Concern. Clin Infect Dis. 2021.
321. Stadlbauer D, Amanat F, Chromikova V, Jiang K, Strohmeier S, Arunkumar GA, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. Curr Protoc Microbiol. 2020;57(1):e100.
322. Woldemeskel BA, Kwaa AK, Garliss CC, Laeyendecker O, Ray SC, Blankson JN. Healthy donor T cell responses to common cold coronaviruses and SARS-CoV-2. J Clin Invest. 2020;130(12):6631-8.
323. VanderWeele TJ, Shpitser I. On the definition of a confounder. Annals of statistics. 2013;41(1):196.
324. Gordis L. More on causal inferences: bias, confounding, and interaction. Epidemiology. 2000;4:247-64.

325. Squires KE, Johnson M, Yang R, Uy J, Sheppard L, Absalon J, et al. Comparative gender analysis of the efficacy and safety of atazanavir/ritonavir and lopinavir/ritonavir at 96 weeks in the CASTLE study. Journal of Antimicrobial Chemotherapy. 2011;66(2):363-70.
326. Ofotokun I, Chuck SK, Hitti JE. Antiretroviral pharmacokinetic profile: a review of sex differences. Gender medicine. 2007;4(2):106-19.
327. Molina J-M, Andrade-Villanueva J, Echevarria J, Chetchotisakd P, Corral J, David N, et al. Once-daily atazanavir/ritonavir compared with twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1–infected patients: 96-week efficacy and safety results of the CASTLE study. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2010;53(3):323-32.
328. Hawkes S, Buse K, Yoon S-Y, editors. Gender-responsive tobacco control: Evidence and options for policies and programmes. Secretariat of the WHO Framework Convention on Tobacco Control; World Health Organization: Geneva, Switzerland; 2018.
329. Reitsma MB, Fullman N, Ng M, Salama JS, Abajobir A, Abate KH, et al. Smoking prevalence and attributable disease burden in 195 countries and territories, 1990–2015: a systematic analysis from the Global Burden of Disease Study 2015. Lancet. 2017;389(10082):1885-906.
330. CDC COVID Response Team, Food and Drug Administration. Allergic Reactions Including Anaphylaxis After Receipt of the First Dose of Moderna COVID-19 Vaccine—United States, December 21, 2020–January 10, 2021. Morbidity and Mortality Weekly Report. 2021;70(4):125.
331. Nguyen K, Srivastav A, Razzaghi H, Williams W, Lindley M, Jorgensen C, et al. COVID-19 vaccination intent, perceptions, and reasons for not vaccinating among groups prioritized for early vaccination, United States, September and December 2020. Morbidity and Mortality Weekly Report. 2021;70.

CURRICULUM VITAE

JANNA R. SHAPIRO

PERSONAL DATA

***Business address**:* Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, room E2202, Baltimore MD, 21205, USA
E-mail: jshapi30@jhmi.edu

EDUCATION

PhD	2018-present	Johns Hopkins Bloomberg School of Public Health, Department of International Health, Global Disease Epidemiology & Control *(Anticipated graduation: May 2022)*
M.Sc.	2017-2018	McGill University, Department of Microbiology & Immunology
B.Sc.	2014-2017	McGill University, Department of Microbiology & Immunology (First Class Honors)

HONORS & AWARDS

2021	Gordis Teaching Fellowship, Johns Hopkins University Undergraduate Department of Public Health Studies
2020-2022	Doctoral Training Award, Fonds de recherche santé de Québec - Santé
2019	Program in Applied Vaccine Experiences (PAVE) internship stipend support, Johns Hopkins Vaccine Initiative
2019	Global Established Multidisciplinary Sites (GEMS) Award, Johns Hopkins University, Center for Global Health
2018	Clements-Mann Fellowship Fund in Vaccine Sciences, Johns Hopkins Bloomberg School of Public Health, Department of International Health
2018	Wilfred Yaphe Award, McGill University
2017	Internship award, Mitacs Accelerate
2017-2018	Master's Training Award, Fonds de recherche santé de Québec
2016	Emily R Crawford Scholarship for high academic merit, McGill University
2016	BSc/MSc Recruitment Award, McGill University
2016	Tomlinson Engagement Award for Mentorship, McGill University
2015	Novartis Scholarship Award, Novartis Pharma Canada Inc.

RESEARCH EXPERIENCE

09/19- present ***Doctoral Student,*** Johns Hopkins Bloomberg School of Public Health

- Advisor: Dr. Sabra Klein; Co-adviser Dr. Rosemary Morgan
- Trainee in the Sex and Gender Analysis Core of the Sex and Age Differences in Immunity to Influenza (SADII) Specialized Center of Research Excellence (SCORE)
- Led collaborative laboratory, quantitative, and qualitative studies on the impact of sex, gender, and age on vaccine responses and attitudes
- Contributed to studies on the use of convalescent plasma as early treatment for COVID-19
- Thesis: The intersection of sex, gender, and aging on influenza and COVID-19 vaccine outcomes

06/19 – 07/19 ***Research Fellow***, Center for Infectious Diseases Research in Zambia (CIDRZ) & Johns Hopkins Bloomberg School of Public Health

- Investigators: Dr. Amanda Debes & Dr. Roma Chilengi
- Facilitated practical and didactic training on immunology assays at research facility in Lusaka, Zambia
- Implemented LPS ELISA at CIDRZ lab for cholera vaccine study

02/19 – 06/19 ***Graduate Research Assistant***, Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health

- Investigator: Dr. Kawsar Talaat
- Administered in-person and phone surveys to understand indirect benefit and risk perception among healthy volunteers who had previously participated in phase I in-patient vaccine trials.

01/19 – 06/19 ***Graduate Research Assistant,*** Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health

- Investigator: Dr. David Sack
- Immunologic assay optimization to facilitate production of a novel oral killed whole-cell cholera vaccine candidate

05/17– 07/18 ***Masters Student***, McGill University
Advisor: Dr. Brian Ward

- Projects included pre-clinical evaluation of a norovirus vaccine candidate, clinical trial of a jet injector device, case study of a rare adverse event following immunization, and rubella sero-epidemiology
- Thesis: Case studies in vaccine evaluation

05/16 – 04/17 ***Undergraduate Honors Student,*** McGill University
Advisor: Dr. Brian Ward

- Thesis: Case study of phrenic nerve paralysis following immunization with Gardasil

PROFESSIONAL EXPERIENCE

08/19 – 12/19 ***Intern***, World Health Organization, Department of Immunization, Vaccines and Biologicals, Initiative for Vaccine Research; Geneva, Switzerland

- Supervisor: Birgitte Giersing
- Contributed to the development of a framework to collect input from low- and middle-income country stakeholders to inform the global vaccine research & development agenda
- Tracked clinical development and characteristics of vaccine candidates for key pathogens

05/14 – 09/16 ***Weekend Manager & Lab Technician***, Feldman & Messias Pharmacy; Montreal, Canada

- Independently managed a team while acting simultaneously as a pharmacy lab technician and a customer service representative
- Ensured the accurate processing of prescriptions

TEACHING EXPERIENCE

01/21 – 05/21 ***Instructor,*** Johns Hopkins University, Dept. of Public Health Studies
Course: Introduction to Vaccinology

- Teaching mentor: Dr. Kawsar Talaat
- Duties: Designed syllabus and course materials, taught lectures, and graded assignments
- Supported by Gordis Teaching Fellowship

03/20 – 05/20 ***Teaching Assistant,*** Johns Hopkins Bloomberg School of Public Health

- Course: The Practice of Public Health through Vaccine Case Studies
- Faculty: Drs. Daniel Salmon & Matthew Dudley
- Duties: Moderated course website, contributed to design of materials, and moderated class discussions in virtual 'Live Talks'

03/20 – 05/20 ***Teaching Assistant,*** Johns Hopkins Bloomberg School of Public Health

- Course: Good Clinical Practice: A vaccine trials perspective
- Faculty: Drs. Naor Bar-Zeev & Jessica Atwell
- Duties: Held office hours, graded assignments, moderated course website, and contributed to design of materials

01/20 – 03/20 ***Teaching Assistant,*** Johns Hopkins Bloomberg School of Public Health

- Course: Vaccine Policy Issues
- Faculty: Dr. Daniel Salmon & Lois Privor-Dumm
- Duties: Scheduled and facilitated logistics for guest speakers, moderated course website, and contributed to design of materials

01/20 – 03/20 ***Teaching Assistant,*** Johns Hopkins Bloomberg School of Public Health

- Course: Infectious Diseases & Child Survival
- Faculty: Drs. Andrea Ruff and Kawsar Talaat
- Duties: Contributed to development and grading of assessments

01/16 – 04/16 ***Teaching Assistant & Peer Mentor***, McGill University

- Course: Introductory Immunology
- Faculty: Dr. Joaquim Madrenas
- Duties: Held office hours, moderated online discussion boards, and prepared practice question sets

EDITORIAL ACTIVITIES

Ad hoc journal reviewer

Nature Medicine
CHEST
American Journal of Preventive Medicine
Frontiers in Psychology
BMJ Global Health
Vaccine
Biology of Sex Differences

PUBLICATIONS

Peer-Reviewed Articles

Shoham S, Bloch EM, Casadevall A, Hanley D, Lau B, Gebo K, Cachay E, Kassaye SG, Paxton JH, Gerber J, Levine AC, Currier J, Patel B, Allen ES, Anjan S, Appel L, Baksh S, Blair PW, Bowen A, Broderick P, Caputo CA, Cluzet V, Cordisco ME, Cruser D, Ehrhardt S, Forthal D, Fukuta Y, Gawad AL, Gniadek T, Hammel J, Huaman MA, Jabs DA, Jedlicka A, Karlen N, Klein S, Laeyendecker O, Lane K, McBee N, Meisenberg B, Merlo C, Mosnaim G, Park HS, Pekosz A, Petrini J, Rausch W, Shade DM, **Shapiro JR**, Singleton JR, Sutcliffe C, Thomas DL, Yarava A, Zand M, Zenilman JM, Tobian AAR, Sullivan D. Randomized controlled trial transfusing convalescent plasma as post-exposure prophylaxis against SARS-CoV-2 infection. *Clinical Infectious Diseases*, in press

Park H-S*, **Shapiro JR***, Sitaras I*, Woldemeskel BA, Garliss CC, Dziedzic A, Sachithanandham J, Jedlicka A, Caputo C, Rousseau KE, Thakar M, Suwanmanee S, Hauk P, Aliyu L, Majewska N, Koley S, Patel B, Broderick P, Mosnaim G, Heath SL, Sydnor ES, Shenoy A, Bloch EM,

Gniadek TJ, Shoham S, Casadevall A, Hanley D, Cox A, Laeyendecker O, Betenbaugh MJ, Cramer SM, Mostafa HH, Pekosz A, Blankson JN, Klein SL, Tobian AAR, Sullivan D, Gebo KA. Adaptive immune responses in vaccinated patients with symptomatic SARS-CoV-2 Alpha infection. *JCI Insight 7(5)* 2022, doi: 10.1172/ jci.insight.155944.
*Co-first authors

Shapiro JR, Morgan R, Leng SX, and Klein SL. Roadmap for sex-responsive influenza and COVID-19 vaccine research in older adults. *Frontiers in Aging 7* 2022, doi: 10.3389/fragi.2022. 836642.

Shapiro JR, Li H, Morgan R, Chen Y, Kuo H, Ning X, Shea P, Wu C, Merport K, Saldanha R, Lio S, Abrams E, Chen Y, Kelly DC, Sheridan-Malone E, Wan L, Zeger SL, Klein SL, Leng SX. Sex-specific effects of aging on humoral immune responses to repeated influenza vaccination in older adults. *npj vaccines 6(1)* 2021, doi: 10.1038/s41541-021-00412-6.

Morgan R, Smith J, Baker P, Griffith D, Klein SL, Logie CH, Mwiine A, Scheim A, **Shapiro JR**, Wenham C, White A. Beyond a zero-sum game: How does the impact of COVID-19 vary by gender? *Frontiers in Sociology 6* 2021, doi: 10.3389/fsoc.2021.650729.

Kini A, Morgan R, Kuo H, Shea P, **Shapiro JR**, Leng SX, Pekosz A, Klein SL. Differences and Disparities in Seasonal Influenza Vaccine, Acceptance, Adverse Reactions, and Coverage by Age, Sex, Gender, and Race. *Vaccine 40(11)* 2021, doi: 10.1016/j.vaccine.2021.04.013.

Kuo H*, **Shapiro JR***, Dhakal S, Morgan, R, Fink AL, Lui H, Westerbeck JW, Sylvia KE, Park HS, Ursin RL, Shea P, Shaw-Saliba K, Fenstermacher K, Rothman R, Pekosz A, Klein SL. Sex-specific effects of age and body mass index on antibody responses to seasonal influenza vaccines in healthcare workers. *Vaccine 40(11)* 2021, doi: 10.1016/j.vaccine .2021.02.047.
*Co-first authors

Klein SL, Pekosz A, Park HS, Ursin R, **Shapiro JR**, Benner S, Littlefield K, Kumar S, Naik HM, Betenbaugh M, Shrestha R, Wu A, Hughes R, Burgess I, Caturegli P, Laeyendecker O, Quinn T, Sullivan D, Shoham S, Redd A, Bloch E, Casadevall A, Tobian A. Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population. *Journal of Clinical Investigation 130(11)* 2020, doi: 10.1172/JCI142004.

Shapiro JR, Hodgins B, Hendin HE, Patel A, Menassa K, Menassa C, Menassa M, Pereira JA, Ward BJ. Needle-free delivery of influenza vaccine using the Med-Jet® H4 is efficient and elicits the same humoral and cellular responses as standard IM injection: A randomized trial. *Vaccine 37(10)* 2019, doi: 10.1016/j.vaccine.2019.01.039.

Gilbert NL, Rotondo J, **Shapiro JR**, Sherrard L, Fraser WD, Ward BJ. Seroprevalence of rubella antibodies and determinants of susceptibility to rubella in a cohort of pregnant women in Canada, 2008–2011. *Vaccine 35(23)* 2017, doi: 10.1016/j.vaccine.2017.04.057.

Articles in review

Shapiro JR, Park H-S, Aytenfisu TY, Caputo C, Lee J, Johnston TS, Li H, Hauk P, Jacobsen H, Li Y, Abrams E, Kocot AJ, Yang T, Huang Y, Cramer SM, Betenbaugh MJ, Debes AK, Morgan R, Milstone AM, Karaba AH, Leng SX, and Klein SL. Association of frailty, age, and biological sex with SARS-CoV-2 mRNA vaccine-induced immunity in older adults. medRxiv (pre-print), doi: 10.1101/2022.03.11.22272269.

Shapiro JR, Privor-Dumm L, Rosser EN, Leng SX, Klein SL, Morgan R. The intersection of gender and race in older adults' decision to receive COVID-19 vaccines. SSRN (pre-print), https://ssrn.com/abstract=4085528

Editorials and Letters

Shapiro JR, Kelin SL, Morgran R. Stop 'controlling' for sex and gender in global health research. Commentary. *BMJ Global Health 6(4)* 2021, doi: 10.1136/bmjgh-2021-005714.

Shapiro JR, Kelin SL, Morgran R. COVID-19: use intersectional analyses to close gaps in outcomes and vaccination. Correspondence. *Nature* 591.7849 2021, doi: 10.1038/d41586-021-00577-z.

Ursin RL*, **Shapiro JR***, Klein SL. Sex-biased immune responses following SARS-CoV-2 infection. Spotlight. *Trends in Microbiology 28(12)* 2020, doi: 10.1016/j.tim.2020.10.002.
*Co-first authors

POSTERS & PRESENTATIONS

Scientific Meetings

Association of Frailty, Age, and Biological Sex with SARS-CoV-2 mRNA Vaccine-Induced Immunity in Older Adults. SeroNet Investigators Meeting, virtual. March 2022.

Sex-specific effects of aging on the response to SARS-CoV-2 mRNA vaccines in older adults. Poster presentation at the Specialized Centers of Research Excellence on Sex Differences Annual Meeting, virtual. December 2021.

Durability and breadth of the humoral immune response to SARS-CoV-2mRNA vaccines in older adults. Oral presentation at International Precision Vaccines Conference, virtual. September 2021.

Sex, age, and frailty intersect in the immunogenicity and reactogenicity of seasonal influenza vaccines in older adults. Oral presentation at Annual Conference on Vaccinology Research, virtual. April 2021.

Sex, age, and frailty intersect to impact the durability of antibody responses to repeated seasonal influenza vaccination in older adults. Poster presentation at Sex Differences in the Immune System Inaugural Meeting, virtual. April 2021.

The intersection of sex and frailty in humoral immune responses to influenza vaccine among community-dwelling older adults. Oral Presentation at Centers of Excellence for Influenza Research and Surveillance (CEIRS) Annual Meeting, virtual. January 2021.

Sex, age and frailty interact to impact the humoral response to seasonal influenza vaccine in older adults. Poster and flashtalk presenation at the Specialized Centers of Research Excellence on Sex Differences Annual Meeting, virtual. December 2020.

The intersection of sex and frailty in humoral immune responses to influenza vaccine among community-dwelling older adults. Oral presentation at Gerontological Society of America Annual Scientific Meeting, virtual. November 2020.

Needle-free delivery of influenza vaccine using the Med-Jet® H4 is efficient and elicits the same humoral and cellular responses as standard IM injection: A randomized trial. Oral presentation at Annual Conference on Vaccinology Research, Baltimore, MD. April 2019.

Bilateral Phrenic Nerve Paralysis After Human Papilloma Virus Immunization. Oral presentation at Canadian Association for Immunization Research and Evaluation (CAIRE) Research Sponsor Advisory Board Meeting, Montreal, QC. November 2017

Seroprevalence of rubella antibodies and determinants of susceptibility to rubella in Canadian pregnant women, 2008-2011. Poster presentation at Student and Young Professionals Global Health Summit, Ottawa, ON. October 2017.

Seroprevalence of rubella antibodies and determinants of susceptibility to rubella in Canadian pregnant women, 2008-2011. Oral presentation at Canadian Immunization Research Network (CIRN) AGM, Halifax, NS. May 2017.

Immunologic Investigation of a Possible Vaccine-Associated Adverse Event: Bilateral Phrenic Nerve Paralysis After Human Papilloma Virus Immunization (Gardasil™). Oral presentation at the Ontario-Quebec Undergraduate Immunology Conference, Toronto, ON. May 2017. [Second place speaker].

MEDIA & SOCIAL MEDIA

Quoted in “The Danger of a ‘Dudes Only’ Vaccine”, The Atlantic, 04/16/2021. [link]

Quoted in “Why Do Mostly Women Report "COVID Vaccine Arm"?”, Psychology Today, 04/16/2021. [**link**]

Panelist for “Track the Vax: The COVID-19 Gender Gap” hosted by MedPage Today. [link]

Panelist for “Johns Hopkins Students Answer the Buzziest COVID-19 Vaccine Questions on Social Media.” [link]

MENTORSHIP & SUPERVISION

Undergraduate Research Assistants

Jonathan Liu, Major: Biomedical Engineering; Project: Surveillance for breakthrough influenza-like illness in vaccinated older adults; 10/2020 – 04/2021

Rachel Barros, Major: Medicine, Science & the Humanities; Project: Surveillance for breakthrough influenza-like illness in vaccinated older adults; 10/2020 – present

Rayna Saldanha, Major: Molecular and Cellular Biology; Project: Sex-Specific effects of age, in the humoral response to seasonal influenza vaccine in older adults; 09/2020 – 12/2020

Katherine Merport, Major: Public Health Studies; Project: Sex-Specific effects of age, in the humoral response to seasonal influenza vaccine in older adults; 09/2020 – present

Masters Students

Jenny Lee, Masters of Health Science in Molecular Microbiology & Immunology lab rotation; Project: Humoral immune response to SARS-CoV-2 vaccination in rheumatoid arthritis patients; 11/2021 – present.

Anna Yin, Masters of Public Health; Project: Comparison of magnitude and duration of antibody response in hospitalized and non-hospitalized COVID-19 patients; 11/2021 – present.

PhD students

Jill Hakim, PhD in Molecular Microbiology and Immunology lab rotation; Project: Prevalences of SARS-CoV-2 seropositivity in Zambia; 03/2022 – present.

CERTIFICATIONS

2021 Johns Hopkins Teaching Academy, certificate of completion
2020 Johns Hopkins Teaching Institute, certificate of completion
2020 Teaching Assistantship Training (JHSPH)
2019 Vaccine Science and Policy Certificate (JHSPH)
2019 Human Research Good Clinical Practice and ICH (CITI)
2019 Basic Human Subjects Research (CITI)
2019 Health Privacy Issues for Researchers (CITI)
2019 Information Privacy Security for Researchers (CITI)
2019 Good Clinical Practice: A Vaccine Trials Perspective (JHSPH)

Intended to be blank.