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MAJOR ARTICLE

Sex Differences in the Immunogenicity and Efficacy of Seasonal Influenza Vaccines: A Meta-analysis of Randomized Controlled Trials

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Background. Sex impacts individuals' response to vaccination. However, most vaccine studies do not report these differences disaggregated by sex. The aim of this study was to assess sex differences in the immunogenicity and efficacy of influenza vaccine.

Methods. We performed a meta-analysis using phase 3 randomized controlled trial data conducted between 2010 and 2018. Using hemagglutination inhibition antibody titers for each strain, differences in geometric mean ratios (GMRs) were calculated by sex. Risk ratios (RRs) comparing seroconversion proportions were pooled for females and males using random-effects models. Vaccine efficacy (VE) was assessed. Data were analyzed by age group (18–64 vs ≥65 years).

Results. A total of 33 092 healthy adults from 19 studies were included for immunogenicity analysis, and 6740 from 1 study for VE. Whereas no sex differences in immunogenicity were found in adults <65 years old, older females had a significantly greater chance to seroconvert compared to older males for all strains: RR_{H1N1} = 1.17 [95% confidence interval {CI}, 1.12–1.23]; RR_{H3N2} = 1.09 [95% CI, 1.05–1.14]; $RR_{Victoria}$ = 1.23 [95% CI, 1.14–1.31]; $RR_{Yamagata}$ = 1.22 [95% CI, 1.14–1.30]. GMRs were also higher in older females for all strains compared to older males. VE in preventing laboratory-confirmed influenza was higher in older females compared to older males with VEs of 27.32% (95% CI, 1.15%–46.56%) and 6.06% (95% CI, −37.68% to 35.90%), respectively.

Conclusions. Our results suggest a higher immunogenicity and VE in females compared to males in older adults. These differences in immunogenicity and VE support the disaggregation of vaccine data by sex in clinical trials and observational studies.

Clinical Trials Registration. CRD42018112260.

Keywords. efficacy; immunogenicity; influenza vaccine; meta-analysis; sex differences.

Influenza is a respiratory illness caused by influenza viruses and is an important cause of morbidity and mortality, despite being vaccine-preventable [1–3]. Globally, influenza is estimated to result in up to 5 million cases of severe illness and 290 000–650 000 deaths annually [1]. Due to changes in the virus (ie, antigenic drift), annual vaccination is recommended and is an effective means to reduce the global burden of disease [1–3]. Despite annual reformulation to match circulating strains, mismatches can lead to low influenza vaccine effectiveness [4]. Host-specific factors are also known to impact individuals' response to vaccines [5].

Sex refers to the biological differences between males and females. There is a growing body of evidence suggesting that sex factors play a major role in the immune response to pathogens and vaccines [6–9]. Genes and hormones affect the immune response to viruses, and females tend to mount stronger innate and adaptive immune responses compared to males, especially in reproductive ages [9–13]. This sexual dimorphism was also observed following influenza vaccination, with a lower antibody response in males and a higher occurrence of adverse events in females [14, 15]. Data on influenza vaccines' efficacy and effectiveness by sex are scarce, but it has been reported that vaccinated females have a lower risk of hospitalizations and deaths compared to vaccinated males [5].

We have previously conducted a systematic review to assess published data on sex differences in the response to seasonal influenza vaccines. Unfortunately, the heterogeneity and paucity of data prevented us from drawing clear conclusions [16]. Although both sexes are represented in clinical trials that assess

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influenza vaccination outcomes, findings are rarely disaggregated by sex [5].

While influenza vaccine recommendations are often tailored to account for age and health status [4], less consideration is given to sex. An improved quantification of sex differences in influenza vaccine outcomes would help inform vaccination policies and enable more targeted use of the various vaccine formulations for different subgroups in the population. We therefore conducted meta-analyses using data from recent published and unpublished phase 3 randomized controlled trials (RCTs) to assess sex differences in the immunogenicity and efficacy of influenza vaccines in healthy adults.

METHODS

Search Strategy and Selection Criteria

In this meta-analysis, individual patient data (IPD) were requested from published and unpublished studies, which were eligible for inclusion if they met all of the following criteria: (1) phase 3 RCT, conducted from January 2010 onward (ie, after introduction of the 2009 H1N1 strain) and published or completed by September 2018; (2) participants were healthy males and females ≥18 years old; (3) intervention was seasonal influenza vaccine, regardless of route of administration, dosage, and formulation. Antibody titers had to be measured before and 2–4 weeks postvaccination. We excluded studies that assessed a pandemic influenza vaccine alone (A/H1N1 or A/H5N2). Finally, immunogenicity and/or efficacy data had to be available for both males and females.

Eligible studies were identified through a 2-step process. We first searched the Cumulative Index to Nursing and Allied Health Literature, PubMed, Embase, and Web of Science, to identify published studies. Then, the following clinical registries were searched to retrieve unpublished studies: ClinicalTrials.gov, Clinical Study Data Request, European Organisation for Research and Treatment of Cancer Clinical Trials Database, European Union Clinical Trials Register, the World Health Organization International Clinical Registry Platform, and Health Canada's Clinical Trials Database. The original search strategy was developed with a research librarian [16]. It was restricted to studies in French or English, published between 1 January 2010 and 3 October 2018. We searched PubMed using a combination of the following terms and their derivatives: "influenza" OR "seasonal influenza"; "vaccine" OR "immunization"; "immunogenicity" OR "efficacy" OR "effectiveness" OR "safety" OR "AEFI" OR "SAE"; "adults"; and "controlled randomized trials." The search strategy was adapted for each database and clinical registry and is presented in the Supplementary Material I. Two reviewers (F. T. and A. A.) independently screened unique records at the title/abstract level, then assessed for eligibility at the full-text level using DistillerSR version 2.35 [17]. Discrepancies were resolved through consensus. F. T. and A. A. contacted study sponsors and authors to request data sharing. All data were made available by 28 January 2022.

The study protocol is registered on the International Prospective Register of Systematic Reviews (PROSPERO; CRD 42018112260) (Supplementary Material II) and was approved by Centre Hospitalier Universitaire Ste-Justine and the University of Alberta Research Ethics Board.

Patient Consent Statement

Patients' written consent was obtained for participating in trials included in this meta-analysis.

Data Collection

Eligible published and unpublished studies were compared, and duplicates removed. IPD were requested by contacting the study corresponding author or sponsor via email, and up to 3 follow-up messages were sent. If no answer could be obtained, the study was excluded. In case IPD were not available, authors were asked to reanalyze their data as per protocol and provide study characteristics and aggregate results, stratified by sex and age group for each outcome. If neither IPD nor aggregate data could be shared, the study was excluded from meta-analysis. An electronic form was used to extract the following study characteristics: RCT identifier; number of participants in the immunogenicity and/or efficacy set; sex; age; vaccine type (trivalent influenza vaccine [TIV] or quadrivalent [QIV] influenza vaccine) and formulation; route of administration (intramuscular or intradermal); influenza season; laboratory test for antibody titers; definition of influenza illness and number of influenza cases; hemisphere; country/region; and underlying medical conditions. Data on previous influenza vaccination, influenza illness history, and race and ethnicity were also abstracted, if available. Investigators were contacted if further information was needed.

Data Analysis

This was a 2-stage meta-analysis of IPD and aggregate data [18, 19]. In the first stage, crude estimates were computed using IPD for each study and outcome, separately. Immunogenicity was assessed in vaccinated participants, using log-transformed hemagglutination inhibition (HI) and microneutralization (MN) antibody titers. Pre- and postvaccination HI and MN geometric mean titers (GMTs) were extracted for each influenza strain (A/H1N1, A/H3N2, B/Yamagata, and B/Victoria). The primary outcome was the seroconversion proportion by sex. Unadjusted risk ratios (RRs) with 95% confidence intervals (CIs) comparing the proportion of seroconverted females versus males were calculated. In HI tests, seroconversion corresponds to a postvaccination HI ≥1:40 if prevaccination HI <1:10; or a 4-fold increase, if prevaccination HI >1:10 [20]. In MN tests, seroconversion was defined as a 4-fold increase in postvaccination titers [21]. The geometric mean ratio

(GMR) from the ratio of GMTs was computed and the mean difference (MD) (95% CI) was used to compare GMR in females versus males. GMR was a secondary immunogenicity outcome. Finally, influenza VE was defined as the relative reduction in the incidence of influenza-like illness (ILI) or laboratory-confirmed influenza (LCI) in vaccinated versus unvaccinated participants. Crude VE (95% CI) was computed using the following formula: $100 \times (1 - RR)$.

In the second stage, effect measures were pooled using the Mantel-Haenszel method and a random-effects model, to allow for between-study heterogeneity [19]. We used the I^2 statistic to assess the variability in effect estimates that was not attributable to chance alone. Heterogeneity was deemed negligible, moderate, or considerable if I^2 was <40%, 40%–75%, or >75%, respectively [19].

F. T. and M. K. independently assessed the risk of bias arising from confounding, selection, classification of interventions, missing data, and measurement of outcomes, using the Risk of Bias in Non-Randomized Studies of Interventions (ROBINS-I) tool [22].. We used a tool for nonrandomized studies as the included RCTs were not initially conducted to assess the effect of sex on vaccination outcomes. Finally, we evaluated the quality of evidence and strength of recommendations for seroconversion using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) tool [23].

Analyses were performed using RStudio 2022.12.0.353 and Review Manager (RevMan version 5.4). Statistical significance was set at $P < .05$ and $P < .10$ for meta-analyses and subgroup differences tests, respectively [24]. Corrections for multiple testing were applied using the Holm-Bonferroni procedure [25]. Findings are reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [26, 27].

Our main analyses were stratified by age, as sex differences in vaccine-induced immunity may differ over the life course [8, 28]. We initially planned to use the following age groups: 18–49, 50–64, and ≥65 years. However, data on participants' age could only be shared in quartiles for all trials conducted by 1 sponsor, due to data anonymization procedures. Thus, we used broader age groups and participants were classified as "younger" (18–64 years) and "older" (≥65 years). Immunogenicity analyses were carried out for each influenza strain.

We performed subgroup analyses by vaccine type (TIV vs QIV); influenza vaccination history (none vs at least 1 vaccine dose); influenza illness history (none vs at least 1 episode); and risk of bias (low/moderate vs serious). A sensitivity analysis was performed using preplanned age groups by excluding studies for which relevant age groups were not available. Finally, we assessed the robustness of our findings for seroconversion by fitting generalized linear mixed models (GLMMs) with logit link and binomial distribution, for each influenza strain. GLMMs were adjusted for sex, age, vaccination history (fixed effects), and sex per study (random effect), to allow the effect of sex to vary between studies. This analysis was conducted using IPD from 1 sponsor, as data on both age and vaccination history were available.

RESULTS

Search Results

A total of 985 unpublished records were assessed for eligibility, and 141 studies were deemed eligible (Figure 1) and checked against 40 eligible published studies (Supplementary Figure 1), leading to the exclusion of 64 duplicate studies. We requested IPD for the remaining 77 eligible studies. IPD were shared by 3 sponsors for 13 trials [29–39], whereas 1 sponsor agreed to share aggregate data for 6 studies [40–45]. No response could be obtained for 36 studies, and data could not be shared for 22 studies (Figure 1).

Study Characteristics

A total of 19 studies were included in our meta-analysis [29–47], and are summarized in Table 1. Overall, studies were conducted from the 2010–2011 to 2017–2018 influenza seasons, mainly in the Northern Hemisphere. Immunogenicity was assessed in all trials [29–47] and VE in 1 trial [41]. Overall, IPD and aggregate immunogenicity data were available for 33 092 vaccinated adults. Aggregate VE data were available for 6740 vaccinated and unvaccinated participants aged ≥65 years (Figure 1). Immunogenicity was assessed using HI tests in all studies, while 2 studies additionally used MN tests for a subgroup of their participants. Influenza vaccines were quadrivalent or trivalent, egg-based, cell-based, or adjuvanted and were administered intramuscularly, except in 1 study where it was intradermal. Included participants were all medically stable adults. Data on influenza vaccination history were provided for 93% of participants with IPD and documented for up to 4 previous seasons (Table 1). Vaccine formulations and study exclusion criteria based on participants' medical condition are provided in Supplementary Table 1.

Quality Assessment

Overall, 5 studies were deemed at low risk of bias (26.3%), 6 at moderate risk (31.6%), and 7 (36.8%) at serious risk of bias for this meta-analysis. Confounding was the main source of bias. Studies were deemed at serious risk of bias when data could not be stratified according to our prespecified age groups (ie, 18–49, 50–64, and ≥65 years). When data on participants' influenza vaccination history were not available or aggregated, studies were deemed at moderate risk of bias. Remaining domains were all deemed at low risk of bias (Supplementary Figure 2).

Main Analyses

Our main analysis using HI titers showed a slightly greater overall chance for seroconversion in females compared to

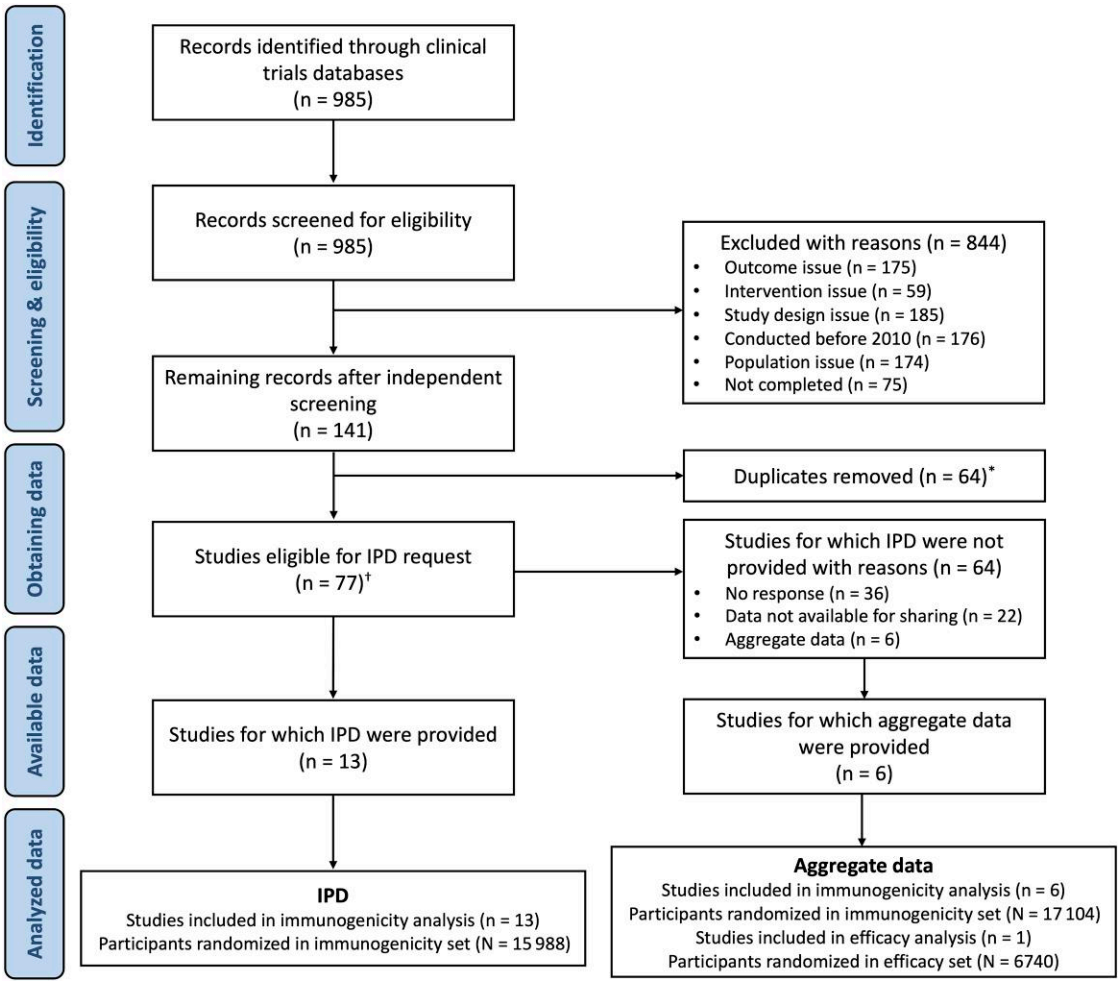

Figure 1. Study selection flowchart (from clinical registries and literature search). *Duplicates removed after comparison with studies found through literature search (Figure S1). †Including the 40 studies found through literature search (published studies identification process detailed in Appendix B). Abbreviation: IPD, individual patient data.

males, for all influenza strains. Tests for subgroup differences (ie, age groups) were significant for influenza A and B strains ($P \leq .005$). Older females had a significantly greater chance for seroconversion for influenza A ($RR_{H1N1} = 1.17$ [95% CI, 1.12–1.23], $I^2 = 68\%$; $RR_{H3N2} = 1.09$ [95% CI, 1.05–1.14], $I^2 = 54\%$) and influenza B ($RR_{Victoria} = 1.23$ [95% CI, 1.14–1.31], $I^2 = 54\%$; $RR_{Yamagata} = 1.22$ [95% CI, 1.14–1.30], $I^2 = 28\%$) ($P < .0001$). No sex differences were observed in younger adults (Figures 2 and 3).

MDs in GMR were also higher in older females compared to older males: $MD_{H1N1} = 1.62$ [95% CI, 1.1–2.14]; $MD_{H3N2} = 0.88$ [95% CI, .49–1.26]; $MD_{Victoria} = 0.57$ [95% CI, .33–.80]; $MD_{Yamagata} = 0.48$ [95% CI, .26–.70], with considerable heterogeneity ($I^2 \geq 74\%$) (Table 2). Similar seroconversion and GMR findings were observed using MN titers in a subgroup of 795 participants, with a null heterogeneity within subgroups (Supplementary Tables 2 and 3).

Finally, crude VE was assessed in a population of 4166 and 2576 older females and males, respectively. Overall, VE in preventing LCI was higher in older females compared to older males with absolute VEs of 27.32% (95% CI, 1.15%–46.56%) and 6.06% (95% CI, −37.68% to 35.90%), respectively. The lack of precision in those estimates was mainly due to the small proportion of LCI cases. No differences were observed for VE in preventing ILI, as VE values overlapped and 95% CI included negative values for females and males (Table 3).

Sensitivity and Subgroup Analyses

Subgroup analyses were performed for seroconversion proportions (ie, main outcome). Our findings remained unchanged with regard to vaccine type, risk of bias assessment, influenza illness history (Supplementary Table 4), and the use of prespecified age groups (Figures 4 and 5). However, tests for subgroup differences were significant for influenza vaccination history in influenza A/H3N2 and B strains ($.0001 \leq P \leq .01$). Whereas no sex differences existed in participants with no vaccination history, previously vaccinated females had greater chance of seroconversion compared to males of their respective age group

Table 1. Summary of Study Characteristics

				Intervention			Outcome			Population					
Study ID [Ref]	Author, Year	Country/ Region	Hemisphere	Season	Vaccine Type	Route	VE	HI	MN	Sex	No.[a] (N = 33 092)	Age Groups, y, as Analyzed[b] (%)	Available Age Groups, y[c]	Influenza Vaccination History, No. (%)[d,e]	Risk of Bias[f]
GQM01 [34]	Pepin, 2013	Western/Eastern Europe	Northern	2011–2012	IIV4/IIV3	IM	✗	✓	✓	F	910	<65 (50.1) ≥65 (49.9)	18–39; >39–59; >59–67; >67–89	445 (48.9)	Serious
										M	647	<65 (50.0) ≥65 (50.0)	18–47; >47–62; >62–68; >68–91	336 (53.2)	
GQM04 [33][g]	Cadorna-Carlos, 2015	Australia, Philippines	Northern Southern	2011–2012 2012	IIV4/IIV3	IM	✗	✓	✗	F	909	<65 (75.2)	>22–35; >35–47; >47–60	392 (43.0)	Serious
										M	645	<65 (75.0)	>18–28; >28–43; >43–60	232 (36.0)	
GQM07 [32]	Choi, 2017	Korea	Northern	2014–2015	IIV4/IIV3	IM	✗	✓	✗	F	220	<65 (100)	19–28; >28–35; >35–41; >41–60	92 (41.8)	Serious
										M	79	<65 (100)	18–29; >29–34; >34–40; >40–59	26 (32.9)	
GQM11 [31]	Sesay, 2018	Western/Eastern Europe	Northern	2014–2015	IIV4/IIV3	IM	✗	✓	✓	F	1198	<65 (50.0) ≥65 (50.0)	18–38; >38–58; >58–68; >68–91	537 (44.8)	Serious
										M	1018	<65 (51.6) ≥65 (48.4)	18–42; >42–62; >62–69; >69–88	485 (47.6)	
QID01 [35]	Gorse, 2015	USA	Northern	2012–2013	QIV/TIV	ID	✗	✓	✗	F	1340	<65 (100)	18–49; 50–64	553 (41.3)	Low
										M	840	<65 (100)	18–49; 50–64	284 (33.8)	
QIV03 [29]	Not published	USA	Northern	2010–2011	IIV4/IIV3	IM	✗	✓	✗	F	398	<65 (25.9) ≥65 (74.1)	30–67; >67–70; >70–75; >75–92	NA	Serious
										M	331	<65 (24.8) ≥65 (75.2)	18–67; >67–71; >71–76; >76–94	NA	
QIV06 [47]	Not published	India	Southern	2015	IIV4	IM	✗	✓	✗	F	34	<65 (64.7) ≥65 (35.3)	18–64; ≥65	NA	Serious
										M	66	<65 (72.7) ≥65 (27.3)	18–64; ≥65	NA	
RPV03C [30]	Zimmermann, 2013	France, Germany	Northern	2010–2011	IIV3/ REPEVAX	IM	✗	✓	✗	F	520	≥65 (100)	60–67; >67–92	323 (62.2)	Serious
										M	412	≥65 (100)	60–67; >67–88	257 (62.3)	
V211–062 [46]	Levin, 2018	USA	Northern	2015–2016	IIV4	IM	✗	✓	✗	F	492	<65 (73.2) ≥65 (26.8)	50–64; 65–89	NA	Moderate
										M	330	<65 (67.9) ≥65 (32.1)	50–64; 65–88	NA	
CSLCT-QIV-13–01 [40]	Treanor, 2017	USA	Northern	2014–2015	IIV4/IIV3	IM	✗	✓	✗	F	1946	<65 (50.8) ≥65 (49.2)	18–49; 50–64; ≥65	1764 (88.6)	Moderate
										M	1449	<65 (47.6) ≥65 (52.4)	18–49; 50–64; ≥65	1257 (85.5)	
V118–18 [41]	Not published	Bulgaria, Colombia, Czech Republic, Estonia, Latvia, Lithuania, Malaysia, Philippines, Poland, Romania, Thailand, and Turkey	Northern Southern	2016–2017 2017	aQIV/Boostrix	IM	✓	✓	✗	F	775[h]	≥65 (100)	≥65	648 (30.8)	Moderate
										M	549[h]	≥65 (100)	≥65	343 (26.6)	
V58–23 [42]	Not published	USA	Northern	2014–2015	TIVc/TIVf	IM	✗	✓	✗	F	1144	<65 (100)	18–49	293 (31.2)	Moderate
										M	381	<65 (100)	18–49	165 (26.6)	
V70–27 [43]	Frey, 2014	USA	Northern	2010–2011	aTIV/TIV	IM	✗	✓	✗	F	4225	≥65 (100)	≥65	114 (2.5)	Moderate
										M	2261	≥65 (100)	≥65	39 (1.6)	
V118–20 [44]	Beran, 2021	USA	Northern	2017–2018	aQIV/aTIV	IM	✗	✓	✗	F	983	≥65 (100)	≥65	880 (87.4)	Moderate
										M	758	≥65 (100)	≥65	661 (85.7)	
V130–01 [45]	Bart, 2016	USA	Northern	2013–2014	QIVc/TIVc	IM	✗	✓	✗	F	1499	<65 (50.9) ≥65 (49.1)	18–49; 50–64; ≥65	413 (27.2)	Moderate
										M	1134	<65 (49.1) ≥65 (50.9)	18–49; 50–64; ≥65	253 (21.7)	

Table 1. Continued

				Intervention			Outcome			Population					
Study ID [Ref]	Author, Year	Country/ Region	Hemisphere	Season	Vaccine Type	Route	VE	HI	MN	Sex	No.[a] (N = 33 092)	Age Groups, y, as Analyzed[b] (%)	Available Age Groups, y[c]	Influenza Vaccination History, No. (%)[d,e]	Risk of Bias[f]
112963 [39]	Tinico, 2014	USA, Mexico, Canada	Northern	2010–2011	IIV4/IIV3	IM	✗	✓	✗	F	1028	<65 (71.2) ≥65 (28.8)	18–49; 50–64; ≥65	710 (69.1)	Low
										M	651	<65 (63.0) ≥65 (37.0)	18–49; 50–64; ≥65	442 (67.9)	
114269 [38]	Kieninger, 2013	USA, Korea, Germany, Romania, Spain, and Taiwan	Northern	2010–2011	IIV4/IIV3	IM	✗	✓	✗	F	1711	<65 (56.6) ≥65 (43.4)	18–49; 50–64; ≥65	1337 (78.1)	Low
										M	1304	<65 (46.6) ≥65 (53.4)	18–49; 50–64; ≥65	1000 (76.7)	
117036 [37]	Schwarz, 2017	USA, Germany, Canada	Northern	2013–2014	IIV4/$Hz_{/su}$	IM	✗	✓	✗	F	429	<65 (59.4) ≥65 (40.6)	50–64; ≥65	310 (72.3)	Low
										M	399	<65 (59.1) ≥65 (40.6)	50–64; ≥65	284 (71.2)	
201251 [36][i]	Claeys, 2018	Germany	Northern	2014–2015	IIV4	IM	✗	✓	✗	F	0	NA	NA	NA	Low
										M	18	<65 (100)	18–49	9 (50.0)	

Abbreviations: aQIV, adjuvanted quadrivalent influenza vaccine (egg-derived); aTIV, adjuvanted trivalent influenza vaccine (egg-derived); Boostrix, diphtheria, tetanus, and pertussis (acellular, component); F, female; HI, hemagglutination inhibition; $HZ_{/su}$, adjuvanted herpes zoster subunit vaccine; ID, intradermal; IIV3, trivalent split-virion inactivated influenza vaccine (egg-derived); IIV4, quadrivalent split-virion inactivated influenza vaccine (egg-derived); IM, intramuscular; M, male; MN, microneutralization; NA, not available; QIV, quadrivalent influenza vaccine (zonal purified subvirion); QIVc, cell-derived trivalent influenza vaccine; REPEVAX, diphtheria, tetanus, pertussis (acellular, component), and poliomyelitis (inactivated) vaccine; TIV, trivalent influenza vaccine (zonal purified subvirion); TIVc, cell-derived trivalent influenza vaccine; TIVf, egg-derived trivalent influenza vaccine; USA, United States; VE, vaccine efficacy.

[a]Study participants included in the immunogenicity subset (all received influenza vaccine).

[b]Age groups as used in the main analysis.

[c]Age groups as available for each study.

[d]A history of at least 1 previous influenza vaccination, where available.

[e]Influenza vaccination history available as follows: GQM01: 2008–2009, 2009–2010, and 2010–2011 seasons. GQM04: From 2009–2010, 2010–2011, and 2011–2012 seasons. GQM07: 2014–2015 season. GQM11: 2011–2012, 2012–2013, and 2013–2014 seasons. QID01: 2011–2012. RPV03C, 112963, 114269, 117036, 117276: 3 previous seasons. 201251: 4 previous seasons. CLSCTQIV-13–01/V118-01/V118-20: Past 5 years. V58-23/V70-27/V130-01: Previous influenza vaccination without precision.

[f]Risk of bias within this meta-analysis was assessed using the ROBINS-I tool, with respect to sex-related outcomes.

[g]Children were excluded from analysis.

[h]Immunogenicity population sample size.

[i]Study could not be included in meta-analyses as data for females (comparator) were not available, but these data were used in the generalized mixed-effects model as part of sensitivity analysis.

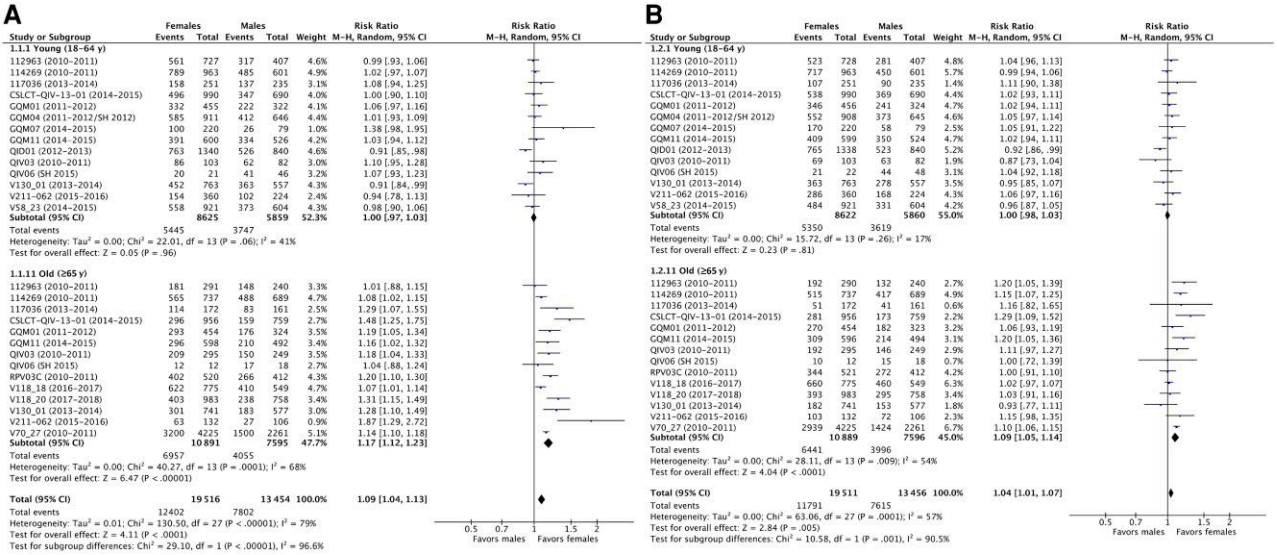

Figure 2. Relative risk for seroconversion following influenza vaccination in females vs males for influenza A strains, by age group. *A*, Influenza A/H1N1 strain. *B*, Influenza A/H3N2 strain. Seroconversion was defined as either a prevaccination hemagglutination inhibition (HI) titer <1:10 and a postvaccination HI titer >1:40 or a prevaccination HI titer ≥1:10 and a minimum 4-fold rise in postvaccination HI antibody titer (European Medicines Agency definition). Abbreviations: CI, confidence interval; df, degrees of freedom; M-H, Mantel-Haenszel; SH, Southern Hemisphere.

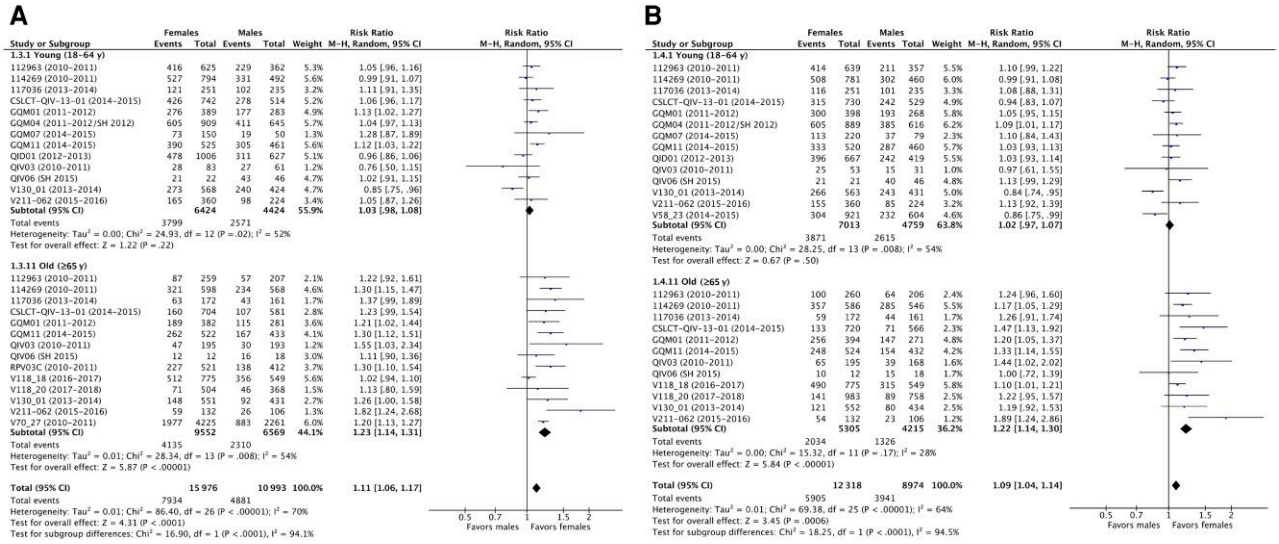

Figure 3. Relative risk for seroconversion following influenza vaccination in females vs males for influenza B strains, by age group. *A*, Influenza B/Victoria strain. *B*, Influenza B/Yamagata strain. Seroconversion was defined as either a prevaccination hemagglutination inhibition (HI) titer <1:10 and a postvaccination HI titer >1:40 or a prevaccination HI titer ≥1:10 and a minimum 4-fold rise in postvaccination HI antibody titer (European Medicines Agency definition). Abbreviations: CI, confidence interval; df, degrees of freedom; M-H, Mantel-Haenszel; SH, Southern Hemisphere.

(H3N2: $RR_{younger}$ = 1.11 [95% CI, 1.03–1.19], RR_{older} = 1.13 [95% CI, 1.05–1.23]; B/Victoria: $RR_{younger}$ = 1.25 [95% CI, 1.06–1.46], RR_{older} = 1.37 [95% CI, 1.24–1.51]; B/Yamagata: $RR_{younger}$ = 1.22 [95% CI, 1.07–1.40], RR_{older} = 1.25 [95% CI, 1.14–1.38]) (Supplementary Table 4). Finally, data were analyzed by season, to ensure that seasonal variation and level of preexisting immunity do not confound our results. Findings were consistent with those presented in the main analysis (data not shown).

We further investigated these results by fitting GLMM for the odds of seroconversion (Supplementary Table 5). Results were in accordance with those presented in the main analyses. Finally, adjusting for multiple testing did not change our conclusions (Supplementary Table 6).

Table 2. Geometric Mean Ratio Difference[a] Between Females and Males With 95% Confidence Intervals

Vaccine Strain	Younger: GMR Difference (95% CI) (No., I^2 Statistic as %)	*P* Value[b]	Older: GMR Difference (95% CI) (No., I^2 Statistic as %)	*P* Value[b]	All Age Groups: GMR Difference (95% CI) (No., I^2 Statistic as %)	*P* Value[b]	*P* Value for Subgroup Differences
A/H1N1	−0.18 (−.81 to .44) (14 480, 50%)	.56	1.62 (1.1–2.14) (18 486, 81%)	<.05	0.82 (.41–1.22) (32 966, 74%)	<.05	<.0001
A/H3N2	0.03 (−.48 to .53) (14 477, 65%)	.92	0.88 (.49–1.26) (18 485, 74%)	<.05	0.47 (.18–.77) (32 962, 69%)	<.05	.009
B/Victoria[c]	−0.05 (−.47 to .37) (11 495, 66%)	.81	.57 (.33–.80) (16 887, 78%)	<.05	0.32 (.12–.53) (28 382, 74%)	<.05	.01
B/Yamagata[c]	−0.10 (−.39 to .19) (12 514, 55%)	.51	0.48 (.26–.7) (10 281, 78%)	<.05	0.22 (.05–.39) (22 795, 70%)	<.05	.002

Abbreviations: CI, confidence interval; GMR, geometric mean ratio.

[a]Compares females to males (reference).

[b]*P* value for the overall effect.

[c]In the CSLCT-QIV-13-01 and V130-01 studies, antibody titers for B strains were aggregated for participants who received trivalent influenza vaccine (TIV) with either B/Victoria or B/Yamagata strains. GMR could not be extracted only for those receiving the TIV with corresponding strain.

Table 3. Crude Vaccine Efficacy Estimates Against Laboratory-Confirmed Influenza and Influenza-like Illness in Females and Males in Older Age Group (≥65 Years)

Sex	LCI VE (95% CI)	ILI VE (95% CI)
Female	27.32% (1.15–46.56)	5.65% (−6.97 to 16.79)
Male	6.06% (−37.68 to 35.90)	−8.40% (−28.51 to 8.56)

Abbreviations: CI, confidence interval; LCI, laboratory-confirmed influenza; VE, vaccine efficacy.

Furthermore, we tried to evaluate the proportion of participants with an ongoing hormone replacement therapy (HRT) when data were available, as HRT may affect immune response to vaccination [48]. HRT was used in <6% of participants (data not shown). Thus, no further sensitivity analyses were done by HRT status, as it was deemed unlikely to impact our findings.

Certainty of Evidence

We used the GRADE methodology to assess the certainty of seroconversion evidence from included studies, in the overall population and older adults. We found that evidence was of low certainty for influenza A/H1N1, due to serious risk of bias and moderate heterogeneity. However, certainty was moderate for remaining 3 strains, as findings were consistent (Supplementary Table 7). Certainty of VE evidence was not assessed, as data were from a single study.

DISCUSSION

In this meta-analysis of 19 RCT and >33 000 participants' data, we found sex differences in influenza vaccine–induced immunogenicity. For all influenza strains and irrespective of their age, females had slightly higher chances to seroconvert compared to males. Seroconversion is an in vitro parameter used as surrogate to clinical VE, that is, associated with a reduction in influenza illness incidence [21, 49]. Antibody ratios (GMRs) were also higher in females of all ages. These findings are consistent with several nonclinical studies that outlined a more robust immune response in females, following influenza vaccination or infection [6, 49–52]. Genes and hormones seem to be key factors to this female-biased antibody response [15, 49, 52]. Aging is associated with important changes in the endocrine and immune systems, commonly referred to as immunosenescence [8, 48, 53]. Although not fully understood, the sexual dimorphism in immune functions seems to persist during immunosenescence [54]. It was suggested elsewhere that aging females lose their immunological advantage in response to vaccines, including influenza [8, 48], yet our age-stratified analyses demonstrated an opposite effect. Indeed, subgroup analyses showed that sex differences in the immunogenicity of influenza vaccines were restricted to older populations. The absolute risk increase for strain-specific seroconversion ranged from 47 to 91 additional cases per 1000 vaccinees in

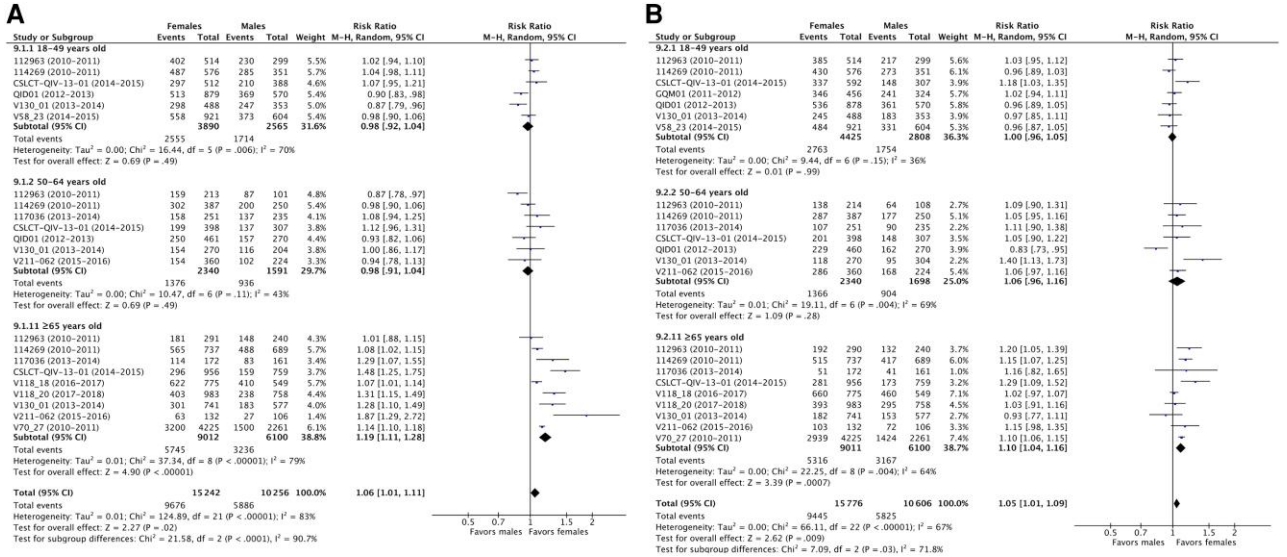

Figure 4. Relative risk for seroconversion following influenza vaccination in females vs males for influenza A strains, by predefined age groups (18–49, 50–64, and ≥65 y). *A*, Influenza A/H1N1 strain. *B*, Influenza A/H3N2 strain. Seroconversion was defined as either a prevaccination hemagglutination inhibition (HI) titer <1:10 and a postvaccination HI titer >1:40 or a prevaccination HI titer ≥1:10 and a minimum 4-fold rise in postvaccination HI antibody titer (European Medicines Agency definition). Abbreviations: CI, confidence interval; df, degrees of freedom; M-H, Mantel-Haenszel.

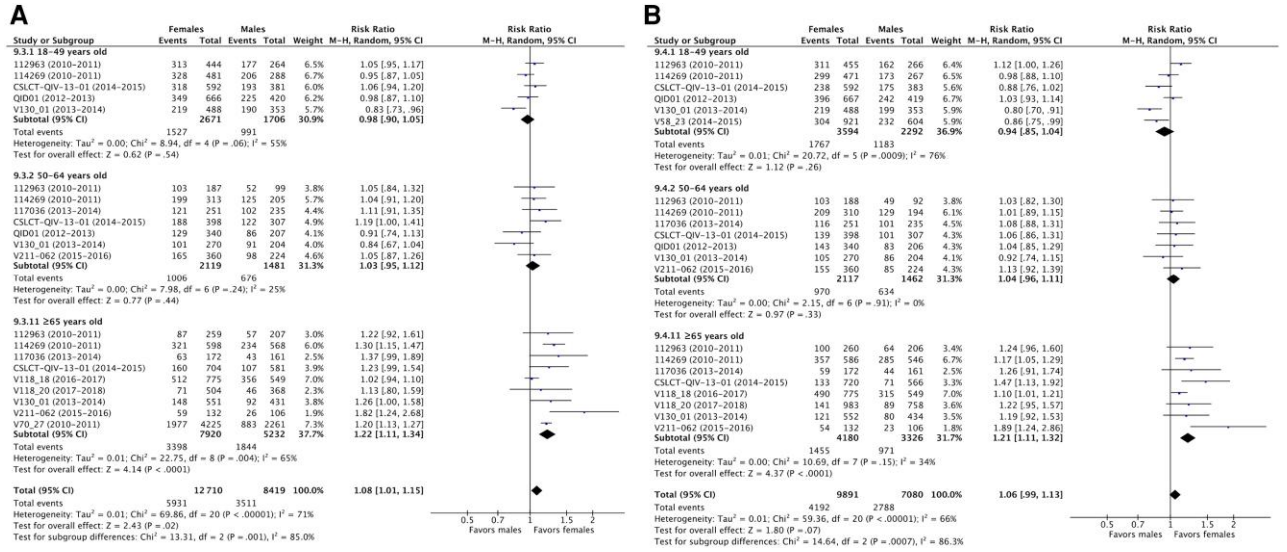

Figure 5. Relative risk for seroconversion following influenza vaccination in females vs males for influenza B strains, by predefined age groups (18–49, 50–64, and ≥65 y). *A*, Influenza B/Victoria strain. *B*, Influenza B/Yamagata strain. Seroconversion was defined as either a prevaccination hemagglutination inhibition (HI) titer <1:10 and a postvaccination HI titer >1:40 or a prevaccination HI titer ≥1:10 and a minimum 4-fold rise in postvaccination HI antibody titer (European Medicines Agency definition). Abbreviations: CI, confidence interval; df, degrees of freedom; M-H, Mantel-Haenszel.

older females, with the highest increase observed for the H1N1 strain. We further investigated the impact of preexisting immunity on sex differences in the immunogenicity of influenza vaccines for each age group. Sánchez-de Prada et al reported similar findings in their retrospective study, where 2243 adults who received influenza vaccine between 2006 and 2018 were enrolled and HI antibody titers were measured before and 28 days after vaccination [55]. While no differences were observed in younger adults, elderly females (≥65 years) displayed a greater humoral response against influenza A/H1N1 and B/Victoria, compared to elderly males [55]. These results are consistent with our findings. One possible explanation to this

phenomenon could be that elderly males experience a more dramatic decrease in their total numbers of T and B cells, compared to elderly females [13, 55, 56].

Surprisingly, in both younger and older adults, influenza vaccination history was associated with significantly higher seroconversion proportions in females for influenza H3N2 and B strains. Sex differences were not observed in adults with no previous influenza vaccination, regardless of their age. Similar results were reported by Engler et al in their RCT investigating the effects of age, sex, and dose on the immunogenicity of intramuscular TIV [57]. The authors reported significantly higher antibody titers and GMR in younger females compared to males of the same age group (ie, 18–64 years), with self-reported receipt of at least 1 dose of TIV within the past 3 years [57]. This might be explained by a higher B-cell activity, including antibody production and activity of memory B cells, in females [58]. While the higher immune response following influenza vaccination in females is thought to correlate with an improved clinical efficacy, sex-disaggregated VE data are still scarce. In this meta-analysis, VE was only assessed in 1 study, which was done in older populations. Influenza vaccine seemed to provide more protection against LCI in older females compared to their male counterparts. Similarly, in an analysis of historical databases of the Canadian Sentinel Practitioner Surveillance Network across 7 influenza seasons, adjusted VE against medically attended influenza illness was higher in females compared to males, with greater effects in those aged 50 years and over [59].

Influenza vaccination is an effective means for preventing influenza infections, yet, its efficacy is highly variable, ranging between 10% and 60%, depending on the season [5]. There is a growing interest in improving influenza vaccination outcomes, to mitigate the global burden of disease [5, 49]. One of the main barriers to achieving optimal VE is the mismatch caused by antigenic drift that yearly vaccine reformulation often fails to circumvent [6]. Although often neglected, host-related factors (eg, age, sex, and preexisting immunity) are also important determinants of vaccination outcomes [5]. In the present study, we demonstrated that age and preexisting immunity do impact the immune response to influenza vaccine when assessed by sex.

This meta-analysis has some limitations. First, we were limited in our ability to adequately account for the effect of age in our analyses. However, this was unlikely to change our conclusions as demonstrated in sensitivity analyses. Second, data on influenza vaccine and illness history were not available for all participants. Preexisting immunity is an important determinant of the response to vaccination [5], and this was further confirmed with subgroup analyses. Fitting GLMM on a subset of studies with complete data allowed us to further validate our results while accounting for the effect of age and vaccination history for seroconversion. Another limit was that only 24.7% of eligible studies could be included in our meta-analysis, mainly due to data-sharing constraints. However, we believe this does not affect our findings, as included RCTs are unlikely to have different results compared to those excluded. Finally, RCT participants are usually different from the general population, as they are medically stable or with no underlying medical conditions. Yet, we believe this does not hinder the generalizability of our findings, due to the biological plausibility of the effect of sex on the immune response following vaccination [5, 49].

To our knowledge, this is the first meta-analysis with IPD and aggregate data to assess sex differences in the immunogenicity and efficacy of seasonal influenza vaccine. Despite the scarcity of data on vaccine efficacy, our study provided considerably robust evidence of sex differences in influenza vaccine–induced immunogenicity. Using data from phase 3 RCTs, we were able to fill important knowledge gaps regarding the impact of sex on the immune response following influenza vaccination. The main advantage of using RCT data is the reduced risk of bias compared to observational studies, although randomization could no longer be considered as the original analyses were not done by sex.

In this meta-analysis, we showed that influenza vaccines' immunogenicity—and, potentially, efficacy—is higher in females compared to males in older populations. While it might be premature to call for routine vaccine dosing recommendations to be tailored for sex, there is a clear signal that the "one size fits all" approach is not optimal. It is therefore necessary for researchers to generate sex-disaggregated data on vaccination outcomes. Further methodological considerations need to be made when assessing sex-specific vaccine effectiveness (ie, real-world VE due to biologic attributes of sex), as well as behavioral differences related to gender. Indeed, the test-negative design is the preferred method to assess vaccine effectiveness as it minimizes selection bias arising from differential health-seeking behaviors in vaccinated and nonvaccinated individuals. In test-negative design studies, cases and controls are originally derived from a population of subjects who seek care for acute respiratory illness [60]. Thus, caution is needed when assessing sex-specific VE, as men tend to show a delayed health-seeking behavior when experiencing illness compared to women [61]. Finally, further studies assessing the administration of different doses to females and to males will help inform policy and recommendations.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. F. T., M. K., C. Q., E. R., M. S., and S. E. M. conceived the study. F. T. conducted the search. F. T. and A. A. screened all studies. F. T., with guidance from M. K. and C. Q., performed the

analysis. F. T. wrote the first draft of the article. All authors critically reviewed the manuscript for intellectual content and approved the final version prior to submission.

Data availability. Individual patient data are available upon request through Vivli and Clinical Study Data Request portals.

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Potential conflicts of interest. M. S. has been an investigator on projects funded by GlaxoSmithKline, Merck, Moderna, Pfizer, Sanofi Pasteur, CSL-Seqirus, Symvivo, and VBI Vaccines, all paid to institution. All other authors report no potential conflicts.

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