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Investigating the Legacy of the 1918 Influenza Pandemic in Age-Related Seroepidemiology and Immune Responses to Subsequent Influenza A(H1N1) Viruses Through a Structural Equation Model

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A(H1N1) strains of *Influenzavirus* were responsible for 2 pandemics in the last 100 years. Because infections experienced early in life may have a long-lasting influence on future immune response against other influenza strains, we drew on previously collected seroincidence data from Singapore ($n = 2{,}554$; June–October 2009) to investigate whether the 1918 pandemic influenza virus and its early descendants produced an age-related signature in immune responses against the A/California/7/2009(H1N1)pdm09 virus of 2009. Hemagglutination inhibition assays revealed a J-shaped relationship; the oldest birth cohort (born in 1911–1926) had the highest titers, followed by the youngest (born in 1987–1992). Differential response by vaccination history was also observed, with seasonal influenza vaccine being associated with higher titers mainly in the oldest birth cohort. On the assumption that antibody titers are a correlate of protection, structural equation modeling predicted that a titer-mediated effect by the vaccine could, on its own, account for a negative association with seroconversion equivalent to a risk reduction of 23% (relative risk = 0.77, 95% confidence interval: 0.60, 0.99) in the oldest birth cohort. A subset of 503 samples tested against the A/Brisbane/59/2007(H1N1) and A/Puerto Rico/8/1934(H1N1) strains also revealed different age-related antibody profiles. The effectiveness of seasonal influenza vaccines against future pandemic strains could thus be age-dependent and related to early-life exposures.

antibodies; cell-mediated immunity; cohort studies; cross-protection; influenza; serology; surveillance; vaccines

Abbreviations: CI, confidence interval; GMT, geometric mean titer; HI, hemagglutination inhibition; LTCF, long-term care facilities; RR, relative risk; SEM, structural equation model.

A(H1N1) subtypes of *Influenzavirus* were responsible for 2 of the 4 naturally occurring influenza A pandemics in the last 100 years. The influenza A(H1N1) pandemic of 1918 was associated with widespread mortality (1, 2) several orders of magnitude worse than that of seasonal influenza. Conversely, the influenza A(H1N1)pdm09 pandemic that occurred in 2009 had an impact not dissimilar to that of seasonal influenza epidemics (3). However, both pandemics were associated with an age-related pattern of morbidity and mortality that differed from seasonal influenza (3, 4), with the elderly being relatively protected as compared with adults in younger age groups.

Differences in age-related morbidity and mortality between seasonal and pandemic influenza may be due to differential exposures to related influenza viruses (5). Indeed, while vastly different in severity, the influenza A(H1N1)pdm09 virus of 2009 bore antigenic similarities to the virus that caused the 1918 pandemic (6). If so, it would be interesting to explore whether survivors of the 1918 influenza pandemic, and those exposed to early descendants of the 1918 virus, differed from younger persons in their immune response to the influenza A(H1N1)pdm09 virus of 2009. Indeed, there were indications of age-related differences in the levels of cross-neutralizing antibodies to influenza A(H1N1)pdm09 among individuals. However, data on the role of pre-2009 seasonal influenza vaccine in accounting for this were equivocal (7). Estimates of the effectiveness of seasonal influenza vaccine against influenza A(H1N1)pdm09 have also been highly variable. Some studies demonstrated weak protection (8), while others found that vaccination increased the risk of symptomatic infections (9, 10). One age-stratified analysis suggested nonsignificant protection in

older age groups (11). However, the small numbers of older persons and the age cutoff point (of ≥50 years) in that study did not permit more detailed assessment of whether this could be attributed to differential responses to the vaccine in birth cohorts exposed to the 1918 influenza A(H1N1) virus and its early descendants.

In this study, we investigated age-related differences in antibody levels against influenza A(H1N1)pdm09 and the association of the seasonal influenza vaccine with antibody titers and risk of seroconversion. We used a structural equation modeling framework to assess whether age-related differences in the vaccine's association with titers could cause age-related differences in vaccine effectiveness. In the Discussion we suggest how this approach could, in the future, be used to predict the age-specific effectiveness of a seasonal influenza vaccine against the emergence of a new pandemic virus. Finally, we discuss whether the age-related signatures identified in our data are more compatible with exposure to influenza A(H1N1) viruses associated with the 1918 pandemic or whether they generally represent early-childhood exposures to influenza A(H1N1) viruses, given that influenza A(H1N1) viruses were reintroduced into the human population (following an absence of about 20 years starting from the late 1950s) in 1977 (12) and were circulating in the human population just before the 2009 pandemic.

METHODS

Study populations and design

We drew on samples and data from our previous study investigating influenza A(H1N1)pdm09 transmission in a community cohort, military personnel, and staff and residents of 2 long-term care facilities (LTCFs) in Singapore (June 22, 2009–October 15, 2009) (13). We also included 2 additional LTCFs for which data were unavailable at the time of (and hence not reported in) the original study, to increase the number of elderly persons for analysis.

The study design involved measuring antibody levels by means of hemagglutination inhibition (HI) assays to influenza A(H1N1)pdm09 in a "baseline" sample (i.e., the earliest available sample for each participant) and up to 2 follow-up samples (Figure 1). In the community cohort, baseline samples were predominantly archived sera collected before the first appearance of the pandemic influenza strain in Singapore in late May 2009 (14). Other baseline samples were taken after local transmission had been detected but before widespread epidemic activity, and were assumed to reflect preexisting antibody titers in the respective populations. The intraepidemic sample (community and military cohorts) was taken from mid-August 2009, and the postepidemic sample was taken from late September 2009 (all groups). Seroconversion (i.e., ≥4-fold increase in titers to A/California/7/2009(H1N1)pdm09 between any pair of samples) was taken as evidence of serological infection during the influenza A(H1N1)pdm09 epidemic, with the change in titers between these samples being used to infer the "survival time" contributed by a given individual.

We considered the 6 weeks on either side of August 1, 2009 (when the epidemic peaked) to be the at-risk period (June 20, 2009–September 12, 2009). These time points coincided with early reports of community-based transmission and the week in which the incidence of influenza-like illness fell to less than 10% of all acute respiratory illness consultations, respectively (15, 16). Participants contributed time starting from their respective start dates, defined as the baseline sample date or June 20, 2009 (whichever was later). However, since the exact timing of infection was unknown, we imputed "survival time" on the basis of when seroconversion occurred (see Figure 1B).

Specimen collection and processing and laboratory methods

HI assays were performed according to standard protocols at the World Health Organization Collaborating Centre for Reference and Research on Influenza in Melbourne, Victoria, Australia (17) as previously described (13). All samples were tested against A/California/7/2009(H1N1)pdm09 pandemic virus. Baseline samples from LTCF staff and residents (which had the broadest age distribution among samples) were also tested against A/Brisbane/59/2007(H1N1), the last A(H1N1) strain used in seasonal vaccine formulations before mid-2009, and A/Puerto Rico/8/1934(H1N1), a historical A(H1N1) strain. Titers are expressed as the reciprocal of the highest dilution at which hemagglutination was prevented.

Outcomes and exposures of interest

The study had 2 key outcomes of interest: HI titers in baseline samples and seroconversion to A/California/7/2009(H1N1)pdm09. The primary exposures of interest were birth cohort and history of influenza vaccination, and the interaction between these 2 factors. We based birth cohort on the participant's birth year, in approximately 10-year bands, starting from 1911 (oldest participant) to 1926, followed by 1927–1936, and ending with the youngest participants, born in 1987–1992. Influenza vaccination history was based on self-reported receipt of vaccine up to 1 year before June 30, 2009 (and supplemented with institutional vaccination records in LTCF residents). Where vaccination history could not be reliably ascertained, participants were assumed not to have had influenza vaccination in the past year. A small number of community cohort participants had baseline samples banked before their most recent vaccination and thus were excluded from our analyses. In addition, samples banked before January 1, 2009, were omitted from analyses of the association between HI titers and vaccination history, since titers can decline substantially 6 months after vaccination (18).

Besides sex and study cohort, we also adjusted for several potential confounders. LTCF and military participants were recruited at the facility and unit levels, and a subgroup identifier in conjunction with a latent variable was used to model the degree of clustering of observations within facilities and units. Additionally, because seasonal influenza vaccination was bundled with other outbreak mitigation measures in "essential" and "health-care" military units (19), a binary variable was used to distinguish "essential"/"health-care" units from "normal" units.

Statistical methods

To investigate age-related signatures in immune profiles, we performed visualization of key outcome variables by birth cohort

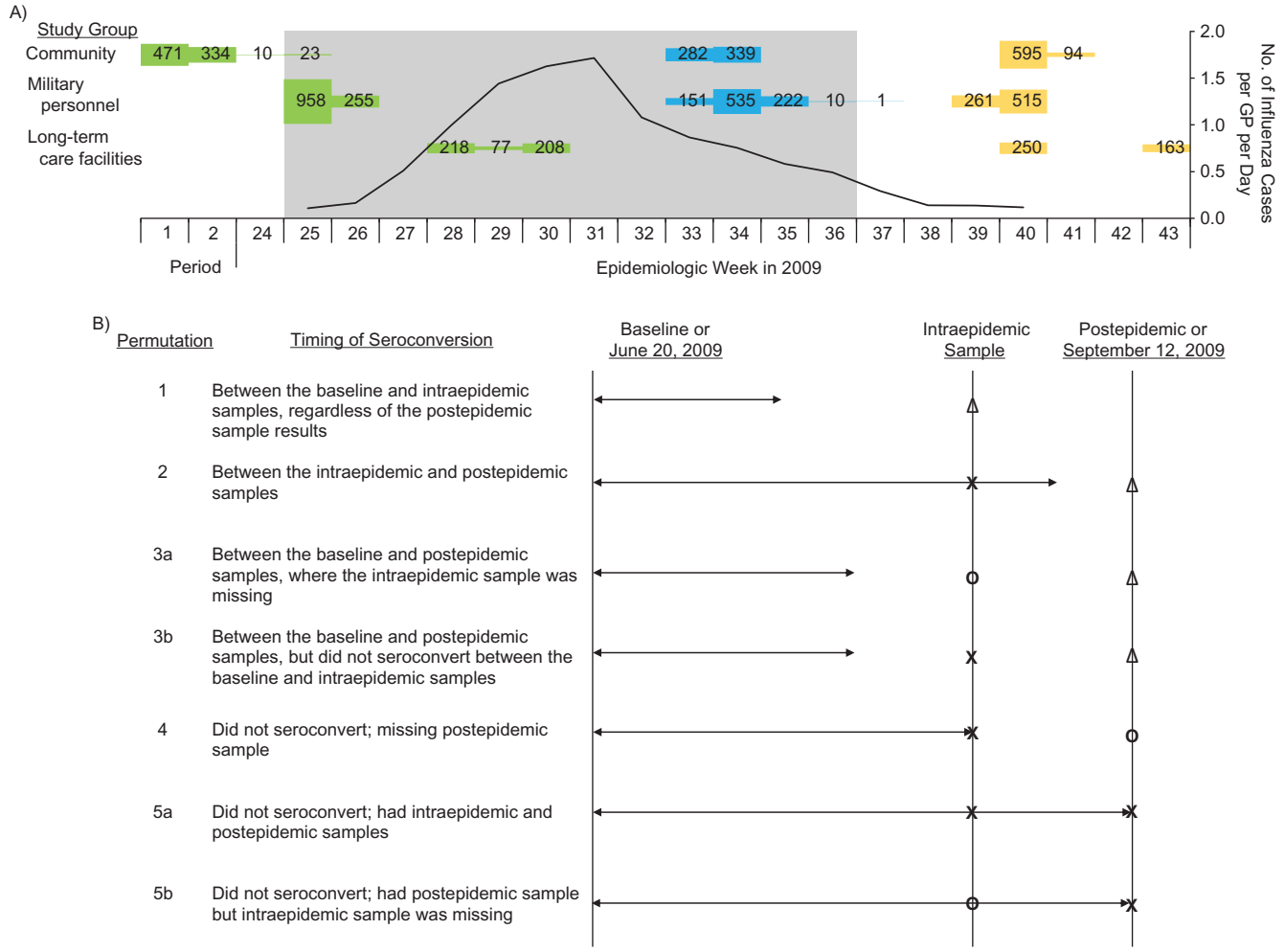

Figure 1. Timing of the collection of blood samples relative to the epidemic of influenza A(H1N1)pdm09 in 2009 (A) and imputation of survival time (B), Singapore, 2009. In part A, baseline (green), intraepidemic (blue), and postepidemic (yellow) samples taken from the community cohort, military personnel, and staff/residents of long-term care facilities (LTCF) are shown relative to influenza A(H1N1)pdm09 activity, represented by the sentinel surveillance cases (black line). No intraepidemic sample was collected from the LTCF cohort. The heights of the colored boxes denote the number of samples collected during a specific time period or epidemiologic week in 2009 (between June 14, 2009, and October 31, 2009). The gray shaded area represents the period which contributed survival time to the failure time regression model. Periods 1 and 2 denote samples collected between the years 2005 and 2008 and between January 1, 2009, and June 13, 2009, respectively. In part B, survival time is determined by the changes in the hemagglutination inhibition antibody titers between any 2 samples, with seroconversion defined as ≥4-fold increased titers to A/California/7/2009(H1N1)pdm09, the marker X denoting the absence of seroconversion, the marker O denoting either missing intraepidemic samples or missing postepidemic samples, the marker Δ denoting seroconversion, and the respective time points being represented as a single vertical line. GP, general practitioner.

and vaccination history, presenting geometric mean titers (GMTs) and the proportion of baseline samples with HI titers above a particular cutoff point and the proportions which seroconverted by baseline sample titer and birth cohort (see Web Appendix 1, available at https://academic.oup.com/aje).

Since HI titers potentially mediate protection (20), if a greater proportion of vaccinated persons had higher titers, vaccinated persons would experience a relative risk (RR) of infection that was decreased relative to nonvaccinated persons ("titer-mediated" RR^M; Web Appendix 2, Web Figure 1). However, a vaccine may cause an additional "titer-independent" effect that further reduces the risk equally among vaccinated persons with different antibody titers (RR^I), and the "combined effect" (RR^C) may thus be stronger. However, in observational studies, potential confounders must be considered. For instance, age affects risk of infection but is also associated with vaccine uptake and different antibody titer distributions.

A structural equation model (SEM) with 2 halves was hence used to investigate the relationship between the vaccine and risk of seroconversion while adjusting for potential confounders (Web Appendix 2, Web Figure 2). Firstly, we grouped titers into 3 categories (<10, 10, and ≥20) and used ordinal logistic regression to estimate the vaccine's association with titers (β_V^T). The second half of the SEM investigated the association of titers (titers of 10 and ≥20 vs. <10 as $\beta_{T_1}^S$ and $\beta_{T_2}^S$, respectively) and vaccination (β_V^S) with the risk of seroconversion

through failure time regression. Multiplying $\beta^S_{T_1}$ and $\beta^S_{T_2}$ by the proportion in each titer category according to vaccination history then gives the respective risk by vaccination history. This allows estimation of the titer-mediated effects (Web Appendix 2; Web Table 1, equations 1 and 2) and, together with the vaccine's titer-independent effect, β^S_V, the combined effect of the vaccine in protecting against seroconversion. We also present a "titer-unadjusted" estimate of the vaccine's association with seroconversion, where the term for titers is omitted. This reflects what studies on vaccine effectiveness that did not measure HI titers might observe. The above analyses were repeated with and without incorporating terms for interaction between vaccination history and birth cohort (as a categorical variable), with stratum-specific coefficients for the relationship between vaccination and seroconversion presented where appropriate.

We also performed structural equation modeling on a synthetic data set (a simplified representation of our actual data; Web Appendix 3, Web Table 2) to assist in the interpretation of our findings. All statistical analyses were performed in Stata 15 (StataCorp LLC, College Station, Texas), with $P < 0.05$ as the cutoff for statistical significance.

RESULTS

The study included 838, 1,213, 211, and 292 participants from the community, military personnel, LTCF staff, and LTCF residents, respectively (Table 1). Sample collection was completed by June 27, 2009, and July 1, 2009, for the community and military cohorts, respectively, and by July 28, 2009, for LTCF staff and residents. In the community cohort, only 367 (43.8%) samples postdated January 1, 2009, and 37 participants reported vaccination after the date of sample collection. Adults born after 1936 were reasonably represented in the community cohort, while military personnel were mostly young males, and LTCF staff were working-age adults. LTCF residents included several younger adults from a home for destitute persons, but 269 of 292 (92.1%) had been born before 1957. Only 47 (5.6%) community cohort participants reported receiving seasonal influenza vaccine, while other groups included a fair mix by vaccination history (42.6% (517/1,213) in military personnel, 42.2% (89/211) in LTCF staff, and 68.5% (200/292) in LTCF residents). However, vaccine uptake varied widely between military units, being 7.4% (35/472) in "normal" units and 64.0% (362/566) and 68.6% (120/175) in "essential" and "health-care" units, respectively. The risk of seroconversion was 13.5% (98/727) in the community cohort, 29.4% (312/1,060) in military personnel, and 4.2% (7/168) and 4.5% (11/245) among LTCF staff and LTCF residents, respectively.

Figure 2A shows that most participants (79.0%) had no detectable antibodies (titers <10) to A/California/07/2009(H1N1)pdm09. The proportion with titers ≥10 was significantly higher in participants who reported vaccination than in those who did not (25.2% and 18.3%, respectively; $P < 0.001$), and likewise for titers ≥20 (15.5% and 12.2%, respectively; $P = 0.029$), but not so for titers ≥40 (9.0% and 7.1%, respectively; $P = 0.129$). Figure 2B suggests an inverse relationship between HI titer and risk of infection, regardless of vaccination history. GMTs by age were J-shaped, being lowest in the 1957–1966 and 1967–1976 birth cohorts and highest in those born before 1927 (Figure 2C). While differences in GMTs by vaccination history varied from 1.0- to 1.1-fold in younger birth cohorts, this difference was 1.4-fold in persons born before 1937. Persons born from 1911 to 1926 also had the greatest difference in antibody prevalence at titers ≥10 (Figure 2D; 33.5% vs. 58% in other age groups) and ≥20 (Figure 2E; 20.6% vs. 2.5%) by vaccination history, although not so for titers ≥40 (not shown). After excluding military participants, the risk of seroconversion (Figure 2F) was similar by vaccination history across the younger birth cohorts. Among persons born in 1911–1926, vaccinated participants were less likely to be infected than nonvaccinated participants, but the overall number of infections was very small, and this difference was not statistically significant (3.3% and 12.5%, respectively; $P = 0.230$).

When using ordinal logistic regression to model HI titers while adjusting for birth cohort and other factors (Web Appendix 4, Web Figure 3), the J-shaped relationship persisted (model A in Table 2). Beta coefficients for titers (β^T) were highest in the oldest birth cohort, born in 1911–1926 (compared with the youngest cohort, $\beta^T = 0.89$, 95% confidence interval (CI): −0.16, 1.94; $P = 0.098$), followed by the second-oldest cohort and then the youngest birth cohort (1987–1992). Persons who reported vaccination had a significantly higher beta coefficient ($\beta^T = 0.60$, 95% CI: 0.32, 0.88; $P < 0.001$) than those who did not. The only other significant association was study group (LTCF staff and residents lower than community participants).

Compared with the youngest birth cohort, all other age groups had a reduced risk of seroconversion (model B in Table 2). Military personnel were at increased risk compared with the community cohort. Being from essential and health-care units in the military was protective. Adjustment for baseline titers through model C did not substantively change the associations. However, having titers of 10 (RR = 0.89, 95% CI: 0.62, 1.28; $P = 0.521$) and ≥20 (RR = 0.42, 95% CI: 0.29, 0.60; $P < 0.001$) was nonsignificantly and significantly associated with decreased risk of infection, respectively. Vaccination history showed little association with risk of infection.

The diamonds in Figure 3 show the association between vaccination and risk of infection by birth cohort, unadjusted for titers. While the titer-adjusted estimates were associated with an increased risk of seroconversion in all participants except the oldest and youngest birth cohorts, the relative risks had wide confidence intervals overlapping with 1. However, the titer-mediated estimate was associated with reduced risk of infection across the age groups, which in the oldest group (RR = 0.77, 95% CI: 0.60, 0.99) had a confidence interval below 1. The vaccine's titer-independent effect (without stratifying by birth cohort; Table 2, model C) was weakly protective. This, combined with the birth-cohort–stratified titer-mediated effect, strengthened the protection associated with vaccination (e.g., in the oldest birth cohort, RR = 0.74, 95% CI: 0.50, 1.09) but resulted in wider confidence intervals overlapping with 1. An analysis in which the vaccine's titer-independent effect was allowed to vary by birth cohort had even wider confidence intervals (Web Appendix 5, Web Figure 4).

Analysis of the LTCF group's baseline samples (using wider 20-year birth cohort intervals) showed HI titer profiles distinct from those for the A/California/7/2009(H1N1)pdm09 strain. The GMT against A/Brisbane/59/2007(H1N1) was highest in

Table 1. Demographic Characteristics, History of Vaccination, and Serological Evidence of Infection Among Participants in a Study of Influenza A Immunity, Singapore, 2009

Characteristic	Participant Group									
	Community Members[a] (*n* = 838)		Military Personnel[b] (*n* = 1,213)		LTCF Staff[c] (*n* = 211)		LTCF Residents[d] (*n* = 292)		All Participants (*n* = 2,554)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Timing of blood sample collection										
Baseline sample	June 29, 2005–June 27, 2009		June 22, 2005–July 1, 2009		July 16, 2005–July 28, 2009		July 16, 2005–July 28, 2009		June 29, 2005–July 28, 2009	
Intraepidemic sample	August 20, 2009–August 29, 2009		August 20, 2009–September 14, 2009		N/A[e]		N/A		August 20, 2009–September 14, 2009	
Postepidemic sample	October 6, 2009–October 11, 2009		September 30, 2009–October 9, 2009		October 5, 2009–October 30, 2009		October 5, 2009–October 30, 2009		September 30, 2009–October 30, 2009	
No. of blood samples tested										
Baseline sample	838	100	1,213	100	211	100	292	100	2,554	100
Intraepidemic sample	621	74.1	919	75.8	N/A	N/A	N/A	N/A	1,540	60.3
Postepidemic sample	689	82.2	776	64	168	79.6	245	83.9	1,878	73.5
At least 2 samples	727	86.8	1,060	87.4	168	79.6	245	83.9	2,200	86.1
Birth cohort										
1911–1926	N/A	N/A	N/A	N/A	N/A	N/A	52	17.8	52	2
1927–1936	4	0.5	N/A	N/A	N/A	N/A	88	30.1	92	3.6
1937–1946	27	3.2	N/A	N/A	3	1.4	91	31.2	121	4.7
1947–1956	153	18.3	15	1.2	14	6.6	38	13	220	8.6
1957–1966	289	34.5	22	1.8	18	8.5	18	6.2	347	13.6
1967–1976	185	22.1	43	3.5	58	27.5	4	1.4	290	11.4
1977–1986	156	18.6	229	18.9	98	46.4	1	0.3	484	19
1987–1992	24	2.9	904	74.5	20	9.5	N/A	N/A	948	37.1
Male sex	353	42.1	1,175	96.9	57	27	182	62.3	1,767	69.2
Receipt of influenza vaccine										
No/not known	791	94.4	696	57.4	122	57.8	92	31.5	1,701	66.6
Yes	47	5.6	517	42.6	89	42.2	200	68.5	853	33.4
Seroconversion[f]	98	13.5	312	29.4	7	4.2	11	4.5	428	19.5

Abbreviations: LTCF, long-term care facilities; N/A, not applicable.

[a] Of the baseline samples, 33 (4.0%) were purposively collected for the study; 367 (43.8%) postdated January 1, 2009, with 12 of these participants (1.4%) receiving seasonal influenza vaccine after sample collection.

[b] Recruited from 15 military units, with 5 normal units contributing 472 personnel (38.9%), 5 essential units contributing 566 personnel (46.7%), and 5 health-care units contributing 175 personnel (14.4%).

[c] Included 24 (11.4%), 116 (55.0%), 35 (16.6%), and 36 (17.1%) staff from LTCF A, B, C, and D, respectively; facilities A and B were part of our previous study (13).

[d] Included 68 (23.3%), 92 (31.5%), 42 (14.4%), and 90 (30.8%) residents from LTCF A, B, C, and D, respectively.

[e] No samples were collected.

[f] As a proportion of all participants who provided at least 2 samples.

the youngest birth cohort (1976–1989; Figure 4A) and lower in other birth cohorts. The vaccine was associated with a difference in GMTs that was greatest in the oldest birth cohort (1911–1936; 2.4-fold, 95% CI: 1.7, 3.4) and smallest in those born in 1957–1976 (1.5-fold, 95% CI: 0.9, 2.4). For A/Puerto Rico/8/1934(H1N1) (Figure 4B), GMTs increased in persons born before 1950 and were highest in the oldest birth cohort (1911–1936). The difference in GMT by vaccination history was widest in the 2 oldest birth cohorts (1.4-fold in those born in 1911–1936 and 1937–1956 vs. 1.2-fold in other birth cohorts).

DISCUSSION

Our study corroborates how early-life exposures—in this case, exposure to the 1918 influenza A(H1N1) virus and its

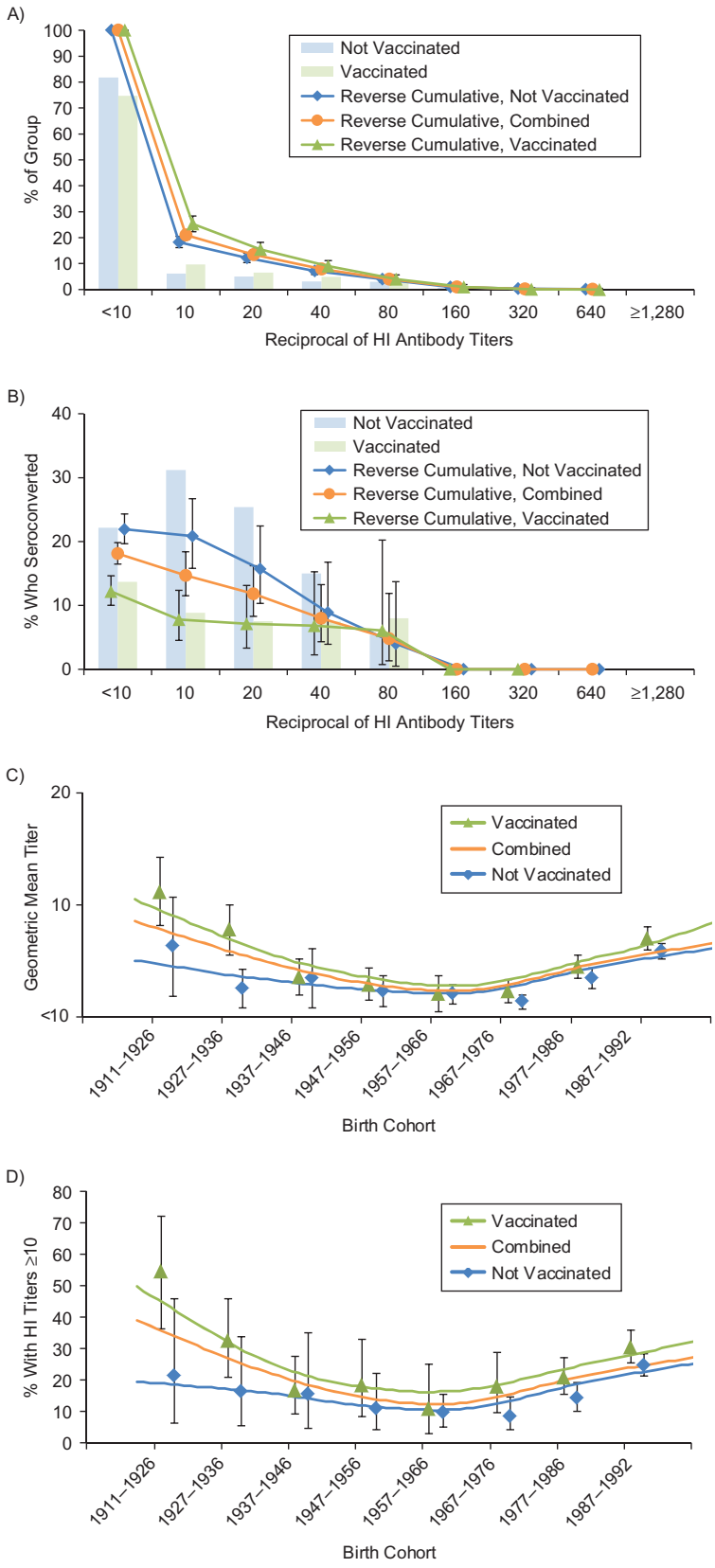

Figure 2 continues

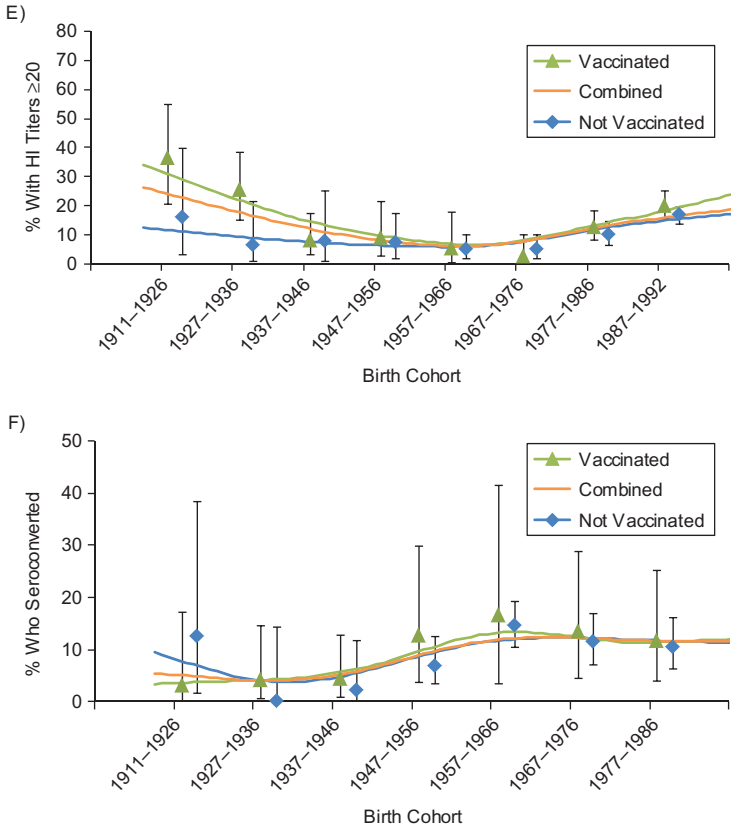

Figure 2. Hemagglutination inhibition (HI) titers for influenza A(H1N1)pdm09 and seroconversion in serum samples collected in Singapore, 2009. A) Distribution of baseline HI titers by vaccination history; B) risk of seroconversion by baseline HI antibody titer and vaccination history; C) distribution of geometric mean titers by birth cohort and vaccination history; D) proportion of participants with HI titers ≥10 by birth cohort and vaccination history; E) proportion of participants with HI titers ≥20 by birth cohort and vaccination history; F) risk of seroconversion after exclusion of the military cohort, by birth cohort and vaccination history. In parts A and B, the bars show the proportions for that HI titer, while lines connect the point estimates for the reverse cumulative distribution for antibody titers and seroconversions, respectively. In parts C–F, the point estimates represent the geometric mean titer, the proportions with HI titers ≥10 and ≥20, and the proportion with seroconversion, respectively; the lines were generated using the moving average/aggregate for a window of 25 years centered around each year, and then further smoothed to aid visualization using a locally weighted scatterplot smoothing (LOESS) plot with a span of 0.4. Error bars in all panels denote the 95% confidence intervals for the point estimates. In parts A and B, the denominators were 1,029, 77, 63, 40, 37, 9, 2, 2, and 0, respectively, for those not vaccinated and 607, 79, 53, 40, 25, 7, 1, 0, and 0, respectively, for those vaccinated in the corresponding titer categories (<10 . . . ≥1,280); in parts C–E, the denominators were 19, 31, 26, 56, 140, 124, 222, and 641, respectively, for those not vaccinated and 33, 59, 72, 44, 38, 68, 197, and 301, respectively, for those vaccinated in the corresponding birth cohorts (1911–1926 … 1987–1992); and in part F, the denominators were 16, 24, 45, 144, 262, 176, and 163, respectively, for those not vaccinated and 30, 47, 66, 31, 18, 37, and 43, respectively, for those vaccinated in the corresponding birth cohorts (1911–1926 … 1977–1986).

early descendants—may have profound and long-lasting implications for subsequent immune response to other influenza A(H1N1) strains. We observed a J-shaped relationship, with the 2 oldest birth cohorts (1911–1926 and 1927–1936) having the highest antibody titers, followed by the youngest (1987–1992). Titers to the A/California/7/2009(H1N1)pdm09 strain were 1.1-fold higher in participants who reported receiving a seasonal influenza vaccine containing an antigenically distant influenza A(H1N1) strain than in those who did not. However, titers were 1.4-fold higher in persons born nearest to the advent of the 1918 influenza pandemic. This potentially could have produced age-related differences in vaccine effectiveness, by mediating some protection in the oldest birth cohorts. However, the small number of infections among those born before 1937 and other limitations in our data set make it difficult to verify whether the vaccine was truly effective in those groups. Finally, in the LTCF subset of baseline samples, the HI titer profiles by birth cohort against 2 other influenza A(H1N1) strains from different time periods differed substantially from each other and from that observed for A/California/7/2009(H1N1)pdm09.

Other investigators have previously demonstrated a high prevalence of cross-reactive antibodies to the A/California/7/2009(H1N1)pdm09 strain in older populations (7, 21, 22). However, Hancock et al. (7) reported that receipt of the seasonal influenza vaccine did not result in a substantial increase in cross-reactive antibodies to A/California/7/2009(H1N1)pdm09 among adults aged 60 years or more, nor did that age group exhibit a greater cross-reactive response than younger age groups. One possible explanation for the discrepant findings between their study and ours is our reliance on cross-sectional samples and self-reported vaccination history (while their study drew

Table 2. Results From an Ordinal Logistic Regression Model of Factors Associated With Baseline Hemagglutination Inhibition Titers to A/California/7/2009(H1N1)pdm09 (Model A) and With Serological Infection Before (Model B) and After (Model C) Adjustment for Baseline Titers, Singapore, 2009

Characteristic	Model								
	Model A: Ordinal Logistic Regression Model of Associations With HI Titers in Baseline Sample[a]			Association With Seroconversion					
				Model B: Exclusion of Baseline Titers[a]			Model C: Inclusion of Baseline Titers[a]		
	β^T	95% CI	*P* Value	RR	95% CI	*P* Value	RR	95% CI	*P* Value
Birth cohort (vs. 1987–1992)									
1911–1926	0.89	−0.16, 1.94	0.098	0.39	0.08, 1.82	0.229	0.43	0.09, 2.04	0.288
1927–1936	0.15	−0.85, 1.16	0.763	0.15	0.03, 0.83	0.029	0.16	0.03, 0.87	0.034
1937–1946	−0.56	−1.57, 0.45	0.275	0.17	0.05, 0.58	0.005	0.16	0.04, 0.56	0.004
1947–1956	−0.72	−1.45, 0.00	0.051	0.33	0.17, 0.64	0.001	0.3	0.15, 0.60	0.001
1957–1966	−1.23	−1.85, −0.60	<0.001	0.54	0.33, 0.90	0.018	0.51	0.30, 0.86	0.011
1967–1976	−0.99	−1.54, −0.44	<0.001	0.46	0.27, 0.80	0.006	0.39	0.22, 0.69	0.001
1977–1986	−0.52	−0.87, −0.18	0.003	0.63	0.43, 0.94	0.022	0.61	0.41, 0.90	0.014
Female sex (vs. male)	−0.28	−0.65, 0.08	0.127	0.85	0.59, 1.21	0.355	0.9	0.62, 1.31	0.589
Study group (vs. community members)									
Military personnel	−0.23	−0.74, 0.28	0.378	2.08	0.93, 4.64	0.073	2.29	0.95, 5.53	0.066
LTCF staff and residents	−0.58	−1.13, −0.03	0.039	0.45	0.16, 1.26	0.127	0.48	0.16, 1.43	0.187
Essential or health-care military unit (vs. normal)	−0.30	−0.63, 0.04	0.080	0.39	0.25, 0.62	<0.001	0.41	0.25, 0.66	<0.001
LTCF resident (vs. LTCF staff)	−0.06	−0.99, 0.87	0.896	2.12	0.61, 7.40	0.239	2.18	0.62, 7.60	0.223
Receipt of seasonal influenza vaccine (vs. no/unknown)	0.6	0.32, 0.88	<0.001	1.04	0.78, 1.39	0.792	0.96	0.71, 1.30	0.787
Baseline HI titer (vs. <10)									
10							0.89	0.62, 1.28	0.521
≥20							0.42	0.29, 0.60	<0.001

Abbreviations: CI, confidence interval; LTCF, long-term care facilities; RR, relative risk.

[a] For illustration of the main effects, the above models exclude the terms for interaction between influenza vaccine and age group. Coefficients for the cutpoints in the ordinal logistic regression model were 0.56 (95% CI: −0.84, 1.22) and 1.12 (95% CI: −0.29, 1.77). *P* values for the term for clustering of observations within military units/LTCFs were 1.000, 0.027, and 0.012 for models A, B, and C, respectively.

on sera previously used for testing vaccine formulations, which would be more robust).

However, an alternative explanation may lie in the age groups analyzed. While Hancock et al. grouped persons aged 60 years and above, we observed that age-related differences for the association of the vaccine with titers were only apparent in cohorts born before 1937. Analyzing our data by age suggests that an increased response to the vaccine may only occur in persons aged 70 years or older. For instance, the differences in GMTs by vaccination history were 1.0-fold, 1.3-fold, and 1.4-fold for those aged 60–69, 70–79, and ≥80 years, respectively. Interestingly, mouse model and epitope modeling work suggests that both the 1934 A/Puerto Rico/8/34(H1N1) strain and the 1947 A/Fort Monmouth/1/47(H1N1) strain had greater potential than the 1957 A/Denver/1/57(H1N1) strain to generate cross-reactive antibodies to the 2009 pandemic virus (23). Since initial exposure to influenza mostly occurs between 2 and 4 years of age (24), those whose earliest exposure was to A/Fort Monmouth/1/47(H1N1) and earlier strains would have been in their mid-60s or older in mid-2009. Age-related differences were even more pronounced in the subset of samples tested against 1934 A/Puerto Rico/8/34(H1N1). There was an apparent peak in antibody titers for persons born around 1930, with the peak difference by vaccination history also being around that point (Figure 4B).

Taken together, our observations provide support for how the 1918 pandemic influenza A(H1N1) strain and its early descendants may have influenced the immune response of the older generation and consequently the epidemiology of subsequent influenza A(H1N1) viruses. This is in line with the "original antigenic sin" hypothesis (25), particularly the refinement proposed by Lessler et al. (26), which they termed "antigenic seniority." Although the study by Lessler et al. was based on serological data on influenza A(H3N2) (26), our data support their hypothesis that subsequent repeat exposures are interacting with the immune memory to elicit higher antibody titers to "more senior" strains. While Lessler et al.'s work did not address the potential role of vaccination in eliciting such a response, our data suggest that influenza vaccine formulations containing an antigenically distant strain may boost antibody levels, but

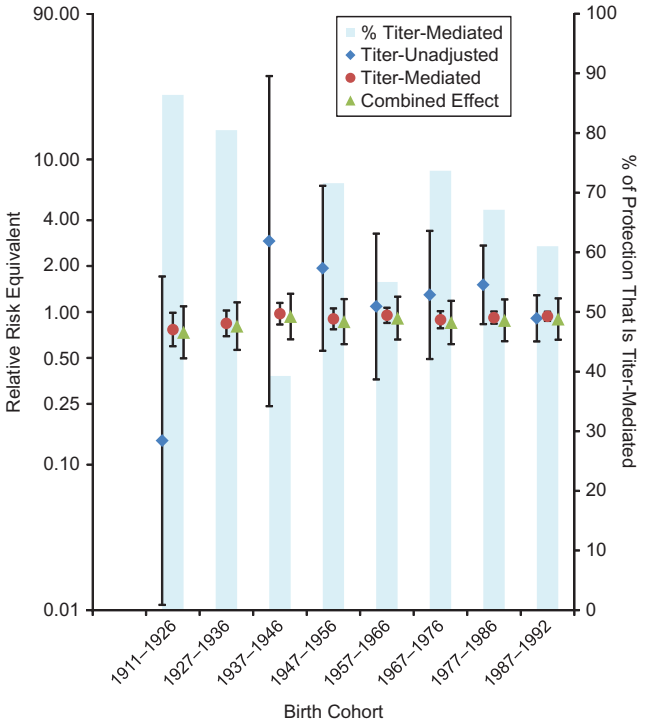

Figure 3. Age-stratified association between receipt of influenza A(H1N1)pdm09 vaccine and the risk of influenza infection, Singapore, 2009. Blue diamonds show titer-unadjusted relative risks, red circles show titer-mediated relative risks, and green triangles show combined relative risks. The light blue bars show the proportional contributions of the titer-mediated estimates to the combined effects. Error bars denote the 95% confidence intervals. For relative risk equivalents of 0.01, 0.10, 0.25, 0.50, 1.00, 2.00, 4.00, 10.00, and 90.00, the corresponding coefficients were −4.50, −2.30, −1.39, −0.69, 0, 0.70, 1.40, 2.30, and 4.50, respectively. The titer-unadjusted estimates (blue diamonds) show an increased risk in all birth cohorts except the oldest and youngest cohorts but have wide confidence intervals. The titer-mediated estimates (red circles) have the narrowest confidence intervals, with all age cohorts showing a reduced risk of infection, which is significant in the oldest birth cohort. The combination of titer-mediated and titer-independent estimates (green triangles), unstratified by age, enhanced the protection of vaccination, although the resulting confidence intervals widened.

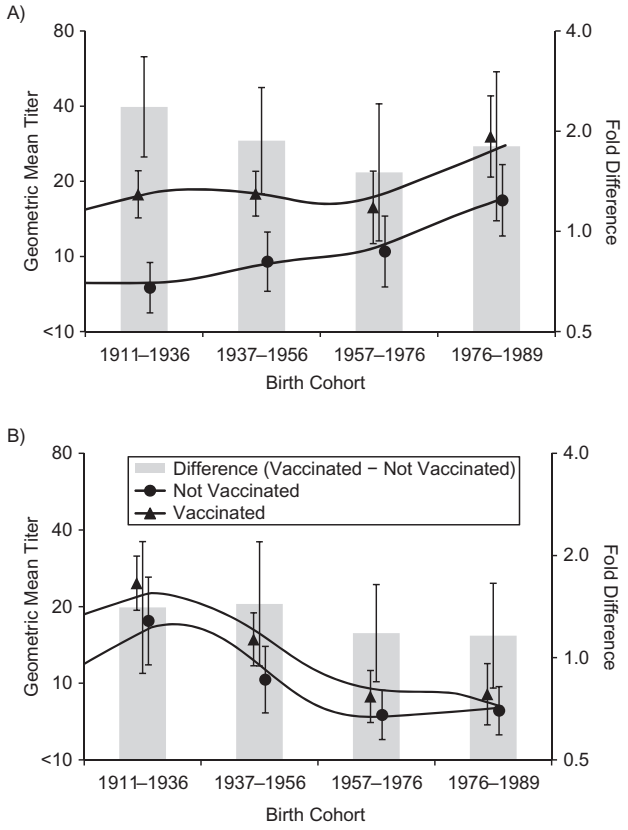

Figure 4. Geometric mean titers against the A/Brisbane/59/2007(H1N1) (A) and A/Puerto Rico/8/1934(H1N1) (B) strains of *Influenzavirus* according to vaccination history and birth cohort, Singapore, 2009. The markers show the point estimates for the geometric mean titers; the lines were generated using the moving average/aggregate for a window of 25 years centered around each year, and then further smoothed to aid visualization using a locally weighted scatterplot smoothing (LOESS) plot with a span of 0.4. Error bars in all panels denote the 95% confidence intervals for the point estimates. The *z*-axis represents the multiple of difference between vaccinated persons and unvaccinated persons. The denominators in parts A and B were 48, 43, 46, and 67, respectively, for those not vaccinated and 92, 103, 52, and 42, respectively, for those vaccinated in the corresponding birth cohorts (1911–1936 … 1976–1989).

only in age groups previously primed with an antigenically similar virus (27, 28). The key question, then, is whether such responses also correlate with protection.

In our titer-unadjusted estimates, the vaccine was associated with a nonsignificant reduction in risk of seroconversion in the oldest birth cohort. However, the confidence intervals were wide, and we could not adequately adjust for potential confounders, such as membership in LTCF with differences in the risk of exposure to the virus. The finding that adjusting for antibody levels did not substantively change the association of the vaccine with seroconversion was also somewhat surprising. To investigate further, we generated synthetic data simulating a titer-mediated effect similar to what we estimated (Web Appendices 3, 4, and 6; Web Table 2) with varying levels of titer-independent effects (Web Appendix 6, Web Figures 5–7). Briefly, the conclusions were that, at the sample size for the oldest birth cohort available to us, it would not have been possible to accurately estimate age-stratified titer-independent and titer-unadjusted effects conferred by the vaccine. On the other hand, the SEM approach correctly estimated the level of age-stratified titer-mediated effect that was simulated.

Were the SEM to be used in a predictive manner, then on the basis of the titer-mediated effect alone, we anticipate that the vaccine would confer negligible protection in younger age groups but would be protective in the oldest birth cohort and equivalent to a vaccine effectiveness of about 23% (1 – RR). Such an effect size could be of public health relevance in a severe pandemic but might be challenging to estimate reliably without large sample sizes, particularly in birth cohorts where infection rates are low. However, it must be acknowledged that our prediction of the titer-mediated effect was affected by how the SEM modeled titer distributions. The ordinal logistic

regression model grouping titers of ≥20 produced a reasonable fit to the observed distribution of titers (Web Appendix 4, Web Figure 3). However, using more categories or linear regression to model titers would have produced more strongly protective estimates for the titer-mediated effect in the oldest birth cohort (data not shown).

However, the degree to which HI titers mediate protection may not be the point here. There has been other evidence suggesting that both the incidence of infection and the severity of the 2009 influenza pandemic virus were lower in older age groups than in younger adults (29, 30). Potential mediators not accounted for here include memory B-cell and memory T-cell responses. These cells could have been primed to respond in older adults by exposure to past influenza A(H1N1) viruses descended from the 1918 pandemic virus (31, 32). Therefore, age-related differences in antibody levels and in the relationship between the vaccine and antibody titers are potentially an imperfect but still useful proxy for other mechanisms that also mediate protection. They point us to birth cohorts in which a seasonal influenza vaccine may be beneficial in the event of a subsequent influenza A pandemic.

We have already highlighted several limitations of the current study (the reliability of vaccination history and the low number of infections in some subgroups). In addition, antibody titers can wane substantially (18, 33, 34) over the time frame of about 6 months to 1 year. While we limited assessment of seroconversion to a 12-week period, we did not account for differences in the time from vaccination to antibody measurement, which could have compromised estimates of the correlation between vaccination and baseline titers, and in turn biased downwards the titer-mediated effect of the vaccine via baseline titers. Additionally, instead of different cohorts, we would ideally have used a large reasonably representative community-based cohort with key age groups and freshly collected (instead of banked) samples. Moreover, a ceiling effect could have prevented us from detecting seroconversion in persons with higher baseline antibody titers (35). We thus compared baseline titer distributions in a set of virologically determined infections (36) with those in nonseroconverting, asymptomatic participants from our community cohort. The resulting estimates for titer-mediated protection against infection were not dissimilar to those presented in Table 2 and Web Tables 3 and 4 (Web Appendices 7 and 8).

In conclusion, our data show how the influenza pandemic virus of 1918 and its early descendants potentially affected birth-cohort–related immune profiles and responses to a seasonal influenza vaccine formulation administered during the 2009 influenza A(H1N1) pandemic. While survivors of the original 1918 pandemic virus are unlikely to be a consideration in the next influenza pandemic, the principle that a past pandemic can interact with another over the space of decades remains relevant. In particular, our analysis suggests that a seasonal vaccine containing an antigenically distant strain can be useful, but only in age groups previously primed to respond. While some studies have already formulated approaches to prediction of a vaccine's effectiveness based on the correlates of protection (37, 38), we propose here how these can be combined with a SEM to simultaneously disentangle some effects from confounders while estimating the contribution of a particular correlate of protection. While the approach met with some challenges, we believe it remains possible to obtain some prediction of the anticipated effect using appropriately collected data, such as measurements of cross-reactive immune responses in age-stratified samples from vaccine trials. While the vaccine effectiveness estimated in the oldest age group based on the titer-mediated effect (via boosting of antibodies) was weak, an intervention with such levels of effectiveness would still be relevant if we were to again encounter a pandemic as severe as the influenza pandemic of 1918.

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