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A randomized controlled trial of antibody response to 2018–19 cell-based vs. egg-based quadrivalent inactivated influenza vaccine in children☆

Krissy K. Moehling[a], **Richard K. Zimmerman**[a], **Mary Patricia Nowalk**[a,*], **Chyongchiou Jeng Lin**[a], **Judith M. Martin**[b], **John F. Alcorn**[b,c], **Michael Susick**[a], **Ashley Burroughs**[d], **Crystal Holiday**[d], **Brendan Flannery**[d], **Min Z. Levine**[d]

[a]Department of Family Medicine, University of Pittsburgh, Pittsburgh, PA, USA

[b]Department of Pediatrics, University of Pittsburgh, Pittsburgh, PA, USA

[c]Department of Immunology, University of Pittsburgh, Pittsburgh, PA, USA

[d]National Center Immunizations and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta GA, USA

Abstract

Background: Current influenza vaccine effectiveness (VE) improvement efforts focus on minimizing egg adaptation mutations during manufacture. This study compared immune response of two FDA-approved quadrivalent inactivated influenza vaccines in an unblinded randomized controlled trial.

Methods: Participants were 144 community dwelling, healthy children/adolescents aged 4–20 years, randomized 1:1 in blocks of 4 to a vaccine grown in cell culture (ccIIV4 [Flucelvax®]; n = 85); or in egg medium (IIV4 [Fluzone ®]; n = 83). Blood was drawn at day 0 prevaccination and at day 28 (19–35 days) post vaccination. Hemagglutination inhibition (HI) assays against A/H1N1 and both B strains and microneutralization (MN) assays against egg-based and cell-based A/H3N2 strains were conducted. The primary outcome measure was seroconversion (day 28/day 0 titer ratio ≥ 4 with day 28 titer ≥ 40). Secondary outcomes were elevated titers (day 28 HI titer ≥ 1:110), geometric mean titers (GMTs) and mean fold rise (MFR) in titers. Outcomes were compared for 74 ccIIV4 recipients and 70 IIV4 recipients, and for those vaccinated and unvaccinated the previous year. Only the HI and MN laboratory analysis team was blinded to group assignment.

*Corresponding author at: Department of Family Medicine, 4420 Bayard St., Ste. 520, Pittsburgh, PA 15260, USA. tnowalk@pitt.edu (M.P. Nowalk).

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Declaration of Competing Interest

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Results: In this racially diverse (81% non-white) group of children with a median age of 14 years, baseline demographics did not differ between vaccine groups. At day 0, half or more in each vaccine group had elevated HI or MN titers. Low seroconversion rates (14%−35%) were found; they did not differ between groups. Among 2018–19 ccIIV4 recipients, those unvaccinated in the previous season showed significantly higher MFR against A/H1N1 and A/H3N2 cell-grown virus than the previously vaccinated. Similar results were found for MFR against B/Victoria among 2018–2019 IIV4 recipients.

Conclusion: In mostly older children with high baseline titers, no differences in seroconversion or other measures of antibody titers were found between ccIIV4 and IIV4 recipients against egg- and cell-grown influenza vaccine viruses.

Keywords

Immunogenicity; RCT; Influenza; Vaccine; Children; Adolescents

1. Introduction

In response to variable vaccine effectiveness and improvements in biotechnology, the types of influenza vaccines available, licensed and recommended for use, and some of the formulations of those vaccines have been changing with increasing rapidity. Furthermore, viral mutations either naturally occurring in the community, such as the significant genetic drift of 2014–2015 [1], or as a result of the manufacturing process have been documented. Increasing glycosolation of the wild influenza virus over time has reduced effectiveness of vaccines grown in eggs in general, and certain mutations, such as T160K, have markedly reduced the protection from egg-derived vaccines [2]. Of clinical significance, Chen et al. (2019), found a strong negative correlation between passage of A/H3N2 viruses in eggs and vaccine efficacy [3]. Thus, measuring the immune response to new vaccine formulations and comparing them with older, widely-used vaccines are warranted.

Furthermore, the immunological responses to new and reformulated vaccines across population subgroups and in the context of influenza vaccination history have not been thoroughly explored. While older adults are the group most susceptible to influenza-related morbidity and mortality [4], children are of particular interest because there is evidence that they serve as the major mode of influenza disease transmission in communities [5]. Moreover, they have the advantages of relatively short vaccination histories, and typically robust immune systems [5]. Thus, a study of immune response of children who were vaccinated two years in a row, compared with those without vaccination in the previous season may increase our understanding of repeated vaccination.

The purpose of this study was to compare serological responses of a racially diverse group of healthy children 4–20 years of age receiving ccIIV or egg-based quadrivalent influenza vaccine (IIV4) in the 2018–2019 influenza vaccination season. 2017–2018 was the first season in which one vaccine was manufactured which substituted an A/H3N2 strain grown using a non-egg, cell-based process (ccIIV) for the standard egg-based A/H3N2 strain while maintaining the A/H1N1 strain and B lineages grown in eggs. In 2018–2019, the ccIIV4

included three cell-based seed strains, namely B/Yamagata, B/Victoria and A/H3N2; A/H1N1 was still derived from an egg-based seed, leading to a 3:1 formulation.

Antibody titers were measured in participants who were known to have received no vaccine or had received the standard egg-based inactivated influenza vaccine in the previous season (2017–2018). We hypothesized that there would be no difference in immunological response between the two vaccines with respect to A/H1N1, but that there would be a difference between the two vaccines with respect to A/H3N2 and the two B lineages. Additionally, participants who were unvaccinated the previous season would have a greater change in antibody response following vaccination than those who had been vaccinated in the previous season.

2. Methods

The Institutional Review Boards at the University of Pittsburgh and the Centers for Disease Control and Prevention approved this study and it was registered at Clinicaltrials.gov, registration number NCT03614975. Written informed consent and assent, where appropriate, were obtained from all participants (or their parents/legal guardians) prior to beginning study procedures.

3. Study design and participants

This study was a randomized controlled clinical trial (RCT) that compared the serologic antibody response to two quadrivalent influenza vaccines: ccIIV4 [Flucelvax®] and the egg-based IIV4 [Fluzone®]. Both vaccines included A/H1N1/Michigan/45/2015-pd m09–like virus, A/H3N2/Singapore/INFIMH-16–0019/2016–like virus, B/Colorado/06/2017–like virus (Victoria lineage), and B/Phuket/3073/2013–like virus (Yamagata lineage). As stated above, the ccIIV4 included three cell-based seed strains, namely the two B lineages and the A/H3N2 and one egg-based strain, A/H1N1 leading to a 3:1 cell:egg seed strain formulation. The previous year's (2017–2018) vaccine formulation contained only one cell-based strain, A/H3N2.

A convenience sample of healthy participants aged 4–20 years old was enrolled during the fall of 2018 (September 13, 2018 through November 20, 2018) from five primary care health centers (one pediatric clinic and four family medicine practices). All study visits were completed prior to regional circulation of influenza virus; the final day 28 visit was completed on 12/13/2018.

Eligibility criteria included no allergies to eggs or influenza vaccine components and willingness to be randomized to receive one of the two 2018–2019 FDA approved study influenza vaccines. Exclusion criteria included: weight <37 lbs.; known to be pregnant; having an immunosuppressing health condition or taking immunosuppressant medications; having already received the 2018–2019 influenza vaccine; or were not able to complete all study visits in the appropriate time window. (See Fig. 1 for CONSORT flow diagram.)

Following screening and consent, a blood sample was drawn. Participants were then randomized to receive one of the two vaccines. In order to ensure that each of the five

enrollment sites administered roughly the same number of each type of vaccine, participants were randomized in blocks of four. A computer-generated 1:1 randomization assignment for each influenza vaccine type was created. Sequentially numbered vaccine assignment cards were created using the random assignment. Each participant was randomized by the research assistant using the next available numbered card. The cards instructed the clinical staff which vaccine to administer using standard protocols. Therefore, randomization was not blinded for either the participant or the research team. However, the HAI assay team was unaware of group assignment.

3.1. Demographic data collection

Baseline data were collected either via interview or on paper with entry by the research assistants into REDCap™ (a secure, online database management system). Baseline demographics included age, sex, race, ethnicity, parental educational status, health insurance coverage, and exposure to household smoking. Height and weight from the electronic medical record (EMR) if available, or from self-report were used to calculate body mass index (BMI). Age- and sex-specific BMI percentiles for children were calculated using the CDC growth charts from the year 2000 [6]. Those who were in the 95th percentile for BMI or higher were defined as obese.

3.2. Biological samples

Whole blood samples were obtained on participants pre-vaccination and 28 days (range 19–35 days) post influenza vacci-nation. Serum for hemagglutination inhibition (HI) titers was drawn in BD Vacutainer™ serum separator tubes with polymer gel/silica activator additive (BD 367989). Tubes were held at room temperature and taken to the processing laboratory within 4 h of being drawn. Aliquoted serum samples were frozen at −80°C until assayed.

3.3. Hemagglutination inhibition laboratory methods

Antibody assays were conducted following CDC's protocols [7] by the Influenza Division research laboratory at the CDC, who were blinded to group assignment. Sera were heat inactivated, tested for nonspecific agglutinins, and adsorbed as needed. Sera were then serially diluted 2-fold and incubated with 4 hemagglutination units per 25 μL of virus with erythrocytes for quantification of HI titers. Turkey erythrocytes were used for the testing of A/H1N1 and B influenza viruses, Guinea pig erythrocytes with 20 mM oseltamivir were used for the testing of A/H3N2 viruses. HI titer was defined as the reciprocal of the last dilution of serum that completely inhibited hemagglutination. Antibody titers <10 (initial sera dilution) were reported as 5 for analysis. Sera were tested in HI assays against the four vaccine strains included in the 2018–2019 influenza vaccines (including egg-grown A/Singapore/INFIM H-16–0019/2016–like virus.

3.4. Microneutralization laboratory methods

MN assays were conducted in the Influenza Division research laboratory at the CDC using Madin-Darby canine kidney (MDCK)–SIAT1 cells [8,9]. Sera were heat inactivated, 2-fold serially diluted and mixed with 100 50% tissue culture infective doses of influenza A/H3N2 viruses and incubated at 37°C with 5% CO_2 for 1 h. The virus-sera mixture was used to

infect 1.5×10^4 MDCK-SIAT1 cells/well and incubated overnight at 37°C with 5% CO_2. After cold acetone fixation, the presence of viral protein was quantified by an enzyme-linked immunosorbent assay, using monoclonal antibodies specific to the nucleoproteins of the influenza A viruses. MN antibody titers were measured against influenza A/H3N2 viruses representing the egg- and cell culture-grown A/H3 N2/Singapore/INFIMH-16–0019/2016–like virus.

3.5. HI outcome measures

The primary outcome measure was seroconversion, defined as the HAI titer ratio of day 28/day 0 ≥ 4 and HI titer at day 28 ≥ 40. Secondary outcomes were elevated titers, defined as a HI titer ≥ 1:110[10] at day 28 [10], geometric mean titers (GMT) and geometric mean fold-rise (MFR) in titers from day 0 to day 28 with 95% Confidence Intervals (CI) from repeated measures linear regression with log-transformed titers.

3.6. Statistical analyses

Based on previous research comparing seroconversion rates between IIV and ccIIV, we determined that a sample size of 160 to 356 would be needed to achieve an 80% power to detect a significant difference at alpha = 0.05. Thus, for comparison of two inactivated vaccines, the study would be underpowered with sample sizes of 74 and 70. A post-hoc power calculation on A/H3N2 – A/ Singapore cell grown virus using a Chi-square test revealed a power of 30%.

Summary statistics of demographics were conducted overall and by vaccine group using chi-square/Fisher exact tests for categorical variables and t-tests for continuous variables. For statistical analyses, specimens with reciprocal HI or MN titers of <10 were assigned a titer of 5. Geometric mean titers (GMTs), MFR or increases (i.e., GMT ratios), and 95% confidence intervals (95% CIs) were calculated using repeated-measure linear mixed models as previously described [11]. Fold-rise was calculated as the ratio of the post-vaccination titer to the pre-vaccination titer. HI and MN titers were log transformed to examine correlations.

Linear regression with log-transformed titers was used to examine associations between pre-vaccination, post-vaccination, or fold-rise in titers with vaccine type and prior season (2017–2018) vaccination status, including an interaction term for vaccine type and prior season vaccination. Generalized linear models with log transformed fold-rise as the outcome variable were used to test for significant effects by vaccine type and prior season vaccination, controlling for pre-vaccination titer. All analytical procedures were performed using SAS® 9.4 (Cary, NC). Statistical significance of two-sided tests was set at type I error (alpha) = 0.05.

4. Results

4.1. Demographics

One hundred seventy-one participants were enrolled between September 13 and December 13, 2018. Three persons withdrew after consent and were not randomized. Of those

randomized, 85 were randomized to receive ccIIV4 and 83 were randomized to receive IIV4. Eighteen participants did not complete both study visits or withdrew just after the day 0 blood draw leaving a total of 150 participants. Of those who completed the study, 4 did not have complete bloodwork (including MN) analyzed by the laboratory. This analysis reflects a total completed cohort of 144; 74 who received ccIIV4 and 70 who received IIV4. See Fig. 1.

Characteristics for the cohort including vaccine type received are presented in Table 1. Overall, 87% of participants were 9–20 years of age, median age = 14 years, 54% were female, and 81% were non-White. Most participants were publicly insured (77%) and most parents (83%) had no college education. Nearly one third were obese and a similar number (31%) reported being exposed to household smoking. Sixty percent of the cohort was vaccinated in 2017–2018 with a IIV4, with 40% being unvaccinated that season. The two vaccine recipient groups did not differ in their demographic characteristics.

Table 2 reports rates of seroconversion and elevated HI titers (titers ≥ 1:40 and ≥ 1:110), GMTs and MFR by type of vaccine received. Seroconversion rates were modest: 20–27% for A/H1N1, 15%−30% for A/H3N2 against egg grown virus and 24%−35% for A/H3N2 against cell-grown virus, 34% for B/Victoria, and 14–24% for B/Yamagata. There were no differences between the two vaccine groups in vaccine responses for rates of seroconversion.

Eighty one percent to 100% of participants had elevated titers ≥ 1:40 titers at day 0 and day 28, with no significant differences observed between vaccines. The number of participants with elevated titers (≥1:110) at baseline was high; for A/H1N1, 56%−58% had elevated titers; for A/H3N2 against egg-grown virus, 99% had elevated titers; for A/H3N2 against cell-grown virus, 46%–53% had elevated titers; for B/Victoria, 49% had elevated titers; and for B/Yamagata, 56%−64% had elevated titers. Again, there were no significant differences between the two types of vaccine recipients.

At day 28, the proportion of participants who had elevated titers increased to 80–86% for A/H1N1, 100% for A/H3N2 against egg-grown virus, 66–77% for A/H3N2 against cell-grown virus, 76–79% for B/Victoria, and 74–82% for B/Yamagata. There were no significant differences between vaccine recipient groups.

Similar results were found for day 0 and day 28 GMTs and MFR across all vaccine strains and lineages with one exception. MFR for A/H3N2 against egg-grown virus was significantly higher in egg-based vaccine (IIV4) recipients (2.3; 95% CI = 1.8–2.9) than cell-based vaccine (ccIIV) recipients (1.6; 95% CI = 1.3–2.0; $P = 0.05$).

4.2. Post hoc analyses including prior vaccination

The majority of participants (62% of ccIIV recipients and 57% of IIV4 recipients) had received IIV4 in the prior season, with the remainder having been unvaccinated. MFR responses to each vaccine were stratified by prior year vaccination status (Table 3). Generally speaking, previously (2017–2018) unvaccinated participants, regardless of the vaccine received in 2018–2019 (egg-based or cell-based vaccine) had greater MFR in antibody titers post vaccination than those who were vaccinated with an egg-based vaccine

in 2017–2018. These values were tested using linear regression that controlled for baseline titers. Three signifi-cant differences in MFR were identified. MFR among the previously unvaccinated was significantly higher for ccIIV recipients against A/H1N1 and A/H3N2 cell grown virus compared with previously vaccinated participants and for IIV4 recipients against B/Victoria. That is, compared with children who received the 2017–2018 IIV4, children who were unvaccinated in 2017–2018 and received ccIIV in 2018–2019, had a significantly greater change in antibody levels against A/H1N1 and cell-grown A/H3N2. The previously unvaccinated who received the 2018–2019 IIV4 had a significantly greater change in antibody levels against B Yamagata than those who had been vaccinated with IIV4 both seasons.

5. Discussion

We found that seroconversion, seropositivity, and fold-rise at day 28 did not differ significantly between ccIIV and IIV4 in this diverse group of children. Baseline titers were generally high and may have limited seroconversion to either vaccine. For A/H3N2, ratio of fold rise to cell- versus egg-grown viruses were higher for ccIIV4 than IIV4, however post vaccination titers to egg-and cell-grown A/H3N2 remained similar ($P > 0.05$). We also found that prior IIV vaccination in the 2017–2018 season was associated with reduced response in 2018–2019 for ccIIV against A/H1N1 and A/H3N2 cell-grown virus and for IIV4 against B/Victoria, after controlling for baseline titers.

It is not surprising that titers against A/H1N1 did not vary between ccIIV and IIV4 because both were based on the egg-adapted seed strain selected by WHO for the 2018–2019 vaccines. Mutations can arise during passage in either egg or cell culture and the starting strain was egg-adapted for both. The A/H1N1 viruses that circulated in 2018–2019 in the US were well matched to the vaccine, resulting in a VE of 44% (37–51%) across all age groups in the US FluVE Network, and 38% (18–53%) for ages 5 to 17 years [12]. Accordingly, A/H1N1 vaccine is estimated to have prevented one influenza A/H1N1-associated illness episode for every 46 vaccinees among children 5 through 17 years [12].

Over the last decade, in comparison to A/H1N1 and B influenza viruses, A/H3N2 viruses have become increasingly glycosylated and the resulting adaptations required for growth in eggs leads to antigenic differences from wild virus [2]. These antigenic changes have led to difficulties in agglutination of the red blood cells used in the HAI assay; thus, we used MN to evaluate the A/H3N2 response.

Baseline MN titers (1098–1285) to the egg-adapted A/Singapore virus were very high, with 99% seropositivity at baseline, which may reflect prior vaccination effects. Despite the fact that the fold-rise to the A/Singapore egg-adapted virus was significantly higher for IIV4 than ccIIV4, final MN titers for both vaccines to the egg-adapted virus were about 10-fold higher than to the cell-grown virus. Egg-based vaccines may skew the response towards egg-adapted viruses, with the potential for mismatch from circulating viruses [2].

The A/H3N2 responses to cell grown virus in this study were complex. Response to ccIIV4 was significantly higher for cell-grown than egg-based virus; this was not seen with IIV4.

Baseline GMTs (93–100) and seropositivity (46–53%) for both vaccines against the cell-grown virus were moderate. After controlling for these pre-vaccination titers, we found an association between prior 2017–2018 vaccination status and fold change in cell-grown antibody titer after 2018–2019 ccIIV4 but not IIV4 for some strains. Although experts hope that newer vaccines, such as ccIIV, based on cell-grown virus, would resolve the egg-adaption issues, our data suggest that the immunological response is more complex. Although our post-hoc analyses are not definitive, prior vaccination with egg-based vaccines might have affected ccIIV's response to cell-grown viruses, likely due to shared epitopes in these viruses. More research is needed about the response of egg-free vaccines in those who have previously received egg-based vaccines.

2018–2019 was the first year that the B lineages in ccIIV were derived from the cell grown seed strain from WHO. The serologic response was similar between the two vaccines. However, the clinical difference between these vaccines with the new formulation of ccIIV is unknown because little wild influenza B virus circulated in the US in 2018–2019.

In post-hoc analyses, which by their nature are not definitive, residual effects of prior season (2017–2018) vaccination were suggested by higher MFR found in those unvaccinated one year earlier. However, when regressions controlled for baseline titers, these comparisons were only significant for ccIIV against A/H1N1 and A/H3N2 cell grown virus and for IIV4 against B/Victoria. Inspection of our data (Table 3) suggests the possibility that additional significant comparisons might have been found with larger sample sizes, given the magnitude of some differences. Effects of prior vaccination have been suggested in some studies, especially for A/H3N2 [13] but not recently in children [14] Our data suggest that this is an issue for further investigation and that egg-free vaccines may not completely resolve prior vaccination concerns.

5.1. Strengths and limitations

Our study included a racially diverse group of children with modest dropout in a study that required multiple blood draws. MN was used for A/H3N2 assays to avoid the well-known agglutination problems of HI with A/H3N2. One experimental limitation is the high baseline titers which may be due to prior vaccination or disease. Other limitations were the moderate sample size which may result in underpowered subgroup analyses and conduct of the study in one geographic location.

6. Conclusions

In this racially diverse group of children with high baseline titers, no differences in day 28 seroconversion were found between ccIIV4 and IIV4. For ccIIV4 but not for IIV4, the MN response to A/H3N2 was significantly higher for cell-based over egg-based MN assays.

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Abbreviations:

HI	hemagglutination inhibition assay
MN	microneutralization assay
ccIIV4	cell-culture-based quadrivalent IIV
IIV	Inactivated influenza vaccine
IIV4	Egg-based quadrivalent inactivated influenza vaccine
EMR	Electronic medical record
BMI	Body mass index
RDE	Receptor-destroying enzyme
PBS	Phosphate-buffered saline
CDC	Centers for Disease Control and Prevention
FDA	Food and Drug Administration
GMT	Geometric mean titers
VE	Vaccine effectiveness
MFR	Mean fold rise

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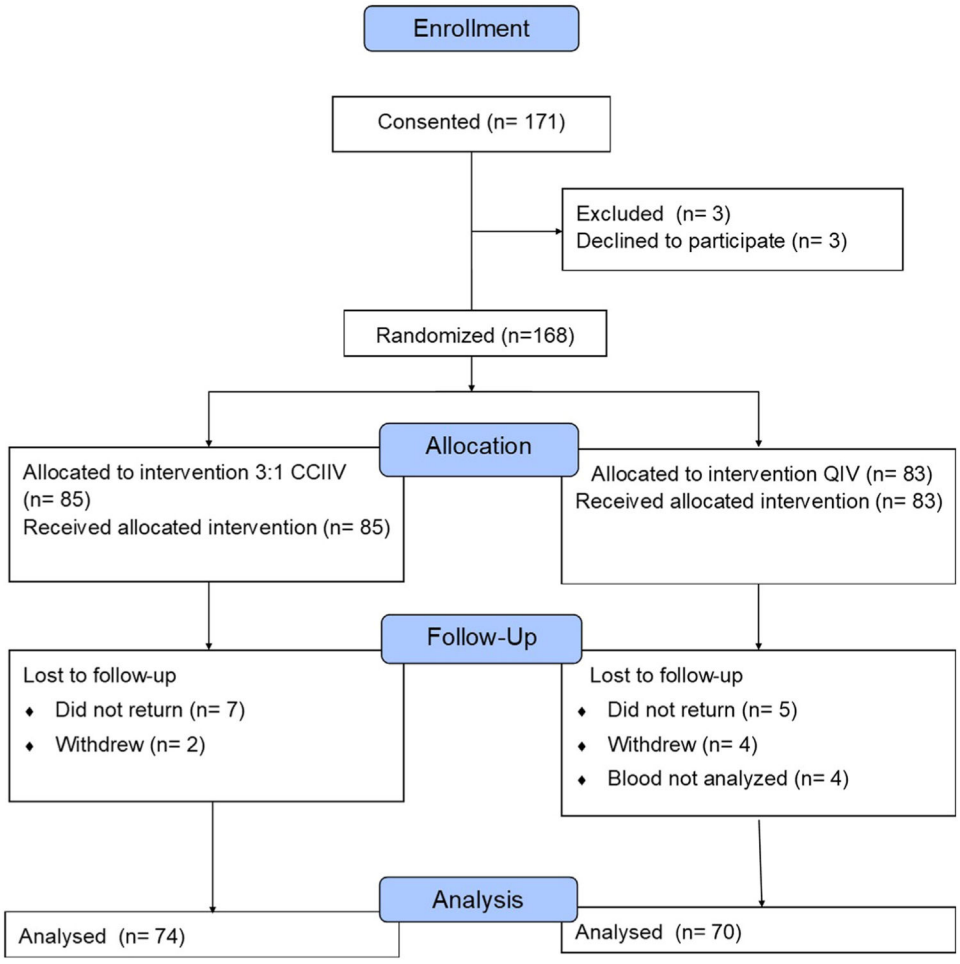

Fig. 1.
Enrollment flow diagram.

Table 1

Demographics by 2018–2019 influenza vaccine type received.

Variable	Overall N = 144	ccIIV4 N = 74	IIV4 N = 70	*P*- value
Age (years), median (Q1, Q3)	14.0 (10.8–16.2)	13.9 (10.7–15.9)	14.0 (11.0–16.6)	0.48
Age 9–20 years, ref. = <9, n (%)	125 (86.8)	66 (89.2)	59 (84.3)	0.38
Female, ref. = male, n (%)	78 (54.2)	39 (52.7)	39 (55.7)	0.72
Non-white race, ref. = white, n (%)	117 (81.3)	60 (81.1)	57 (81.4)	0.95
Non-Hispanic, ref. = Hispanic, n (%)	134 (95.0)	70 (95.9)	64 (94.1)	0.63
Parental education ≤ some college, ref. = ≥college, n (%)	118 (83.1)	59 (80.8)	59 (85.5)	0.46
Public health insurance, ref. = other insurance, n (%)	111 (77.1)	57 (77.0)	54 (77.1)	0.99
BMI ≥ 95th percentile, ref. = <95th percentile, n (%)	44 (30.6)	22 (29.7)	22 (31.4)	0.82
Exposed to household smoking, ref. = no smoke exposure, n (%)	45 (31.3)	23 (31.1)	22 (31.4)	0.96
2017 influenza vaccine status				
IIV receipt	86 (59.7)	46 (62.2)	40 (57.1)	0.54
Not vaccinated/no record	58 (40.3)	28 (37.8)	30 (42.9)	
2016 influenza vaccine status				
IIV receipt	113 (78.5)	58 (78.4)	55 (78.6)	0.98
Not vaccinated/no record	31 (21.5)	16 (21.6)	15 (21.4)	

*By Chi-square/Fisher's Exact for categorical variables, *t*-test for continuous variables.

Nonwhite race = AIAN, Asian, Black, NHPI, multi-race.

Other insurance = Private, non-insured, combined private and public insurance.

ccIIV4 = 3 cell-based to 1 egg-based vaccine component inactivated influenza vaccine.

IIV4 = egg-based quadrivalent inactivated influenza vaccine.

Table 2

Pre- and post vaccination hemagglutination inhibition (HI) and microneutralization (MN) assay titer responses by vaccine type.

Antibody response measure	ccIIV4 recipients N = 74	IIV4 recipients N = 70	*P* value[†]
A/H1N1 - A/Michigan			
Seroconversion, %	27	20	0.32
Day 0 HI titer ≥ 1:40, %	85	89	0.54
Day 28 HI titer ≥ 1:40, %	97	99	0.59
Day 0 HI titer ≥ 1:110, %	58	56	0.77
Day 28 HI titer ≥ 1:110,%	86	80	0.30
Day 0 HI GMT (95% CI)	117 (89–154)	111 (86–145)	0.80
Day 28 HI GMT (95% CI)	292 (238–359)	241 (191–305)	0.22
Mean fold-rise in titer (95% CI)	2.5 (1.9–3.3)	2.2 (1.8–2.6)	0.42
A/H3N2-A/Singapore Egg Grown Virus			
Seroconversion, n (%)	15	30	0.08
Day 0 MN titer ≥ 1:40, %	*100*	*100*	Undefined
Day 28 MN titer ≥ 1:40, %	100	100	Undefined
Day 0 MN titer ≥ 1:110,%	99	99	0.97
Day 28 MN titer ≥ 1:110,%	100	100	Undefined
Day 0 GMT (95% CI)	1285 (9881671)	1098 (8541411)	0.39
Day 28 GMT (95% CI)	2064 (1664–2560)	2497 (2023–3083)	0.21
Mean fold-rise in titer (95% CI)	1.6 (1.3–2.0)	2.3 (1.8–2.9)	**0.05**
A/H3N2 - A/Singapore Cell Grown Virus			
Seroconversion, n (%)	35	24	0.21
Day 0 MN titer ≥ 1:40, %	81	81	0.96
Day 28 MN titer ≥ 1:40, %	95	94	0.94
Day 0 MN titer ≥ 1:110,%	53	46	0.40
Day 28 MN titer ≥ 1:110,%	77	66	0.13
Day 0 GMT (95% CI)	100(75–134)	93 (67–129)	0.73
Day 28 GMT (95% CI)	256 (188–349)	174 (130–231)	0.07
Mean fold-rise in titer (95% CI)	2.6 (1.9–3.5)	1.9 (1.5–2.4)	0.11
B/Victoria - B/Colorado			
Seroconversion, %	34	34	0.95
Day 0 HI titer ≥ 1:40, %	88	86	0.71
Day 28 HI titer ≥ 1:40, %	93	96	0.52
Day 0 HI titer ≥ 1:110, %	49	49	0.99
Day 28 HI titer ≥ 1:110, %	76	79	0.68
Day 0 HI GMT (95% CI)	109 (83–142)	98 (74–131)	0.61
Day 28 HI GMT (95% CI)	232 (176–306)	233 (186–292)	0.97
Mean fold-rise in titer (95% CI)	2.1 (1.6–2.8)	2.4 (1.9–2.9)	0.55
B/Yamagata - B/Phuket			
Seroconversion, %	14	24	0.10

Antibody response measure	ccIIV4 recipients N = 74	IIV4 recipients N = 70	*P* value[†]
Day 0 HI titer ≥ 1:40, %	86	87	0.91
Day 28 HI titer ≥ 1:40, %	95	97	0.45
Day 0 HI titer ≥ 1:110, %	64	56	0.34
Day 28 HI titer ≥ 1:110, %	82	74	0.24
Day 0 HI GMT (95% CI)	130(100–170)	102 (79–133)	0.19
Day 28 HI GMT (95% CI)	193 (156–238)	200 (161–248)	0.80
Mean fold-rise in titer, (95% CI)	1.5 (1.1–1.9)	2.0 (1.6–2.4)	0.10

Note:GMT = geometric mean titer; CI = confidence interval; Seroconversion = HI titer ratio of day 28/day 0 ≥ 4 and HI titer at day 28 ≥ 40.

[*] Primary outcome was seroconversion at day 28.

Paired *t*-test compares log MFR MN titers against A/H3N2(cell) vs A/H3N2 (egg):

[*] ccIIV, P < 0.001;

[**] IIV, *P* = 0.12.

[†] Paired T-test was used to compare log titers; Chi-square test was used to compare rates.

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Table 3

Pre-and post-vaccination antibody titers and mean fold-rise by vaccination status in 2017–18 season.

Antibody response measure	2018–2019 ccIIV recipients		2018–2019 IIV4 recipients	
	Unvaccinated in 2017–2018 N = 29	IIV recipients in 2017–2018 N = 45	Unvaccinated in 2017–2018 N = 30	IIV4 recipients in 2017–2018 N = 40
A/H1N1 - A/Michigan				
Day 0 HI GMT (95% CI)	94 (55–162)	134 (99–182)	95 (64–141)	126 (87–181)
Day 28 HI GMT (95% CI)	385 (274–542)	246 (191–318)	263 (178–388)	226 (167–307)
Mean fold-rise in titer (95% CI)	**4.1 (2.1–8.0)**	**1.8 (1.5–2.3)**	2.8 (2.0–3.9)	1.8 (1.5–2.2)
A/H3N2-A/Singapore Egg Grown Virus				
Day 0 GMT (95% CI)	1,127 (700–1,815)	1,399 (1,018–1,921)	1,040 (641–1686)	1,144 (874–1,496)
Day 28 GMT (95% CI)	2354 (1,680–3299)	1,896 (1,422–2,527)	2975 (2,074–4267)	2,190 (1,694–2833)
Mean fold-rise in titer (95% CI)	2.1 (1.2–3.8)	1.4 (1.2–1.5)	2.9 (1.8–4.6)	1.9 (1.5–2.5)
A/H3N2 - A/Singapore Cell Grown Virus				
Day 0 GMT (95% CI)	117 (76–181)	90 (60–135)	96 (56–164)	90 (59–139)
Day 28 GMT (95% CI)	414 (251–685)	188 (128–275)	209 (130–338)	151 (105–217)
Mean fold-rise in titer (95% CI)	**3.5 (1.9–6.7)**	**2.1 (1.6–2.8)**	2.2 (1.4–3.5)	1.7 (1.3–2.1)
B/Victoria - B/Colorado				
Day 0 HI GMT (95% CI)	115 (70–189)	106(77–145)	71 (43–119)	126 (91–173)
Day 28 HI GMT (95% CI)	283 (168–476)	205 (148–283)	245 (169–355)	224 (167–301)
Mean fold-rise in titer (95% CI)	2.5 (1.3–4.6)	1.9 (1.5–2.5)	**3.4 (2.4–4.9)**	**1.8 (1.4–2.3)**
B/Yamagata - B/Phuket				
Day 0 HI GMT (95% CI)	109 (64–185)	146 (109–195)	64 (43–96)	145 (107–198)
Day 28 HI GMT (95% CI)	200 (141–284)	188 (142–249)	186 (132–261)	211 (158–282)
Mean fold-rise in titer (95% CI)	1.8 (1.1–2.9)	1.3 (0.9–1.8)	2.9 (2.0–4.1)	1.5 (1.2–1.8)

*Bold = $P < 0.05$ for association between 2017 and 2018 vaccination status and fold change in antibody titer from linear regression controlling for pre-vaccination titer.