HHS Public Access

Author manuscript

Vaccine. Author manuscript; available in PMC 2019 January 29.

Published in final edited form as:

Vaccine. 2018 January 29; 36(5): 758–764. doi:10.1016/j.vaccine.2017.12.024.

An updated influenza A(H3N2) vaccine generates limited antibody responses to previously encountered antigens in children

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Abstract

Background—Influenza vaccination may provide a “back-boost” to antibodies against previously encountered strains. If the back-boost effect is common, this could allow more aggressive vaccine updates, as emerging variants would be expected to both elicit de-novo responses and boost pre-existing responses against recently circulating strains. Here we used the emergence of an antigenically novel A(H3N2) strain to determine whether an antigenically updated vaccine boosted antibodies against historical strains.

Methods—We performed hemagglutination-inhibition (HI) assays on pre- and post-vaccination sera from 124 children 5–17 years old who received 2015–2016 inactivated influenza vaccine, containing an antigenically updated A(H3N2) strain. We evaluated the mean fold increase in HI titer against both the 2015–2016 vaccine strain and representative strains from two prior antigenic clusters. Factors associated with post-vaccination titers against historical strains were evaluated using linear regression, adjusting for baseline titer.

Results—Geometric mean titers against each antigen examined increased significantly after vaccination (P <0.0001). Mean fold increase was 3.29 against the vaccine strain and 1.22–1.46 against historical strains. Response to vaccine strain was associated with increased post-vaccination titers against historical strains.

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Conclusions—A vaccine containing an antigenically novel A(H3N2) strain modestly boosted antibody responses against historical influenza strains in children.

Keywords

influenza; vaccine; pediatrics; back-boost

Introduction

Influenza viruses undergo continuous antigenic evolution that is punctuated by the periodic emergence of variants with substantial antigenic differences from previously circulating variants. As a result, influenza virus strains can be grouped into clusters of antigenically similar viruses, where new clusters are created during the expansion of a drift variant [1]. These antigenic drift variants can emerge unpredictably and sometimes suddenly, which complicates vaccine strain selection. Decisions regarding the recommended influenza vaccine composition start 6–7 months before the influenza season [2,3]. During this time an antigenically novel strain can emerge, creating a mismatch between vaccine and circulating strains and reducing vaccine effectiveness. Before the 2014–15 influenza season an antigenic cluster transition created a larger-than-usual mismatch between the circulating A(H3N2) strain and the vaccine strain, A/Texas/50/2012. This virus was antigenically similar to a majority of circulating A(H3N2) viruses in early 2014 [4]. However, increasing prevalence of antigenically drifted A(H3N2) viruses during the 2014–15 season resulted in poor A(H3N2)-specific vaccine effectiveness [5,6].

An individual's repertoire of antibody responses is shaped by each influenza exposure event. It has been shown that pre-existing immunity as well as vaccine effectiveness can affect the response to vaccination [7,8]. However, there have been few studies examining how an influenza exposure can affect the pre-existing antibody repertoire. Recently, Fonville et al. demonstrated how exposure events alter the overall hemagglutination-inhibition (HI) antibody response by simultaneously visualizing antigenic distances among influenza strains and the magnitude of HI antibody responses to create an "antibody landscape" [9]. Intriguingly, they noted that both infection and vaccination boosted antibody responses against not only the vaccine or infecting strain, but also against historical influenza strains, resulting in a "back-boost" effect [9]. This study also noted that vaccination with an updated influenza vaccine in 1997 was capable of boosting responses against the previously circulating antigenic variant A/Wuhan/359/1995 to levels higher than those elicited by the homologous A/Nanchang/933/1995 vaccine [9].

The "back-boost" effect raises the possibility of employing a new vaccine strain selection process in which vaccines can be updated with emerging, antigenically novel strains, even when they do not yet predominate among circulating viruses. The antigenically advanced strain would be relied upon to elicit antibodies against the emerging antigenic variant while also boosting antibodies against historical strains, thus providing coverage against both "new" and "old" strains. The back-boost effect was initially identified in adults and relies on pre-existing influenza immunity; here we determined whether a similar back-boost could occur in children who have comparatively limited influenza exposure histories. The study

recruited children with and without a known prior infection with influenza A(H3N2) viruses, which allowed us to examine factors associated with a back-boost response.

Methods

Study population and serum collection

This study was carried out in fall 2015 before the North American 2015–2016 influenza season. Parents of children 5–17 years old were contacted by mail and telephone inviting their child to participate in a vaccine immune response study before they received their 2015–2016 influenza vaccine. Participants were selected from children who 1) previously participated in similar influenza vaccine immune response studies in the previous two influenza seasons, or 2) lived in Marshfield, Wisconsin where annual studies of influenza vaccine effectiveness were conducted [10]. We restricted recruitment to this population because influenza vaccinations are captured using a validated vaccination registry [11] and medically attended influenza illnesses were prospectively identified and confirmed by RT-PCR. All participants received the 2015–2016 trivalent inactivated influenza vaccine (IIV3) delivered intramuscularly, containing an A/Switzerland/9715293/2013-like A(H3N2) virus, and provided a serum sample before vaccination and 28 days after vaccination. Data on demographics and high-risk conditions were electronically extracted from the medical record.

Study procedures were approved by the Marshfield Clinic Institutional Review Board. Informed consent was obtained from the parents/guardians of all participants and assent was obtained from children aged ≥7 years.

Hemagglutination inhibition (HI) assay

HI assays were performed at the University of Wisconsin-Madison on pre-baseline and post-vaccination serum samples as described by the World Health Organization [12]. Briefly, 1 part serum was mixed with 3 parts receptor-destroying enzyme II (RDEII; Accurate Chemical, Westbury, NY) and incubated at 37°C for 18–20 hours to remove non-specific inhibitors of hemagglutination. RDEII was then inactivated by incubating the samples at 56°C for 30 minutes. To remove non-specific antibodies 1 part packed guinea pig red blood cells (Innovative Research, Novi, MI) was mixed with 10 parts serum and incubated at room temperature for 1 hour, inverting every 15 minutes. The samples were then diluted to a total serum dilution factor of 1:10 with PBS and oseltamivir (final concentration 20nM; ApexBio, Houston, TX) and serial two-fold dilutions were mixed with 4 HA units of A(H3N2) viruses in a U-bottomed microtiter plate. The plates were incubated at room temperature for 30 minutes, then 0.75% guinea pig red blood cells were added with a final oseltamivir concentration of 20nM. The samples were then incubated again for 30 minutes at room temperature. The reciprocal of the dilution at which no inhibition was observed was recorded as the HI antibody titer. If inhibition was not observed a serum dilution of 1:5 was recorded as a negative result to allow for statistical analysis. Samples were tested in duplicate with pre- and post-vaccination samples from the same participants tested at the same time. Wells with 4 HA units of virus and PBS were kept as positive and negative controls for hemagglutination.

Antibody titers were determined against the following viruses: the 2015–2016 vaccine strain A/Switzerland/9715293/2013 (SW/13) (ATCC, Manassas, VA) belonging to the genetic clade 3C.3a; the 2014–2015 vaccine strain A/Texas/50/2012 (TX/12) (ATCC, Manassas, VA) belonging to clade 3C.1; the 2012–2013 vaccine strain A/Victoria/361/2011 (VI/11) (ATCC, Manassas, VA) belonging to clade 3C.1; and A/Wuhan/359/1995 (WU/95) (kindly provided by Dr. Yoshihiro Kawaoka) which represented an A(H3N2) antigenic cluster that circulated from 1995–1997, before the participants were born [13]. Viruses were grown in cell culture using MDCK-SIAT1 cells.

Statistical analyses

The primary outcome was geometric mean fold increase (MFI) in HI titers against TX/12; MFI was also assessed for VI/11, a virus from the same antigenic cluster as TX/12, and for WU/95, which is antigenically distinct from TX/12. An analysis of MFI was stratified based on baseline TX/12 HI titer <40 or ≥40 to account for potential differential antibody response due to baseline TX/12 HI titer. Seroprotection against the previous season vaccine strain, TX/12, was determined for two levels of protection, defined as the proportion with post vaccination HI titer ≥40, the titer level considered seroprotective for regulatory purposes; and ≥110, which has been suggested to correspond to 50% clinical protection in children [14,15].

Linear regression models with $\log_2$-transformed HI titers were used to identify factors associated with post-vaccination HI titer against viruses representing past A(H3N2) antigenic clusters, adjusting for baseline HI titers. Factors examined included SW/13 (vaccine strain) MFI, age group (5–11 and 12–17 years), presence of a high-risk condition, vaccination group (any vs. no vaccination within the last 5 years), and A(H3N2) infection history within the last 5 years. High-risk conditions were defined by presence of a healthcare visit or hospitalization during the previous 12 months with ≥1 ICD-9 diagnosis codes for conditions associated with increased risk of influenza, as previously described [16,17]. Covariates associated with post-vaccination titer at $P<0.05$ in backwards elimination were included in the final linear regression model. Separate regression models were used to assess titers against each historical strain: TX/12, VI/11, and WU/95. Estimates of post-vaccination geometric mean titers (GMT) for each historical antigen were calculated and plotted adjusting for the baseline GMT for each antigen using “predicted marginal means” or “least-squares means,” available in the R package lsmeans [18].

Logistic regression was used to identify predictors of post-vaccination seroprotection against TX/12. For this analysis, participants with baseline titers above the seroprotective level (≥40 or ≥110) were excluded. The same covariates assessed in the linear regression model were examined in the logistic regression models.

All analyses were performed using R version 3.3.0 [19]. Figures were generated using GraphPad Prism 6 and R package ggplot2 [20].

Results

Participant characteristics

There were 124 participants with paired sera from both baseline and post vaccination visits. The mean age was 12 years, and 54 (44%) were female. In the 2014–2015 (prior) season, 77 (62%) received inactivated influenza vaccine (IIV), 13 (11%) received live attenuated influenza vaccine (LAIV), and 34 (27%) were unvaccinated; 22 (18%) had PCR-confirmed influenza A (H3N2) virus infection. At baseline, 95 (77%) had HI titer ≥40 against A/Texas/50/2012 (TX/12), the strain in the prior season vaccine. There were no significant differences between participants who had baseline titers of either <40 or ≥40 against TX/12 with regard to age group, sex, presence of high-risk medical conditions, prior (2014–2015) season vaccination history, or history of PCR-confirmed A(H3N2) influenza infection in 2012–2013 (group 3C.1) or 2014–2015 (group 3C.2a) (Table 1).

Baseline and vaccine response to the vaccine strain SW/13

Baseline responses against the vaccine strain SW/13 could indicate prior exposure to that virus, which might affect back-boost responses. We found that participants with baseline HI titers ≥40 against TX/12 had higher baseline titers against the group 3C.3a A/Switzerland/9715293/2013 (SW/13; 2015–2016 vaccine) strain than participants with baseline HI titers <40 against TX/12 (GMT 68 vs 18, P <0.001; Table 1). Participants with a PCR-confirmed A(H3N2) infection during the 2012–2013 season had a baseline GMT of 75 against SW/13 (95% CI 36 – 153; Table 1). Participants with a PCR-confirmed A(H3N2) infection during the 2014–2015 season, during which SW/13-like viruses first circulated, had a baseline GMT of 60 (95% CI 44 – 83; Table 1). After vaccination, the unadjusted MFI against SW/13 was 3.30 (95% CI 2.36 – 4.63) and 3.28 (95% CI 2.73 – 3.95) for those with baseline TX/12 titer <40 and ≥40, respectively (Figure 1 and Table 2). Thus participants responded to vaccination with SW/13, although for most participants these responses were modest.

Baseline and vaccine response to viruses representing past A(H3N2) antigenic clusters

The central question of our study was whether vaccination with an updated A(H3N2) antigen provides a back-boost to recently circulating strains in children. The back-boost response generated by the SW/13 vaccine strain was evaluated against both the closely related TX/12 and VI/11 strains of recent vaccines and the distant WU/95.

Prior season vaccine strains TX/12 and VI/11—At baseline, all participant groups had mean baseline titers <40 against the previous vaccine strain A/Victoria/361/2011 (VI/11), even those with baseline titers >40 against TX/12 (Table 1). Participants with a PCR-confirmed A(H3N2) infection during the 2012–2013 or 2014–15 seasons had baseline GMTs of >40 against TX/12, but <40 against VI/11 (Table 1). If vaccination provided a back-boost against recent, “antigenically adjacent” viruses in our participants, we would expect to observe a significant increase in mean fold titers against VI/11 and TX/12. The unadjusted MFI in participants with baseline TX/12 titer <40 was 1.84 (95% CI 1.50 – 2.26) against TX/12 and 1.61 (95% CI 1.33 – 1.95) against VI/11 (Figure 1A and Table 2). Similarly, MFI in participants with baseline TX/12 HI titers ≥40 was 1.36 (95% CI 1.25 – 1.48) against TX/12 and 1.37 (95% CI 1.25 – 1.51) against VI/11 (Figure 1B and Table 2).

Thus we observed modest, but statistically significant, increases against both recent vaccine strains VI/11 and TX/12 after vaccination with the new antigenic variant SW/13.

Antigenically distant A/Wuhan/359/1995 (WU/95)—WU/95 circulated before the individuals in this cohort were born; we expect that participants would not have been exposed to this virus and would therefore not mount a back-boost response to it. Consistent with this expectation, all participants had low baseline titers against WU/95, though the baseline titer against this virus trended slightly higher in participants with baseline TX/12 ≥40 (GMT 9 vs. 18, respectively; Table 1). Participants with a PCR-confirmed A(H3N2) infection during the 2012–2013 season had a baseline GMT of 10 (95% CI 6 – 17; Table 1). Likewise, participants with a PCR-confirmed A(H3N2) infection during the 2014–2015 season had a baseline GMT of 15 (95% CI 11 – 20; Table 1). In participants with baseline TX/12 HI titer <40, there was no significant difference in pre- and post-vaccination titers against WU/95 ($P = 0.44$; Figure 1A and Table 2), while participants with baseline TX/12 titer ≥40 had a MFI of 1.24 (95% CI 1.13 – 1.35) against WU/95 (Figure 1B and Table 2).

Factors associated with responses to SW/13 and viruses representing past A(H3N2) antigenic clusters

To determine which factors influence the back-boost response, we examined multiple covariates in a regression model to measure their association with the back-boost responses to the recent antigenic clusters. We found baseline titer and SW/13 response to be associated with the back-boost in antibody titers against TX/12 and VI/11. Adjusting for baseline TX/12 titer, a 2-fold increase in SW/13 response from pre- to post-vaccination was associated with an average increase of 19% (95% CI 13% – 25%) in post-vaccination titer against TX/12 (Figure 2 and Table 3). For VI/11, a 2-fold increase in SW/13 response from pre- to post-vaccination was associated with an average increase in post-vaccination titer against VI/11 of 14% (95% CI 7% – 21%) adjusting for baseline VI/11 titer (Figure 2 and Table 3). For WU/95, a 2-fold increase in SW/13 response from pre- to post-vaccination was associated with an average increase in post-vaccination titer against WU/95 of 11% (95% CI 5% – 17%) adjusting for baseline WU/95 titer (Figure 2 and Table 3). The regression models explained 73%, 74%, and 83% of the variability in post-vaccination titers against TX/12, V11, and WU/95, respectively. Age (Figure S1), high-risk condition, vaccination group, and infection status were examined but did not significantly impact post-vaccination titers against any viruses.

Factors associated with seroprotection against the previous season vaccine strain TX/12

Finally, because the goal of vaccination is to induce a protective level of antibodies, we determined whether back-boost effects could induce seroprotective antibody titers against the previous vaccine strain TX/12, using both definitions of seroprotection described above (≥40 or ≥110). Following vaccination with SW/13, 52% (15 of 29) of participants with baseline TX/12 HI titer <40 were considered seroprotected with TX/12 HI titers ≥40 and 11% (12 of 114) of participants with baseline TX/12 HI titer <110 were considered seroprotected with TX/12 HI titers ≥110. Baseline titer against TX/12 and response against SW/13 were independent predictors of seroprotection against TX/12 using either definition of seroprotection (≥40 or ≥110). Even small increases in baseline titer against TX/12

greatly increased participants' odds of being seroprotected after vaccination: a 2-fold higher baseline titer increased the odds of seroprotection by 40.67 (95% CI 3.38 – 302.54) for TX/12 HI titer ≥40 or 3.52 (95% CI 1.56 – 10.29) for TX/12 HI titer ≥110 (Table 4). Response to the vaccine strain also increased the odds of seroprotection against TX/12: a 2-fold increase in SW/13 response was associated with 5.9 (95% CI 2.0 – 44.1) times higher odds of seroprotection with TX/12 HI titer ≥40 and 2.1 (95% CI 1.4 – 3.4) times higher odds of seroprotection with TX/12 HI titer ≥110, adjusting for baseline TX/12 titer (Table 4).

Discussion

Antigenic variants of influenza A(H3N2) viruses emerged in 2014 and were the predominant A(H3N2) viruses during the 2014–2015 season in the United States. As a result, A/Switzerland/9715293/2013 (SW/13; HA genetic group 3C.3a) was chosen as the A(H3N2) component of the 2015–16 Northern Hemisphere influenza vaccine due to its availability and cross-reactivity with the 3C.2a viruses that circulated during 2014–2015. Vaccination with SW/13 boosted HI antibodies against both the TX/12 and VI/11 reference viruses from the previous A(H3N2) antigenic cluster. For some subjects, we also noted a very small, but statistically significant, increase in antibody titers against WU/95, a virus from a substantially earlier antigenic cluster. The biological significance of this small increase is unclear; WU/95 circulated before almost all participants were born and we therefore do not expect a strong back-boost against this virus. Notably, pre- and post-vaccination titers against WU/95 remained below the threshold of seroprotection in all participants even after vaccination. However, the magnitude of HI antibody titer changes against the earlier A(H3N2) viruses after vaccination (i.e., the back-boost) was small in comparison to the response against the vaccine antigen SW/13, even after adjusting for baseline HI titer. When the mean fold increase against viruses representing past A(H3N2) antigenic clusters is stratified by baseline TX/12 titer it is apparent that the strength of the back-boost is independent of baseline TX/12 titer. Only the response to the vaccine strain SW/13 was significant in determining the magnitude of back-boost against historical antigenic clusters. Overall, these data suggest that children vaccinated with a strain from the novel A(H3N2) antigenic cluster received only a small boost to antibodies against previously circulating A(H3N2) influenza strains.

The value of a back-boost becomes clear if vaccine compositions were to be antigenically updated, while the majority of circulating viruses belong to the "old" antigenic cluster. Currently, strain changes are only made when evidence suggests that a novel antigenic strain is likely to become the dominant strain in circulation, and a suitable candidate vaccine virus is available. However, independent lines of emerging evidence may argue in favor of more frequent updates. First, repeated vaccination with antigenically similar viruses may contribute to reduced vaccine effectiveness [21,22]. The back-boost effect could allow for more frequent updates—newly emerging strains could be selected for vaccine formulations even before they reach a majority of circulating viruses. In this case there would be a need to rely on the vaccine to provide protection against the previous antigenic cluster via the back-boost. We therefore asked whether vaccination with SW/13 provided a back-boost that converted participants' HI titers against viruses from recently circulating antigenic clusters to seroprotective levels, defined both by the standard 40 measure and the more stringent

level of 110, which may be more relevant for children. Approximately half the participants (15 of 29; 52%) with a baseline HI titer <40 against TX/12, representing the previous antigenic cluster, had titers ≥40 against that virus after vaccination. When using the more conservative 110 cutoff this proportion dropped to only 11% 12 of 114). Altogether, our data suggest that influenza vaccination provides a modest back-boost in children that may not be sufficient to provide protection against previous antigenic clusters.

Our study was limited both in the number of participants and the number of virus strains examined. Using additional virus strains would allow for a more complete understanding of the dynamics of the response against past antigenic clusters. Our study was designed to determine whether vaccination with a newly updated vaccine strain was capable of boosting responses against recently circulating, “antigenically adjacent” influenza strains. We focused on A(H3N2) due to the recent emergence of a novel antigenic cluster, but back-boost effects could be different for H1N1 viruses. There were also limitations in the determination of influenza infection status. It is possible some participants were sub-clinically exposed or had undocumented infection, leading to differences in exposure status and HI antibody titer. Undocumented exposures and infection would also explain the lack of effect that infection status had in our model. The ability to mount a back-boost response to a given influenza virus may be impacted by multiple factors, including the nature of one’s first exposure to influenza. It is possible, for example, that the antibody repertoire stimulated by a first exposure to multivalent vaccine is fundamentally different from one stimulated by a first exposure via infection with a single live virus. We could not determine unambiguously whether all individuals in our cohort were first exposed to influenza by vaccination or infection, so future detailed studies will be necessary to explore this question. It is also worth noting that the HI assay is an indirect measure of neutralization; neutralization assays may more sensitively detect back-boosted responses and may also be more relevant to protection. However, few studies have sought to correlate antibody responses measured by neutralization assay with protection, due in part to the relatively higher cost and complexity of neutralization assays [23]. Interestingly, a recent study using neutralization assays detected a modest “forward-boost” in children: 38–45% of participants receiving IIV containing TX/12 seroconverted to 3C.2a or 3C.3a viruses, respectively [24].

In this study we observed a small but statistically significant boost to antibodies against viruses from earlier antigenic clusters in children. One potential factor that could affect the back-boost response is immune imprinting. If the participants initial exposure was to an H3, the participant may see a larger back-boost than if their initial exposure was to an H1 [25,26]. Future studies with larger panels of viruses are needed to address this important possibility. Additionally, the immunological mechanism of the back-boost should be investigated further given the recommendations for annual vaccination. The back-boost likely reflects the activation and expansion of strain-specific memory B cells and/or the expansion of B cell clones that cross-react to both strains. The effects of repeated vaccination on back-boost response are not known and merit further research. Overall, understanding the interactions between pre-existing immune responses and vaccination will improve influenza vaccine design and efficacy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Jennifer Meece, Deanna Cole, Diane Kohnhorst, Tamara Kronenwetter Koepel, Jackie Salzwedel, Suellyn Murray, Rebecca Pilsner, Nan Pan, Wayne Frome, and Erin Donnerbauer from the Marshfield Clinic Research Institute for their contribution to the study data collection. Thanks to Dr. Yoshihiro Kawaoka for providing A/Wuhan/359/1995.

Funding

This work was supported by Centers for Disease Control and Prevention NIH/CDC U01 IP 000471. NIH National Research Service Award T32 GM07215 to NWF. This research was conducted in part at a facility constructed with support from NIH Research Facilities Improvement Program grants RR015459 and RR020141.

HQM, JPK, and EAB have received research support from MedImmune.

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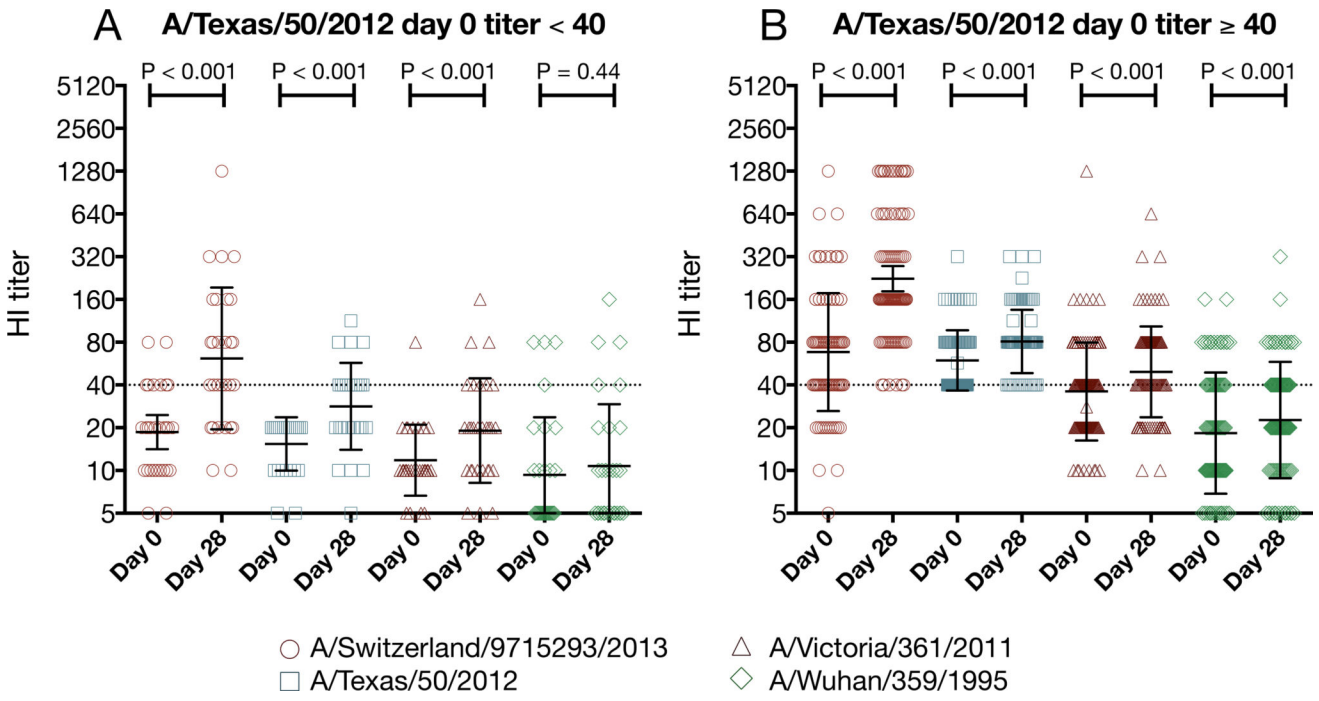

Figure 1.

Pre- and post-vaccination HI antibody titers in participants with high ≥ 40 vs. low <40 baseline A/Texas/50/2012 titers. HI antibody responses were measured against 4 antigens, pre- and post-vaccination. Subjects were stratified into 2 groups based on their baseline response to A/Texas/50/2012. **A**) Subjects with an A/Texas/50/2012 baseline HI titer <40. **B**) Subjects with an A/Texas/50/2012 baseline HI titer ≥40. Bars and error bars represent mean and 95% confidence interval respectively. The horizontal dashed line indicates a seroprotective titer of 40. Statistical significance determined using ANOVA with Bonferroni's correction for multiple comparisons.

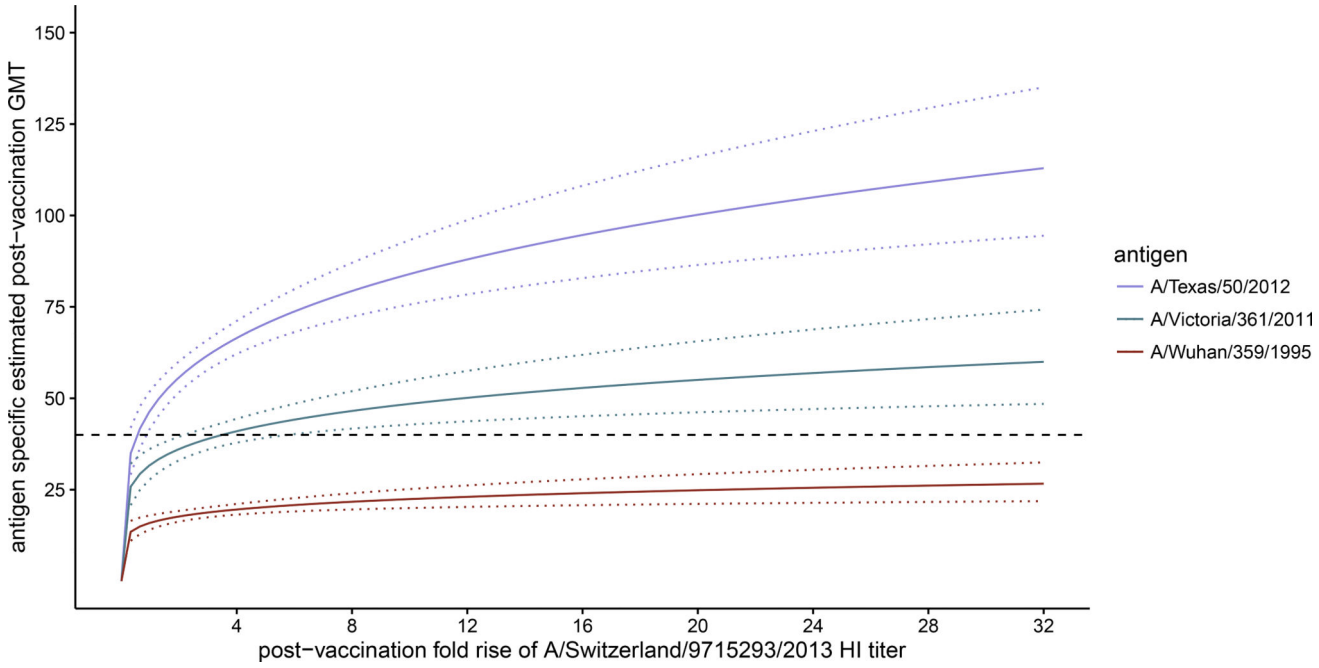

Figure 2.

Estimated post vaccination GMT by increase in A/Switzerland/9715293/2013 titer following vaccination with A/Switzerland/9715293/2013. Post-vaccination HI titer against A/Texas/50/2012, A/Victoria/361/2011, and A/Wuhan/359/1995 were determined using multiple linear regression with the baseline GMT for each antigen (43, 28, and 16), respectively. Solid line indicates post-vaccination GMT while dotted line indicates the 95% confidence interval. The horizontal dashed line indicates a seroprotective titer of 40.

Table 1

Characteristics of study population by baseline HI titer against A/Texas/50/2012 (2014–2015 vaccine strain)

	baseline HI titer against A/Texas/50/2012			
	low HI titer < 40 (n = 29)	high HI titer ≥ 40 (n = 95)	*P* value[a]	all (n = 124)
Age				
Mean ± Std. Dev.	11.93 ± 3.35	11.98 ± 2.81	0.9	11.97 ± 2.93
	No. (%)	No. (%)		No. (%)
5–11 years	11 (38)	44 (46)	0.6	55 (44)
12–17 years	18 (62)	51 (54)		69 (56)
Sex	No. (%)	No. (%)	0.9	No. (%)
Male	17 (59)	53 (56)		70 (56)
Female	12 (41)	42 (44)		54 (44)
High-risk condition [b]	10 (34)	21 (22)	0.3	31 (24)
2014–2015 vaccination status	No. (%)	No. (%)	0.6	No. (%)
unvaccinated	10 (34)	24 (25)		34 (27)
vaccinated – IIV	17 (59)	60 (63)		77 (62)
vaccinated – LAIV	2 (7)	11 (12)		13 (11)
PCR confirmed A(H3N2) infection	No. (%)	No. (%)		No. (%)
2012–2013	1 (3)	9 (9)	0.5	10 (8)
2014–2015	2 (7)	20 (21)	0.1	22 (18)
Baseline A/Switzerland/9715293/2013 titer, GMT (95% CI)	18 (14 – 24)	68 (56 – 83)	<0.001	50 (42 – 61)
Baseline A/Victoria/361/2011 titer, GMT (95% CI)	12 (10 – 15)	36 (31 – 42)	<0.001	28 (24 – 32)
Baseline A/Wuhan/359/1995 titer, GMT (95% CI)	9 (6 – 13)	18 (15 – 22)	0.004	16 (13 – 19)
Baseline titer in subjects with PCR confirmed A(H3N2) infection in 2012–2013				(n = 10)
A/Switzerland/9715293/2013 GMT (95%CI)				75 (36 – 153)
A/Texas/50/2012 GMT (95% CI)				49 (35 – 70)
A/Victoria/361/2011 GMT (95% CI)				30 (20 – 46)
A/Wuhan/359/1995 GMT (95% CI)				10 (6 – 17)
Baseline titer in subjects with PCR confirmed A(H3N2) infection in 2014–2015				(n = 22)
A/Switzerland/9715293/2013 GMT (95%CI)				60 (44 – 83)
A/Texas/50/2012 GMT (95% CI)				55 (42 – 71)
A/Victoria/361/2011 GMT (95% CI)				33 (25 – 43)
A/Wuhan/359/1995 GMT (95% CI)				15 (11 – 20)

Abbreviations: IIV = Inactivated Influenza Vaccine, LAIV = Live Attenuated Influenza Vaccine, GMT – Geometric Mean Titer

[a] Statistical significance calculated with Chi-Square, T-test (log transformed for GMT), or Fishers exact test, *P* values were adjusted using Bonferroni's method of adjusting for multiple comparisons.

[b] Presence of ≥1 medical record–documented high-risk code as defined by the Advisory Committee on Immunization Practices guidance for conditions that increase risk for complications from influenza [16,17].

Table 2

Unadjusted mean fold increase stratified by baseline HI titer against A/Texas/50/2012

	Baseline A/Texas/50/2012 (n = 29)			Baseline A/Texas/50/2012 (n = 95)			All (n = 124)		
	HI titer < 40			HI titer ≥ 40					
Antigens	**MFI**	**95% CI**	***P* value[a]**	**MFI**	**95% CI**	***P* value[a]**	**MFI**	**95% CI**	***P* value[a]**
A/Switzerland/9715293/2013	3.30	2.36 – 4.63	< 0.0001	3.28	2.73 – 3.95	< 0.0001	3.29	2.80 – 3.87	< 0.0001
A/Texas/50/2012	1.84	1.50 – 2.26	< 0.0001	1.36	1.25 – 1.48	< 0.0001	1.46	1.34 – 1.58	< 0.0001
A/Victoria/361/2011	1.61	1.33 – 1.95	0.0002	1.37	1.25 – 1.51	< 0.0001	1.43	1.31 – 1.56	< 0.0001
A/Wuhan/359/1995	1.15	0.97 – 1.37	0.44	1.24	1.13 – 1.35	< 0.0001	1.22	1.13 – 1.31	< 0.0001

Abbreviations: MFI=mean fold increase, CI=confidence interval

[a]Statistical significance calculated with ANOVA using Bonferroni's method of adjusting for multiple comparisons.

Table 3

Factors associated with post-vaccination HI titers against viruses representing past A(H3N2) antigenic clusters in linear regression models.

Factors	Fold increase in post-vaccination HI titer	95% CI	*P* value
A/Texas/50/2012			
A 2-fold increase in A/Switzerland/9715293/2013 response[a]	1.19	1.13 – 1.25	< 0.0001
A 2-fold increase in baseline A/Texas/50/2012 HI titer	1.73	1.62 – 1.84	< 0.0001
A/Victoria/361/2011			
A 2-fold increase in A/Switzerland/9715293/2013 response[a]	1.14	1.07 – 1.21	< 0.0001
A 2-fold increase in baseline A/Victoria/361/2011 HI titer	1.81	1.70 – 1.93	< 0.0001
A/Wuhan/359/1995			
A 2-fold increase in A/Switzerland/9715293/2013 response[a]	1.11	1.05 – 1.17	0.0004
A 2-fold increase in baseline A/Wuhan/359/1995 HI titer	1.90	1.80 – 2.00	< 0.0001

[a] A/Switzerland/9715293/2013 response defined as post-vaccination titer/pre-vaccination titer

Table 4

Factors associated with post-vaccination seroprotection (HI titer ≥ 40 or HI titer ≥ 110) against A/Texas/50/2012 in multiple logistic regression models.

Factors	OR	95% CI	*P* value
Seroprotection with TX/12 titer ≥ 40 (n = 29) [a]			
A 2-fold increase in A/Switzerland/9715293/2013 response[b]	5.92	2.01 – 44.08	0.0128
A 2-fold increase in baseline A/Texas/50/2012 titer	40.67	3.38 – 302.54	0.0223
Seroprotection with TX/12 titer ≥ 110 (n = 114) [a]			
A 2-fold increase in A/Switzerland/9715293/2013 response[b]	2.08	1.41 – 3.36	0.0008
A 2-fold increase in baseline A/Texas/50/2012 titer	3.52	1.56 – 10.29	0.0082

[a] participants with baseline titers above the seroprotective level were excluded

[b] A/Switzerland/9715293/2013 response defined as post-vaccination titer/pre-vaccination titer