Interseason waning of vaccine-induced hemagglutination inhibition antibody titers and contributing factors to pre-existing humoral immunity against influenza in community-dwelling older adults over 75 years of age

Bettina Wunderlich
Johns Hopkins Bloomberg School of Public Health

Thomas Laskow
Johns Hopkins University School of Medicine

Huifen Li
Johns Hopkins University School of Medicine

Li Zhang
Copenhagen University Hospital Amager and Hvidovre

Engle Abrams
Johns Hopkins University School of Medicine

Jing Tian
Johns Hopkins University School of Medicine

Jun Yu
Johns Hopkins University School of Medicine

Yiyin Chen
Johns Hopkins University School of Medicine

Juliette Tavernier
Copenhagen University Hospital Amager and Hvidovre

Yushu Huang
Vassar College

Kawsar Talaat
Johns Hopkins Bloomberg School of Public Health

Jay H. Bream
Johns Hopkins Bloomberg School of Public Health

Qian-Li Xue
Johns Hopkins University School of Medicine

Graham Pawelec
University of Tübingen
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Abstract

**Background:** Seasonal influenza causes significant morbidity and mortality with a disproportionately high disease burden in older adults. Strain-specific hemagglutination-inhibition (HAI) antibody titer is a well-established measure of humoral immunity against influenza and pre-vaccination HAI antibody titer is a valuable indicator of pre-existing humoral immunity at the beginning of the influenza season in highly vaccinated older adults. While vaccine-induced HAI antibody titers are known to wane over time, accurate assessment of their interseason decline has been challenging. This is because pre-vaccination HAI antibody titers are conventionally measured using current season vaccine strain antigens instead of the prior season vaccines with which individuals were immunized, and do not accurately represent residual antibody titers from prior season vaccination. This study took advantage of available pre-vaccination HAI antibody titers measured using prior season vaccine strain antigens in a longitudinal influenza immunization study with participants enrolled for multiple consecutive influenza seasons from 2014 through 2017 during which influenza A virus (IAV) H3N2 and influenza B virus (IBV) strains were changed in 2015 and in 2016. While the IAV H1N1 vaccine strain remained the same from 2014 through 2016 seasons, it was changed in the 2017 season. Finally, we investigated factors contributing to pre-existing humoral immunity.

**Results:** Interseason decline of HAI antibody titers was evident, but rates of decline varied among vaccine strains and study seasons, from 18% (p=0.43) to 61% (p<0.01). Rates of decline were noticeably greater when pre-vaccination HAI titers were measured using the conventional approach, from 33% (p=0.12) to 83% (p<0.01). All p values were adjusted for age at prior study season, sex, race, and education. This was largely because the conventional approach underestimated residual HAI antibody titers from prior season vaccinations. Moreover, interseason antibody decline and prior season post-vaccination HAI antibody titer had significant and independent associations with pre-vaccination HAI antibody titer.

**Conclusions:** The conventional approach overestimates interseason HAI antibody decline as it underestimates residual antibody titers from prior season vaccination, particularly when virus strains in the vaccine formula change. Moreover, interseason antibody decline and prior season post-vaccination HAI antibody titers independently contribute to pre-existing humoral immunity in this highly vaccinated, community-dwelling older adult population.

**Background**

Seasonal influenza causes significant morbidity and mortality each year. The highest burden of severe disease and deaths occurs in older adults, particularly those over 75 years of age [1]. Not only does influenza infection cause acute respiratory illness, it can also lead to exacerbation of comorbid chronic conditions, such as cardiovascular diseases in older adults; substantial evidence indicates protective effects of influenza vaccination against both [2–5]. Therefore, annual immunization with influenza vaccines has been recommended for older adults as the primary prevention against this common viral infection for more than 50 years [6, 7].
While correlates of protection of influenza vaccination are not completely understood, humoral immunity is thought to be very important in preventing infection. This is typically assessed by measuring strain-specific hemagglutination-inhibition (HAI) antibody titers. Post-vaccination HAI titers and vaccine-induced HAI antibody responses (i.e., seroconversion) are the main focus of studies of influenza vaccine immunogenicity. However, older adults typically have significant pre-vaccination HAI titers at the beginning of each influenza season, likely from prior annual vaccinations and/or previous influenza infection(s) [8–10]. Substantial evidence suggests that such pre-existing humoral immunity has a major impact on post-vaccination HAI titers and vaccine-induced antibody responses [10–13]. Since vaccine-induced HAI antibody titers are known to wane over time and older adults are a highly vaccinated population [14–18], it is critically important to accurately assess interseason decline of HAI titers from prior season vaccination. This knowledge will help improve our understanding of waning vaccine-induced humoral immunity over time, a significant problem common to respiratory viral infections including the ongoing pandemic caused by SARS-CoV-2 [19, 20].

To determine interseason decline of HAI antibody titers induced by prior season vaccination, we took advantage of the availability of pre-vaccination HAI antibody titers measured using prior season vaccine strain antigens from 2014 through 2017 influenza seasons during which influenza A virus (IAV) H3N2 and influenza B virus (IBV) strains changed in 2015 and again in 2016. While the IAV H1N1 vaccine strain remained the same from 2014 through 2016 seasons, it changed in the 2017 season. These data were available from Johns Hopkins Longitudinal Influenza Immunization Study of Aging over 75 (JH LIISA 75+), an ongoing study of annual influenza immunization in community-dwelling older adults over 75 years of age [11] with many participants enrolled for multiple consecutive study seasons, making it possible to directly assess interseason decline of HAI antibody titers in the same individuals. We also investigated interseason antibody decline and prior season post-vaccination antibody titer as factors contributing to pre-existing humoral immunity.

Results

Study participants, influenza immunization, and HAI antibody titers

Table 1 shows demographic and clinical characteristics of the participants. The mean age of participants during the study was 82 years for each of the four study seasons, and there is a preponderance of females (over 50% each year). Over 80% of participants considered themselves white, and the majority had completed college. Figure 1 summarizes the study design and the influenza virus strains included in each study season’s vaccine formula. To minimize the impact of breakthrough influenza infection on subsequent season pre-vaccination HAI antibody titers and interseason antibody decline, five confirmed influenza cases identified through post-vaccination influenza surveillance (two cases in 2014, one in 2015, and two in 2016) were excluded from this study. For the purpose of the main analyses included in
this study, participation in two consecutive study seasons was required, yielding a final sample size of 49 for 2014–2015, 52 for 2015–2016, and 78 for 2016–2017 study seasons.

Table 1
Demographic characteristics of the entire study cohort included in the analysis, by individual study seasons

<table>
<thead>
<tr>
<th>Total person-seasons*</th>
<th>2014 (n = 76)</th>
<th>2015 (n = 114)</th>
<th>2016 (n = 92)</th>
<th>2017 (n = 172)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (standard deviation)</td>
<td>83.3 (5.3)</td>
<td>82.4 (5.6)</td>
<td>82.2 (5.6)</td>
<td>82.8 (5.1)</td>
</tr>
<tr>
<td>Sex (male), n (%)</td>
<td>30 (39.5)</td>
<td>51 (44.7)</td>
<td>40 (43.5)</td>
<td>69 (40.1)</td>
</tr>
<tr>
<td>Race (white), n (%)</td>
<td>63 (82.9)</td>
<td>99 (87.6)</td>
<td>79 (85.9)</td>
<td>148 (86.1)</td>
</tr>
<tr>
<td>Education, n (%)</td>
<td>28 (36.8)</td>
<td>36 (31.9)</td>
<td>32 (34.8)</td>
<td>60 (34.9)</td>
</tr>
<tr>
<td>High school</td>
<td>29 (38.2)</td>
<td>43 (38.0)</td>
<td>41 (44.6)</td>
<td>73 (42.4)</td>
</tr>
<tr>
<td>College</td>
<td>19 (25.0)</td>
<td>34 (30.1)</td>
<td>19 (20.6)</td>
<td>39 (22.7)</td>
</tr>
</tbody>
</table>

*Out of total person-seasons, 113 subjects participated in a single season and 124 subjects participated in at least two consecutive seasons from 2014 through 2017.

**Assessment of interseason decline of HAI antibody titers from the immediate prior season vaccination**

First, we evaluated interseason HAI antibody titer decline over a period of approximately one year. We compared current season pre-vaccination HAI titer with post-vaccination HAI titer from the immediate prior season for the same individuals, generating a ratio of the two. The rate of interseason HAI antibody titer decline was derived from the formula (1-ratio) x 100%. As shown in Table 2, all ratios were less than 1 with the lowest ratio being 0.39 indicating highest rate of decline rate of 61%. Statistically significant interseason antibody titer declines were observed for IAV-H3N2 between 2015–2016 (58%), IBV strain between 2014–2015 (61%), and IAV-H1N1 between 2016–2017 (35%), all \( p < 0.01 \), adjusting for age at prior study season, sex, race, and education. Adjusted rates of interseason decline for IAV-H3N2 between 2014–2015 and IBV between 2015–2016 were 18% \( (p = 0.43) \) and 22% \( (p = 0.13) \), respectively. Interestingly, the interseason decline rate for IAV-H3N2 between 2014–2015 seasons was 31% \( (p < 0.01 \) by unadjusted analysis), but biological sex appeared to be a major factor contributing to the decline; adjusting for sex resulted in a non-significantly different rate of 18%.
Table 2
Ratios of pre-vaccination HAI titers over prior season post-vaccination HAI titers among participants who completed the study in the corresponding two consecutive seasons when corresponding virus strains in the vaccine formula changed*

<table>
<thead>
<tr>
<th>Vaccine strain</th>
<th>Consecutive two seasons</th>
<th>Unadjusted</th>
<th>Adjusted**</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAV-H3N2</td>
<td>2014–2015 (n = 49)</td>
<td>0.69 (0.53–0.89) (p &lt; 0.01)</td>
<td>0.82 (0.51–1.34) (p = 0.43)</td>
</tr>
<tr>
<td></td>
<td>2015–2016 (n = 52)</td>
<td>0.50 (0.41–0.60) (p &lt; 0.01)</td>
<td>0.42 (0.28–0.62) (p &lt; 0.01)</td>
</tr>
<tr>
<td>IBV</td>
<td>2014–2015 (n = 49)</td>
<td>0.37 (0.32–0.42) (p &lt; 0.01)</td>
<td>0.39 (0.30–0.50) (p &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>2015–2016 (n = 52)</td>
<td>0.75 (0.64–0.87) (p &lt; 0.01)</td>
<td>0.78 (0.56–1.08) (p = 0.13)</td>
</tr>
<tr>
<td>IAV-H1N1</td>
<td>2016–2017 (n = 78)</td>
<td>0.70 (0.60–0.82) (p &lt; 0.01)</td>
<td>0.65 (0.49–0.85) (p &lt; 0.01)</td>
</tr>
</tbody>
</table>

*Pre-vaccination and prior season post-vaccination HAI antibody titers were both measured using the respective prior season vaccine strain antigens. **Adjusted for age at prior study season, sex, race, and education.

**Associations of interseason antibody decline with current season pre-vaccination HAI antibody titers**

Because pre-vaccination HAI antibody titer is an important indicator of pre-existing humoral immunity against influenza in older adults at each season, we investigated which factors contributed to pre-vaccination HAI antibody titers in this highly vaccinated older adult population. We began with an exploratory analysis of the relationships between interseason antibody decline and pre-vaccination HAI antibody titers. In this analysis, the ratio of pre-vaccination titer to prior season post-vaccination titer shown in Table 2 was employed as the indicator of interseason antibody decline. Figure 2 illustrates the relationships obtained from univariate analysis between current season pre-vaccination HAI titers, also referred to as “residual”, and interseason antibody decline. Of note, a higher ratio (all ratios are less than 1 as shown in Table 2) is an indicator of less interseason decline. As such, a positive association with a significant Spearman correlation coefficient indicates an inverse relationship. That is, a lower interseason HAI antibody decline is associated with a higher pre-vaccination HAI antibody titer. This analysis yielded statistically significant associations between pre-vaccination HAI titers and interseason antibody decline for all vaccine strains and study seasons except for IAV-H3N2 between 2015–2016 (ρ = 0.23, p = 0.10) (Fig. 2A-C).

Consistent with these results from univariate analysis, except for IAV-H3N2 between 2014–2015 or between 2015–2016 (data not shown), estimated effects of interseason antibody decline on pre-vaccination HAI antibody titers were statistically significant for IBV between 2014–2015 and between 2015–2016 (1.34 [1.13–1.59], p < 0.01 and 1.10 [1.02–1.19], p = 0.02, respectively) and IAV-H1N1 between 2016–2017 (1.11 [1.05–1.17], p < 0.01), all adjusted for age at prior study season, sex, race, and education.
Associations of prior season post-vaccination HAI antibody titers with current season pre-vaccination HAI antibody titers

We also evaluated relationships between prior season post-vaccination HAI antibody titers and current season pre-vaccination HAI antibody titers (Fig. 3). The results indicate significant associations across all vaccine strains and study seasons, with the strongest associations seen in IBV ($\rho = 0.89$ in 2014–2015) (Fig. 3A-C).

Consistent with these results from univariate analysis, estimated effects of prior season post-vaccination HAI antibody titers on pre-vaccination HAI antibody titers were statistically significant for IAV-H3N2 between 2014–2015 and between 2015–2016 (1.52 [1.33–1.74] and 1.72 [1.55–1.90], respectively, both $p < 0.01$), IBV between 2014–2015 and between 2015–2016 (2.16 [1.92–2.43] and 1.83 [1.65–2.02], respectively, both $p < 0.01$), and IAV-H1N1 between 2016–2017 (1.82 [1.61–2.04], $p < 0.01$), all adjusted for age at prior study season, sex, race and education. In fact, the effect size for IBV between 2014–2015 was the largest, exhibiting a two-fold increase in prior season post-vaccination HAI titer associated with a 116% increase in current season pre-vaccination HAI antibody titer ($p < 0.01$).

Independent effects of interseason antibody decline and prior season post-vaccination HAI antibody titer on current season pre-vaccination HAI antibody titers

We further explored independent effects of interseason antibody decline and prior season post-vaccination HAI antibody titers on pre-vaccination HAI antibody titers, as summarized in Table 3. In Model 1, significant effects of interseason HAI antibody decline were retained after adjusting against prior season post-vaccination HAI antibody titers, with or without adjusting for additional covariates (i.e., age at prior study season, sex, race, and education), for all vaccine strains and study seasons. In Model 2, significant effects of prior season post-vaccination titers were retained after adjusting against interseason antibody decline, with or without adjusting for additional covariates, for all vaccine strains and study seasons.
Table 3
Independent effects of interseason antibody decline and prior season post-vaccination HAI antibody titers on current season pre-vaccination HAI titers*

<table>
<thead>
<tr>
<th>Vaccine Strain</th>
<th>Consecutive two seasons</th>
<th>Adjusted for prior season post-vaccination titers</th>
<th>Adjusted for additional covariates$^2$</th>
<th>Adjusted for interseason antibody decline</th>
<th>Adjusted for additional covariates$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAV-H3N2</td>
<td>2014–2015 (n = 49)</td>
<td>1.06 (1.04–1.08) ($p$ &lt; .01)</td>
<td>1.06 (1.04–1.08) ($p$ &lt; .01)</td>
<td>1.82 (1.65–1.99) ($p$ &lt; .01)</td>
<td>1.83 (1.67–2.01) ($p$ &lt; .01)</td>
</tr>
<tr>
<td></td>
<td>2015–2016 (n = 52)</td>
<td>1.21 (1.18–1.24) ($p$ &lt; .01)</td>
<td>1.21 (1.18–1.24) ($p$ &lt; .01)</td>
<td>1.98 (1.90–2.07) ($p$ &lt; .01)</td>
<td>1.98 (1.90–2.07) ($p$ &lt; .01)</td>
</tr>
<tr>
<td>IBV</td>
<td>2014–2015 (n = 49)</td>
<td>1.27 (1.22–1.31) ($p$ &lt; .01)</td>
<td>1.26 (1.22–1.31) ($p$ &lt; .01)</td>
<td>2.04 (1.94–2.13) ($p$ &lt; .01)</td>
<td>2.04 (1.95–2.14) ($p$ &lt; .01)</td>
</tr>
<tr>
<td></td>
<td>2015–2016 (n = 52)</td>
<td>1.16 (1.14–1.18) ($p$ &lt; .01)</td>
<td>1.16 (1.14–1.18) ($p$ &lt; .01)</td>
<td>2.02 (1.94–2.10) ($p$ &lt; .01)</td>
<td>2.02 (1.94–2.11) ($p$ &lt; .01)</td>
</tr>
<tr>
<td>IAV-H1N1</td>
<td>2016–2017 (n = 78)</td>
<td>1.17 (1.15–1.19) ($p$ &lt; .01)</td>
<td>1.17 (1.14–1.19) ($p$ &lt; .01)</td>
<td>1.91 (1.81–2.00) ($p$ &lt; .01)</td>
<td>1.92 (1.82–2.03) ($p$ &lt; .01)</td>
</tr>
</tbody>
</table>

*Summary of estimated effects of interseason antibody decline adjusting for prior season post-vaccination HAI antibody titers (Model 1) and vice versa (Model 2) on current season pre-vaccination HAI antibody titers. Notes: Outcome measure is current season pre-vaccination HAI antibody titers measured using prior season vaccine strain antigens. $^1$Estimated effects are expressed as expected fold-change (95% confidence interval) in current season pre-vaccination HAI titers that is associated with a 0.1-unit less interseason antibody decline after adjusting against prior season post-vaccination HAI antibody titers (Model 1) or a two-fold increase in prior season post vaccination HAI antibody titers after adjusting against interseason antibody decline (Model 2), with or without adjusting for additional covariates (both Model 1 and 2). $^2$Additional covariates include age at prior study season, sex, race, and education.

**Discussion**

In this study, the longitudinal nature of the JH LIISA 75+ study cohort with participants enrolled for multiple consecutive influenza seasons has enabled us for the first time to directly assess interseason antibody decline and explore factors that contribute to pre-existing humoral immunity in community-dwelling older adults over 75 years of age. This was made possible by the availability of data on both current season pre-vaccination and prior season post-vaccination HAI titers measured using the prior
season vaccine strain antigens when the corresponding vaccine strains in the vaccine formula changed between the two consecutive study seasons. Our results demonstrate that while interseason antibody decline is evident, the rates of such declines are highly variable depending on vaccine strains and influenza seasons. Moreover, we have identified both interseason antibody decline and prior season post-vaccination HAI antibody titers as major factors that impact pre-existing humoral immunity in this highly vaccinated older adult population.

Intra-(within)season decline of strain-specific HAI antibody titers has been recognized since the first successful trial of inactivated influenza vaccine in 1943 in which Francis and colleagues reported that vaccine-induced HAI antibody titers declined by about one-third 4–5 months after vaccination [21]. However, studies have also showed that vaccine-induced HAI antibody titers could be maintained at high levels in persons ≥ 60 years of age for at least 4 months (summarized in [14]). While interseason antibody decline is likely, its accurate assessment has been challenging. This is because the conventional approach is to employ current season vaccine strain antigens to measure current season pre-vaccination HAI antibody titers, instead of the prior season vaccines with which individuals were immunized. Such conventionally measured pre-vaccination HAI antibody titers do not accurately represent “residual” antibody titers from prior season vaccination, particularly when virus strains in the vaccine formula change. Whether this would lead to overestimating interseason antibody decline had not been determined. Here we assessed current season pre-vaccination HAI titers using the conventional approach for the evaluation of interseason antibody decline, compared with the specific strain approach outlined above. As shown in Supplement Table 1, except for IBV between 2015–2016 (0.67, \( p = 0.12 \)), ratios of current season pre-vaccination HAI antibody titer over prior season post-vaccination HAI antibody titer were lower than those in Table 2, ranging from 0.59 to 0.17. This translates to statistically significant interseason antibody declines from 41–83% (\( p < 0.01 \) for all), after adjusting for age at prior study season, sex, race, and education. In addition, we evaluated and compared conventionally measured current season pre-vaccination HAI antibody titers over those measured using prior season vaccine strain antigens. As shown in Supplement Table 2, conventionally measured pre-vaccination HAI antibody titers were lower than those measured using prior season vaccine strain antigens. Specifically, ratios of conventionally measured pre-vaccination HAI antibody titers over those measured using prior season vaccine strain antigens for IAV-H3N2 in 2015 and 2016, IBV in 2015, and IAV-H1N1 in 2017 were 0.36, 0.56, 0.41, and 0.86, respectively, all statistically significant (\( p < 0.01, 0.03, 0.01 \) and 0.04, respectively), after adjusting for age, sex, race, and education. These results were confirmed when we conducted the same analysis in a larger sample consisting of all participants with both sets of pre-vaccination HAI antibody titer data available in all individual study seasons (Supplement Table 3). Taken together, results from these additional analyses support the notion that the conventional approach, i.e., measuring pre-vaccination HAI antibody titer using current season vaccine strain antigens, overestimates the rate of interseason antibody decline; the explanation for such overestimation is its inaccurate assessment of residual HAI antibody titers from prior season vaccination. An exception is the IBV strain of Victoria lineage in the 2016 season as conventionally measured pre-vaccination HAI antibody titers against this vaccine strain were almost two-fold higher than the residual antibody titers measured using prior season
IBV strain antigens, as shown in Supplement Table 2 (unadjusted) and Supplement Table 3. The reason for this exception is not entirely clear, but may be related to the fact that IBV strains are highly conserved and the repeated exposure to similar strains over the years has instilled robust humoral immunity in older adults [22, 23]. It may also be related to an unusually high cross reactivity of pre-vaccination HAI antibody against the 2016 vaccine IBV strain. As such, the interseason decline of HAI titers against IBV between 2015–2016 was not statistically significant, particularly when current season pre-vaccination HAI antibody titers were measured using the conventional approach (Supplement Table 1).

Because of the clinical and immunological importance of pre-existing humoral immunity characterized by pre-vaccination HAI antibody titers, we sought to identify factors contributing to antibody status. One accepted contributing factor is interseason antibody decline, but our results document that less interseason antibody titer decline is associated with higher current season pre-vaccination HAI antibody titers. Nonetheless, such an association was not consistent across all vaccines strains or study seasons evaluated. Our study participants are drawn from a highly vaccinated older adult population with repeated annual vaccinations throughout many prior influenza seasons. In this context, it is likely that contribution of interseason antibody decline from any particular season vaccination, e.g., the immediate prior season in this case, to the pre-vaccination HAI titers, may represent a portion of the potentially accumulative effect and can be impacted by other covariates and, therefore, may vary. On the other hand, the prior season post-vaccination HAI antibody titer appears to be a strong and consistent contributing factor across all vaccine strains and seasons studied. Theoretically, a high post-vaccination antibody titer in the prior season represents a high starting point for interseason antibody decline, leading to a high residual antibody titer for the next influenza season. Results from further analyses indicate independent effects of both interseason antibody decline and prior season post-vaccination HAI antibody titers, highlighting the importance of both. Nevertheless, further longitudinal studies are warranted to determine whether a low rate of interseason antibody decline and/or a high prior season post-vaccination antibody titer serve as a predictor for a robust pre-existing humoral immunity and whether this protects better against influenza as hypothesized. Additionally, it may be that these parameters could be used as an indicator of certain unmeasured intrinsic immune system characteristics, which might include the maintenance of memory B cells and plasma cells for low-level antibody production to counteract antibody waning and provide for further understanding of influenza immunity to identify vulnerable populations who are unable to mount robust humoral immune responses to vaccination.

The strengths of this study include: 1) a well characterized population of community-dwelling older adults over 75 years of age with participants enrolled for multiple consecutive influenza seasons; 2) a unique opportunity of available pre-vaccination HAI antibody titers measured using prior season vaccine strain antigens for in-depth characterization of interseason antibody decline and contributing factors to pre-existing humoral immunity; and 3) ability to exclude breakthrough influenza cases identified through vigorous post-vaccination influenza surveillance to minimize their impact on pre-existing humoral immunity in the following season and interseason antibody decline.
The study also has limitations. For example, our data do not address vaccine effectiveness, a direct and important clinical outcome measure that would require a large sample size. However, strain-specific HAI antibodies are considered to be the major immune mechanism mediating vaccine-induced protection against influenza infection [24] and serve as the basis of age-specific immunogenicity criteria employed by the regulatory committees of the European Medicines Agency (EMA) and the US Federal Drug Administration (FDA) for the approval of new influenza vaccines [25]. Another limitation is the relatively small study sample size. While JH LIISA 75+ cohort enrolled significant number of participants in each study season, the sample size for this study is limited by the requirement for participation in two consecutive study seasons. To partially address this limitation, the analysis of comparisons of two sets of pre-vaccination HAI antibody titers presented in Supplement Table 2 was repeated and validated among all participants who completed individual study seasons with a larger sample size (Supplement Table 3). Finally, given the nature of the hemagglutination inhibition assay, while pre-vaccination HAI antibody titers measured using prior season vaccine strain antigens are more likely representative of residual antibodies from prior season vaccination, “cross-reactivity” cannot be completely excluded, i.e., such measurements may also indicate HAI antibodies cross-reactive to viral strain antigens from prior influenza vaccinations and/or breakthrough infections accumulating over many previous influenza seasons. Despite these limitations, findings from this study provide a framework for considering a more accurate assessment of residual antibodies from prior season vaccination, interseason antibody decline, and factors contributing to pre-existing humoral immunity in this highly vaccinated older adult population. They also provide initial evidence suggesting that the extent of interseason HAI antibody decline is not as pronounced as conventionally believed to be in this subset of older adults. To further address intra- and interseason waning of vaccine-induced humoral immunity as well as pre-existing humoral immunity in older adults, more in-depth investigations including detailed intra-seasonal HAI antibody analyses with frequent sampling after vaccine administration until the beginning of the next influenza season in longitudinal studies as well as vaccine clinical effectiveness are indicated.

**Conclusions**

Results from this study demonstrate that while interseason strain-specific HAI antibody decline is evident in older adults, the extent of such decline is not as pronounced as conventionally estimated in community-dwelling older adults over 75 years of age. In addition, interseason antibody decline and prior season post-vaccination antibody titers are major factors that independently contribute to pre-existing humoral immunity in this highly vaccinated, oldest old subset of community-dwelling older adult population.

**Methods**

**The study population and protocol**

JH LIISA 75+ is a prospective observational study of influenza immunization in community-dwelling older adults over 75 years of age. The study was started in 2014 and is currently ongoing. Subjects were
recruited via collaborating physicians, community newspaper advertisement and flyers at outpatient clinics, senior centers, and residential areas in Baltimore, Maryland. Candidates who consented to participate were screened by trained clinical research coordinators. Exclusion criteria include a history of allergic reaction to influenza vaccine or egg, currently on oral steroids or immunosuppressive therapy, worsening or new-onset of immune-modulating conditions (e.g., rheumatoid arthritis, hematologic malignancies, etc.), or acute illness such as a viral infection. In each of the study seasons, screening and pre-vaccination evaluation were started in late summer, 4–6 weeks before annual massive influenza vaccination in the Baltimore area. Study participants came to the Clinical Research Unit at Johns Hopkins Institute of Clinical and Translational Research or Biology of Healthy Aging Studies Unit on the Johns Hopkins Bayview Medical Center campus, or study visits were conducted at participants’ homes as needed. Detailed demographic and clinical information were obtained. After a pre-vaccination blood draw, participants received a high dose trivalent inactivated influenza vaccine (HD-IIV3, Fluzone® High-Dose, Sanofi, Swiftwater, PA), one of the FDA-approved influenza vaccines specifically recommended for older adults. A second (post-vaccination) blood sample was collected during the 4th week following vaccine administration, and then, post-vaccination influenza surveillance was conducted in each study season until the end of April. From 2014 to 2017, a total of 237 participants were enrolled in JH LIISA 75+ cohort. Among them, 113 only participated in one study season and 124 participated in at least two seasons. For individual study seasons, there were 74 unique subjects for 2014, 114 for 2015, 92 for 2016 and 172 for 2017, leading to a total sample of 454 season-persons for the entire four-season study period (Supplement Table 4).

Influenza virus strains in the vaccine formula for 2014 through 2017 study seasons

From 2014 through 2017, HD-IIV3 for each season contains 60 µg of hemagglutinin (HA) antigen for each of the three influenza virus strains, namely, IAV H1N1 and H3N2 plus IBV. IAV H1N1 vaccine strain remained the same from 2014 through 2016, A/California/07/2009, but changed to A/Michigan/45/2015 X-275 (pdm-09-like) in 2017. However, IAV H3N2 and IBV vaccine strains were different for each of the 2014, 2015, and 2016 seasons, namely, A/Texas/50/2012, A/Switzerland/9715293/2013 and A/Hong Kong/4801/2014 for 2014, 2015, and 2016, respectively; B/Massachusetts/02/2012 (Yamagata lineage), B/Phuket/3073/2013 ether-treated (Yamagata lineage) and B/Brisbane/60/2008 ether-treated (Victoria lineage) for 2014, 2015, and 2016, respectively (Fig. 1).

Measurement of strain-specific anti-influenza HAI antibody titers

A validated HAI assay was used to quantify antibody titers against study vaccine antigens for the three vaccine strains (H1N1, H3N2 and B) in each year and were performed by Sanofi as previously described (9, 18). Briefly, serum was incubated with type III neuraminidase to eliminate non-specific inhibitors and then with turkey red blood cells to adsorb non-specific agglutinins. Two-fold serial dilutions of sera, beginning at a 1:10 dilution, were then performed in duplicate, and sera were incubated with influenza
virus (4 hemagglutination units/25μl). Turkey red blood cells were then added, and the titer defined as the highest dilution in which hemagglutination of turkey red blood cells was inhibited.

**Data analysis**

Baseline demographics including age, sex, race, and education were compared by influenza season between 2014 and 2017. To analyze inter-season waning in HAI antibody titers, we modeled the ratio of current season pre-vaccination HAI antibody titers ($Y_{current}$) over prior season post-vaccination HAI titers ($Y_{prior}$) using the generalized linear model (GLM) with a log link and a Gamma error distribution, i.e.,

$$\log[E(Y_{current}/Y_{prior})]=\beta_0 + \beta_1Z,$$

where $E(Y_{current}/Y_{prior})$ is the mean value of the ratio; and $Z$ represents a vector of confounders. $\exp(\beta_1)$ therefore can be interpreted as the mean ratio of $Y_{current}/Y_{prior}$ after controlling for covariates $Z$ (i.e., mean age, female sex, white race, and high school or below education). Crude and covariate-adjusted analyses were conducted separately for each influenza vaccine strain (i.e., IAV H1N1 and H3N2 and IBV). We also examined the differences in the estimates of the ratio when current season pre-vaccination HAI antibody titers were measured by current season vaccine strain antigen (conventional approach) versus those measured by the vaccine strain antigens with which the same participant was immunized in the prior season. To directly assess the differences in the measurement of current season HAI antibody titers by using current season versus prior season vaccine strain antigens, we also used GLMs to estimate the crude and adjusted ratio of current season pre-vaccination HAI antibody titers measured by the two types of vaccine strain antigens. Next, we analyzed the crude and adjusted associations of current-season pre-vaccination HAI antibody titers measured using previous season vaccine strain antigens with both (separately and jointly) the interseason decline in HAI antibody titers and prior-season post vaccination HAI antibody titers. In this model, the interseason decline was defined as the ratio of current season pre-vaccination HAI antibody titer over prior-season post vaccination HAI antibody titer. The effect size from this model was expressed as expected fold change in the current-season pre-vaccination HAI antibody titers that is associated with a 0.1 unit increase in the ratio (or two-fold increase in prior-season post vaccination HAI antibody titers). We similarly explored the impact on the associations when the current season HAI antibody titers were measured using conventional approach. All analyses were performed using Stata version 15.

**Declarations**

**Ethics approval and consent to participate**

Written, informed consent was obtained from all participants. The study protocol was approved by the Johns Hopkins School of Medicine Institutional Review Board (ID: NA_00092365).

**Consent for publication**

Not applicable.

**Availability of data and materials**
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

S.X.L and B.W. wrote the main manuscript text and prepared figure 1, and J.T. prepared all tables and figures 2-3. QL.X. was a major contributor to interpreting the data. All authors have either contributed data collection, or read and contributed to the revision of the manuscript.

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**References**


Figures
Study design. High dose (HD) inactivated trivalent influenza vaccine (IIV3) administration and specific influenza virus strains included in the vaccine formula (gray horizontal bar) are shown in each study season. Pre-vaccination strain-specific hemagglutination inhibition (HAI) antibody titers were measured using current season vaccine strain antigens (conventional approach, green bar) and prior season vaccine strain antigens (orange bar).

Figure 2

Relationships between current season pre-vaccination HAI antibody titers and interseason antibody decline. Associations of current season pre-vaccination HAI antibody titers measured using prior season vaccine strain antigens with interseason antibody decline as indicated by the ratios shown in Table 2 were evaluated in univariate analyses. (A) IAV-H3N2: Spearman correlation coefficient between 2014-
2015 (n=49) was 0.30 (p= .04) and that between 2015-2016 (n=52) was 0.23 (p= .10); (B) IBV: Spearman correlation coefficient between 2014-2015 (n=49) was 0.49 (p< .01) and that between 2015-2016 (n=52) was 0.34, (p=.02); (C) IAV-H1N1: Spearman correlation coefficient between 2016-2017 (n=78) was 0.51 (p< .01).

Figure 3

Relationships between current season pre-vaccination HAI titers and prior season post-vaccination HAI antibody titers. Associations of pre-vaccination HAI antibody titers with prior season post-vaccination HAI titers were evaluated in univariate analyses. (A) IAV-H3N2: Spearman correlation coefficient between 2014-2015 (n=49) was 0.72 and that between 2015-2016 (n=52) was 0.82, p< .01 for both; (B) IBV: Spearman correlation coefficient for between 2014-2015 (n=49) was 0.89 and that between 2015-2016 (n=52) was 0.80, p< .01 for both; (C) IAV-H1N1: Spearman correlation coefficient between 2016-2017 (n=78) was 0.66, p< .01.

Supplementary Files

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