Preexisting Immunity Not Frailty Phenotype Predicts Influenza Post Vaccination Titers Among Older Veterans

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Running Head: Frailty and influenza vaccine responses

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Abstract

Both preexisting immunity to influenza and age have been shown to be a correlate of influenza vaccine response. Frailty, an indicator of functional impairment in older adults, has also been shown in one study to predict lower influenza vaccine responses among non-veterans. In the current study we aimed to determine the association between frailty, preexisting immunity and immune response to influenza vaccine in older veterans. We analyzed 117 subjects (age range 62-95 years; median 81) divided into 3 cohorts based on the Fried frailty test: non-frail (NF: N=23; median age 68), pre-frail (PF: N=50; median age 80) and frail (F:N=44; median age 82) during the 2010-2011 and 2011-2012 influenza seasons. Subjects received the seasonal trivalent inactivated influenza vaccine and baseline and post-vaccination samples were obtained. Anti-influenza humoral immunity as measured by hemagglutinin inhibition (HI) and microneutralization (MN) assays were measured for influenza B, A(H1N1)pdm09 and A(H3N2) viruses. Post vaccination-titers to Influenza vaccine were not different between frail and NF subjects overall in this older subset of veterans. However, preexisting HI titers strongly correlated with post-vaccination titers among all functional status groups. When microneutralization titers are compared, the association between preexisting immunity and vaccine response varies by frailty status with the highest correlation observed in the NF subjects. In conclusion, preexisting immunity rather than frailty appear to predict post-influenza vaccine titer in this older veteran cohort.
Introduction

Seasonal Influenza has a disproportionately greater adverse effect on older adults. While persons over the age of 65 comprise roughly 13% of the US population, the overwhelming majority of influenza related deaths and most hospitalizations occur in this population [1-4]. This pattern is even more pronounced among those at the older extreme of this group [2]. Age-associated impaired cell-mediated immunity renders aging older adults increasingly susceptible to infections while, at the same time, resulting in reduced immune responses to vaccinations, the very strategy used to prevent infections [5-7]. Influenza has served as a prime example of this paradigm [8]. While influenza vaccine can reduce complications such as pneumonia and hospitalizations among older adults [9, 10], the magnitude of mortality and morbidity benefit, among the elderly compared to younger adults has been a source of debate [11, 12].

This discrepancy in evidence highlights the heterogeneous nature of this population. One source of variability is preexisting immunity to influenza. In fact, preexisting immunity to influenza has been proposed as a greater correlate of immunity to influenza than age in the elderly [13]. Another potential source of variable response to influenza vaccine is functional decline or frailty. Frailty syndrome, an indicator of functional impairment in older adults, is a strong marker of increased risk for poor health outcomes including falls, disability and death [14]. There has been increasing evidence to suggest that frailty also negatively impacts the immune system, beyond what would be expected as a result of aging. Existence of a dysregulated immune system, marked by a heightened inflammatory state and increase in immunologic markers of T cell senescence, has been identified and described in frail older adults [15]. B lymphocyte diversity has also been shown to decline in frail individuals, making them more susceptible to novel antigens both via natural infection or immunization [16]. Frailty has also been shown to have an impact on the innate immune system. Decreased production of
Toll-like receptor ligand (TLR)-induced interleukin (IL)-12p70 and IL-23, cytokines that play an important role in the protection against pathogens has been associated with frailty rather than age [17]. There are some data to suggest that frailty may adversely affect responses to influenza and pneumococcal vaccinations [18-20]. Further studies are needed to better understand the immune response to influenza and vaccination, particularly among the frail elderly.

In this present study, we sought to determine the relationship between frailty and strain specific influenza hemagglutination inhibition (HI) and microneutralization (MN) assays among older veterans. These results may help identify those older adults who are most likely to have decreased response to influenza vaccination and, as a result, persisting risk for morbidity and mortality from influenza despite vaccination.

**Methods and materials**

Setting, sample, and study design:

This is an observational study in which Veterans 60 years of age or older, receiving care at the Louis Stokes Cleveland VA Medical Center (LSCVAMC) outpatient geriatrics and internal medicine clinics were enrolled over two influenza seasons. Those receiving immunosuppressive medications or with immunosuppressive conditions including HIV, cancer undergoing chemotherapy, and severe anemia were excluded from the study. Subjects in whom influenza vaccine was contraindicated due to allergy to any component of the vaccine were also excluded. Subjects were recruited during the 2010-2011 and 11-12 influenza seasons. Subjects could only participate for one season. Serum samples were obtained at the time of administration of seasonal trivalent inactivated influenza (TIV) vaccine and at 2-4 months post-vaccination. The 2010-2011 TIV contained A/California7/2009-like virus (the pandemic 09 H1N1), A/Perth/16/2009-like virus (H3N2) and B/Brisbane/60/2008 strains. The 2011–2012 TIV contained the same strains and represented no change from the previous season. All subjects
completed frailty testing at the time of admission to the study. We analyzed data from 117 subjects (age range 62-95 years; median 81). Human experimentation guidelines of the Department of Health and Human Services were followed in the conduct of this study. The study was reviewed and approved by the Institutional Review Board at the LSCVAMC and all participants provided informed written consent.

Frailty assessment:
The Fried Frailty Phenotype, a widely accepted and validated instrument of frailty measurement in older adults, was utilized for functional assessments [21]. Study staff administered the five components of the Fried frailty assessment tool: weakness as assessed by grip strength (measured by Jamar dynamometer average of 3 trials with dominant hand), walking speed (15 feet, straight line, one way), unintended weight loss >= 10 pounds in the past year, self-reported exhaustion (two Likert-type questions from CES-D Depression Scale [22], and physical activity based on the Minnesota Leisure Time activity questionnaire [23]. Grip strength was stratified by sex and body mass index, walking speed was stratified by sex and height and physical activity score was stratified by sex as suggested by Fried and colleagues. Subjects were assigned to the non-frail (NF) category for 0 criteria met, pre-frail (PF) for 1-2 criteria and frail (F) for 3 or more criteria met on the Fried frailty instrument. Laboratory measurements:
Hemagglutinin inhibition (HI) and microneutralization assays are measured for influenza B, A(H1N1)pdm09 and A(H3N2) viruses. HI and microneutralization titers were performed using routine methods as previously described [24-27]. The microneutralization assay has been shown to have better sensitivity in detecting serum antibodies than HI and to correlate with protection against seasonal influenza [28-30]. However, HI assays offer several advantages including that they are relatively simple and inexpensive to perform and widely used around the world, allowing for easier comparison.
Results of the assays are expressed as geometric mean titers (GMT). Results of the assays are expressed as geometric mean titers (GMT). Seroconversion is defined as ≥ 4 fold increase in HI GMT from baseline. Seroprotection is defined as post-vaccination HI titer ≥ 1:40.

Statistical analysis:

Analysis was completed in SPSS; Graphpad Prism 6.0 and R 3.2.2 using the ggplot2 package to graphically represent the data. Differences in geometric mean titers (GMT) ratios (pre to post HI or microneutralization titers) among frailty groups were calculated using ANOVA. For pre- to post-vaccination titer comparisons, log2-transformed HI and microneutralization pre and post titers were used as independent and dependent variables respectively. Associations between age and fold increases in titers were examined using Spearman Rank correlations both overall and within frailty groups.

Results

We classified 117 subjects according to the three Fried Frailty Phenotype categories: non-frail (N=23), pre-frail (N=50) and frail (N=44). Prevalence of frailty in this cohort was 43% while pre-frailty was identified in 38% of the subjects. The median age in the NF group (68 years, range 62-90) was significantly lower than the PF (80 years; 62-92) and frail (82 years; 62-92) groups. As expected in an older veteran population, the subjects were primarily male (96%). African Americans accounted for over half (54%) of the study subjects. Pre-vaccination seroprotection rates were similar for all viruses (B: 27%; H1N1: 24%, H3N2: 28%). After vaccination, seroprotection rates were significantly higher for A(H3N2) (64%) compared with A(H1N1)pdm09 (50%) and B viruses (47%) (p=0.02). These rates did not vary based on frailty status (data not shown). 87% of subjects had received the seasonal influenza vaccine in the preceding year.

We compared immune responses to influenza vaccine as measured by GMT ratios (HI and microneutralization assays) across frailty groups. HI fold increases in response to B (means: NF=3.0,
PF=2.3, F=3.1), A(H1N1)pdm09 (NF=2.9, PF=2.3, F=3.1) and A(H3N2) (NF=3.3, PF=5.9, F=6.4) viruses were similar among the three groups (Figure 1A). Microneutralization fold increases after vaccine were also similar between frailty groups (Figure 1B). Additionally, while aging was generally associated with lower immunity to influenza, these correlations were not statistically significant when evaluated separately within frailty groups (data not shown). On the other hand, pre-existing immunity to influenza was found to strongly correlated post-vaccination titer (Figure 2). HI pre-vaccination titers strongly correlated with post-vaccination titers among all functional status groups (B virus: F: $r=0.72$; $p<0.0001$; PF: $r=0.69$; $p<0.0001$; NF: $r=0.80$; $p<0.000$), (H1N1: F: $r=0.60$; $p<0.001$; PF: $r=0.82$; $p<0.0001$; NF: $r=0.53$; $p=0.01$) and (H3N2: F: $r=0.57$; $p<0.001$; PF: $r=0.60$; $p<0.001$; NF: $r=0.58$; $p=0.004$) (Figure 2A). When microneutralization titers are compared, the association between preexisting immunity and post-vaccine titer varied by frailty status with the highest correlation observed in the NF subjects for B virus and H3N2 (B virus: F: $r=0.76$, $p<0.0001$; PF: $r=0.72$, $p<0.0001$; NF: $r=0.94$, $p<0.0001$), (H1N1: F: $r=0.58$, $p=0.001$; PF: $r=0.84$, $p<0.005$; NF: $r=0.47$, $p=0.04$) and (H3N2: F: $r=0.41$, $p=0.02$; PF: $r=0.72$, $p<0.005$; NF: $r=0.75$, $p<0.001$) (Figure 2B).

**Discussion:**

In the present study we investigated relationships between frailty status and influenza vaccine responses in older veterans with a secondary aim of examining the role of preexisting immunity in older adults. Seroprotection rates were higher for A(H3N2) than A(H1N1)pdm09 or B viruses. In our cohort, HI or microneutralization fold increases did not differ by frailty status. When assessed within frailty groups, increased age was not significantly associated with lower influenza vaccine responses. Pre-vaccination strain specific immunity was found to be strongly associated with post-vaccine responses in all frailty groups with the greatest correlations in microneutralization titers observed in the non-frail group.
Overall seroprotection rates were lower for A(H1N1)pdm09 and B viruses than for A(H3N2). These rates did not differ by frailty status. Our observed rates of seroprotection were similar to what has been reported among older adults, including higher immune protection against A(H3N2) [13]. Incidentally, among older adults, A(H3N2) virus has been found to be associated with more severe morbidity and increased mortality than A(H1N1)pdm09 [2, 3]. Notably, there were similar levels of pre-vaccination seroprotection among all three viruses. The 2010-2011 influenza season was preceded by the 2009 A(H1N1) pandemic, which occurred from April 2009 through June 2010 [32]. The circulating virus during this period was predominantly A(H1N1)pdm09 with less than 1% of cases representing A(H3N2) or B viruses. This prompted change in 2010-11 TIV components, with replacement of the A(H1N1) strain to the pandemic A(H1N1)pdm09 strain. The A(H3N2) strain was also changed from A/Brisbane/10/2007 to A/Perth/16/2009. The B strain was the only component preserved during this year. Change in vaccine strains offers an opportunity for greater GMT fold change due to the presence of a new antigen. This effect may be blunted in older adults due to reduced immunity to novel antigens. While immunosenescence is thought to contribute to decreased effectiveness of immunizations among older adults, age alone is likely not the entire explanation for this phenomenon. There is evidence to suggest that a number of factors such as functional status and preexisting immunity also play a role [33]. While there is growing literature on the association between functional decline and immunosenescence, there are limited data on influenza specific responses in frail elderly. There are data from an observational study demonstrating potentially lower clinical efficacy of influenza vaccination in vaccinated frail nursing home residents compared with non-frail as demonstrated by increasing all-cause mortality with increasingly impaired functional status [18]. To the best of our knowledge, there is only one other published report specifically comparing strain specific immunity to
influenza among frailty groups [19]. Our results differ from Yao and colleagues, who found frailty to be associated with impairment in TIV-induced strain-specific immunity to influenza as measured by HI titers. They incidentally also demonstrated increased rates of influenza-like illness and laboratory-confirmed influenza infection. Aside from differences in veteran and non-veteran populations, an important difference between our studies is the influenza seasons studied. Not only was 2007-2008 influenza season documented as moderate in severity by the Centers for Disease Control and Prevention, the TIV A(H3N2) and B viruses were poorly matched to the circulating strains [32, 34]. There is evidence that influenza vaccine is less effective during seasons of antigenic mismatch between the vaccine and circulating strains. In a study of adults older than 65 years of age in a season of antigenic mismatch, significantly higher HI titers were required to achieve the same level of protection as a well-matched vaccine. On the other hand, at low titers some measure of protection was seen against matched virus [35]. Additionally, vaccine effectiveness may be related to risk of developing influenza, with the lowest level of protection evident in highest risk persons during poor match seasons [36, 37]. By contrast, 2011-12 influenza season set a record for the lowest and shortest peak of influenza-like illness [38]. Similarly, according to the CDC, the 2010-2011-influenza season was less severe than both the pandemic year (2009-2010) and the 2007-2008 season [38]. As previously noted, the vaccine components were unchanged during these two years. In fact, estimates for the 2011-2012 TIV effectiveness have demonstrated that with unchanged vaccine components, protection may have extended beyond a single season [39]. All these factors may have contributed to lack of demonstrable differences amongst the frailty groups in the present study. Since the overwhelming majority of our subjects received influenza vaccine in the previous years, this may have resulted in similar immune responses regardless of functional status.
Pre-existing immunity was a strong correlate of post-vaccine immunity in our cohort. This is consistent with a previously published report by Reber and colleagues in which pre-vaccination titers were the best predictor of post-vaccination immunity to influenza in adults older than 50 years of age [13]. In fact, they found age to be only modestly associated with lower vaccine responses. We also did not find age to be a significant negative factor when evaluated within the frailty groups. Even though A(H1N1) and A(H3N2) strains were replaced in the 2010-2011 strains, they were replaced by previously circulating strains. The overwhelming majority of subjects in our study were vaccinated in the prior year. The strains were entirely preserved the following year for the 2010-11 season.

Therefore, either through immunization or natural infection, subjects were likely to have more cross-protective immunity for influenza A. The importance of preexisting immunity was underscored during the 2009 A(H1N1) pandemic when older adults were less severely affected than younger individuals [40, 41]. Young adults had little evidence of cross-reactive antibodies to the pandemic virus while a large proportion of older adults had preexisting cross-reactive immunity to the A(H1N1)pdm09 strain.

Our study had several limitations. This was a small sample size at a single institution and results may not be generalizable. Due to the small sample size we are unable to make any clinical inferences. While pre-vaccination titers correlate with post-vaccination titers, they do not inform us with regards to vaccine effects.

In conclusion, this study highlights the potential of preexisting immunity against influenza to mitigate the ill effects of immunosenescence associated with functional decline. The data underscores the range of factors that can alter post-vaccine titer to Influenza vaccine in older adults and underscores pre-vaccination titer, likely influenced by prior vaccination and antigen exposure history, as an important determinant of seroprotection.
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References:


Figure Legends

Figure 1A&B: Comparison of (A) Hemagglutination Inhibition (HI) and (B) Microneutralization (MN) post/pre vaccination geometric mean titer (GMT) ratios within frailty groups. No statistically significant differences as measured by One Way ANOVA were found between frailty groups.

Figure 2A&B: Correlation of preexisting immunity to post vaccination response as measured by (A) Hemagglutination Inhibition and (B) Microneutralization (MN) assays. Antibody levels were plotted within frailty groups, and correlations were calculated to determine the effect of preexisting on postvaccination antibody titers. The Spearman correlation coefficient and P value are presented for each frailty group.