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RESEARCH PAPER

The effect of frailty on HAI response to influenza vaccine among community-dwelling adults ≥ 50 years of age

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ABSTRACT

The immune response to vaccine antigens is less robust in older adults because of changes in the aging immune system. Frailty, the multi-dimensional syndrome marked by losses in function and physiological reserve, is increasingly prevalent with advancing age. Frailty accelerates this immunosenescence but the consequence of frailty on immune response specific to influenza vaccine among older adults, is mixed. An observational, prospective study of 114 adults was conducted in the fall of 2013 to assess the association of physical frailty with immune response to standard dose influenza vaccine in community-dwelling adults ≥ 50 years of age. Participants were stratified by age (<65 years and ≥65 years), and vaccine strain (Influenza A/H1N1, A/H3N2 and B) was analyzed separately adjusting for body mass index (BMI) and baseline $\log_2$ hemagglutination inhibition (HAI) titers. Overall, immune responses were lower among those ≥65 years of age than those <65 years. Among those ≥65 years there were no significant differences between frail and non-frail individuals in seroprotection or seroconversion for any influenza strain. Frail individuals <65 years of age compared with non-frail individuals were more likely to be seroprotected and to seroconvert post vaccination. Linear regression models show the same pattern of significant differences between frail and non-frail for those <65 years but no significant differences between frailty groups for those ≥65 years. Additional research may elucidate the reasons for the differences observed between younger frail and non-frail adults.

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Introduction

The burden of annual influenza is substantial. Approximately 226,000 hospital admissions[1] and 19,100 deaths[2] are attributed to influenza each year in the United States (U.S.); 90% of deaths occur among adults 65 years of age and older.[3] Annual influenza epidemics are estimated to result in: 3.1 million hospitalized days, 31.4 million outpatient visits, $10.4 billion in direct medical costs and $16.3 billion in lost earnings.[4] Thus, influenza vaccination is recommended for everyone ≥6 months of age.[5]

Older adults are less able to mount a robust immune response to antigens present in vaccines because of age-related changes in the immune system. For example, studies have shown lower humoral and cell-mediated immune system responses specific to influenza vaccination in older adults compared with younger adults.[6] Moreover, antibody responses to influenza vaccine in older adults is associated with altered T-cell function and an overall decline in cell-mediated adaptive immunity response.[7] Advancing age is also associated with increasing prevalence of frailty, the multi-dimensional syndrome marked by losses in function and physiological reserve.[8] Frailty has been shown to accelerate immunosenescence, such that individuals determined to be frail have been shown to mount lower immune responses to antigen stimulation.[6] Physical frailty, characterized by diminished strength, endurance, and reduced physiologic function,[9] leads to increased risk of acute illness, falls, disability, hospitalization, institutionalization and mortality.[8,10]

Relatively few studies of influenza vaccine immunogenicity among frail older adults exist. We found only two studies that specifically measured the impact of frailty on immune response to influenza vaccine using physical frailty measures. One study demonstrated that physical frailty is associated with lessened immunological response to influenza vaccine and greater influenza-like illness among community-dwelling adults >70 years of age.[11] The other found no difference in post-vaccination geometric mean titer ratios between frail and non-frail groups of veterans aged ≥62 years (mean age = 81 years).[12] To our knowledge, similar studies of adults younger than 65 years of age have not been conducted. The purpose of this study was to examine the effect of physical frailty on immune response to influenza vaccine in community-dwelling adults ≥50 years of age and determine if those responses differed by age.

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Table 1. Demographics overall and by frailty status stratified by age groups[‡]

Characteristics	< 65 years (N = 66)			≥ 65 years (N = 40)			
	Frail (N = 29)	Non-frail (N = 37)	*P* value[a]	Frail (N = 18)	Non-frail (N = 22)	*P* value[a]	Overall (N = 106)
Age, Median (q1, q3)	57.3 (54.4–61.8)	58.3 (56.1–61.0)	0.28	70.5 (68.1–74.0)	68.0 (66.6–73.6)	0.24	62.3 (57.3–67.6)
White race, N (%)	15 (52)	19 (51)	0.98	12 (67)	13 (59)	0.75	59 (56)
Non-Hispanic, N (%)	29 (100)	36 (97)	1.00	17 (94)	21 (95)	1.00	103 (97)
Female, N (%)	23 (79)	28 (76)	0.78	12 (67)	17 (77)	0.50	80 (75)
Diabetic, N (%)	11 (38)	12 (32)	0.64	7 (39)	6 (27)	0.44	36 (34)
BMI, N (%) ≥30 (obese)	21 (73)	18 (49)	0.05	12 (67)	9 (41)	0.13	57 (55)
Current smokers, N (%)	11 (38)	7 (19)	0.09	3 (18)	2 (9)	0.64	23 (22)
Socioeconomic Scale, Median (q1, q3)[b]	5 (3–6)	5 (5–7)	0.02*	5 (4–7)	6 (5–7)	0.34	5 (4–7)
EQ VAS Health Scale, (median split), N (%)[c] ≥80% at baseline	10 (36)	23 (62)	0.03*	7 (41)	15 (68)	0.09	55 (53)
Perceived Stress Scale, Median (q1, q3)	5 (2–7)	4 (1–7)	0.27	2 (2–5)	2 (0–4)	0.18	3 (1–6)
PHQ-9 Depression, N (%) Mild to severe depression (score 10–27)	14 (48)	11 (30)	0.12	5 (29)	4 (18)	0.47	34 (32)
Frailty Items, Median (q1, q3)[d]							
Grip Strength T-score, M(SD)	48.7 (11.9)	51.8 (8.8)	0.22	50.6 (12.2)	58.5 (11.7)	0.05	52.2 (11.3)
SF-12 Vitality T-Score	49.1 (39.2–49.1)	58.9 (49.1–58.9)	<.0001	49.1 (39.2–49.1)	58.9 (49.1–58.9)	<.0001	49 (49–59)
SF-12 Physical Functioning T-Score	41.3 (33.5–41.3)	49.2 (49.2–57.1)	<.0001	41.3 (33.5–41.3)	57.1 (49.2–57.1)	<.0001	49 (41–57)
SF-12 PCS T-Score	38.3 (36.2–41.1)	51.8 (45.9–57.0)	<.0001	37.8 (31.2–41.0)	52.1 (50.8–56.7)	<.0001	46 (39–53)

[‡]Numbers may not add to 100% due to rounding
[a]*P* values for tests: Chi-square/Fisher's Exact for categorical variables, Anova/Kruskal Wallis for continuous variables
[b]Socioeconomic scale range is 1–9 where: 1 = Worst off, 5 = Middle, and 9 = Best off
[c]EQ-VAS Health scale range is 0–100 where 0 = Worst imaginable health state and 100 = Best imaginable health state (at baseline)
[d]Physical Frailty items: Grip strength (weakness), SF-12: Vitality scale (exhaustion), Physical Functioning scale (walking time), Physical Component Summary score (physical activity health)
*significant at *P* value <0.05.

Results

Of the 114 enrolled, 8 participants were missing ≥2 of the frailty indicator components, leaving a total sample size for analysis of 106. Characteristics of the participants are presented in Table 1. Overall, participants were predominantly female (75%), White (56%), had a median age of 62.3 years (57.3–67.6), self-reported a median SES score of 5 (4–7), 53% reported baseline health at ≥80%, indicated average levels of stress (median 3), and 68% had low levels or no depressive symptoms. Thirty-four percent of the cohort were diabetic and over half (55%), were obese. Frail and non-frail participants ≥65 years did not differ in demographic or health characteristics. Conversely, among those <65 years of age, frail individuals as compared with non-frail individuals reported a significantly lower health state (64% <80% vs. 38% <80%) and lower SES (5; 3–6 vs. 5; 5–7).

Table 2 shows the percent of individuals in each frailty category by age group who seroconverted (top), were seroprotected at baseline and Day 21 (middle), as well as Geometric Mean Titers (GMTs) for each group (bottom). There were no significant differences between frail and non-frail individuals who were ≥65 years old.

Among those <65 years of age, statistically significant differences were seen between the frailty categories. Notably, a greater percent of frail persons as compared to non-frail seroconverted to the A/H1N1 (34% vs. 8%, $P = 0.008$) and A/H3N2 (59% vs. 22%, $P = 0.002$) vaccine strains. Higher percentages of being seroprotected at Day 21 for each vaccine strain were evident for frail persons as compared to the non-frail in this age category and these differences were statistically significant for each strain (A/H1N1: 79% vs. 40%, $P = 0.002$; A/H3N2: 86% vs. 62%, $P = 0.03$; B: 90% vs. 68%, $P = 0.04$). At Day 21, GMTs were higher for the frail compared with the non-frail; these between-group differences were significant for A/H1N1 13.0 vs. 4.8, $P < 0.001$) and A/H3N2 (14.0 vs. 6.8, $P = 0.01$) vaccine strains.

Table 3 provides logistic regression results for the outcome of seroprotection 21 days post vaccination for each vaccine strain. Among persons <65 years of age, frailty was positively associated with post-vaccination seroprotection with frail individuals having greater odds of being seroprotected than non-frail individuals, adjusting for obesity and baseline $\log_2$ HAI titers. This was significant after multiple comparison adjustment for the A/H1N1 strain (Odds Ratio (OR): 8.79, 95% Confidence Intervals (CI): 1.78–43.31, $P = 0.008$). Adjusting for multiple comparisons, the overall effect of frailty on post-vaccination seroprotection levels did not vary by age group.

Logistic regression models for the outcome of seroconversion for each vaccine strain are shown in Table 3. Frailty was positively associated with seroconversion among those <65 years of age with frail individuals having greater odds of seroconverting than non-frail persons. This was significant after multiple comparison adjustment for the A/H3N2 (OR: 5.85, 95% CI: 1.86-18.40, $P = 0.003$) vaccine strain. The overall effect of frailty on seroconversion status varied by age group and was significant after multiple comparison adjustment for the A/H3N2 ($P = 0.005$) vaccine strain.

Linear regression models for the outcome of log2 HAI titers each vaccine strain are shown in Table 3. Among those <65 years of age, frail individuals as compared to non-frail persons had small, (approximately 1/2 fold) but significant (using multiple comparison adjustment) increases in HAI post-vaccination titer levels for A/H1N1 and A/H3N2 vaccine strains (beta 0.55, $P < 0.001$ and beta 0.50, $P = 0.005$, respectively). The effect of frailty on post-vaccination HAI titers varied by

Table 2. Hemagglutination-inhibition responses to 2013–2014 vaccine strains, overall and by frailty status stratified by age groups

	< 65 years (N = 66)			≥ 65 years (N = 40)			
Immunological response measure	Frail (N = 29) No. (%)	Non-frail (N = 37) No. (%)	*P* value[a]	Frail (N = 18) No. (%)	Non-frail (N = 22) No. (%)	*P* value[a]	Overall (N = 106) No. (%)
Seroconversion (4-fold rise at Day 21)							
H1N1 A/California/07/2009	10 (34)	3 (8)	0.008*	5 (28)	6 (27)	1.00	24 (23)
H3N2 A/Texas/50/2012	17 (59)	8 (22)	0.002*	2 (11)	7 (32)	0.15	34 (32)
B-Yamagata lineage B/Massachusetts/2/2012	11 (38)	8 (22)	0.15	5 (28)	7 (32)	0.78	31 (29)
Seroprotection Day 0 (HI titer ≥ 1:40)							
H1N1 A/California/07/2009	9 (31)	12 (32)	0.90	4 (22)	7 (32)	0.72	32 (30)
H3N2 A/Texas/50/2012	8 (28)	13 (35)	0.51	4 (22)	3 (14)	0.68	28 (26)
B-Yamagata lineage B/Massachusetts/2/2012	11 (38)	13 (35)	0.81	8 (44)	7 (32)	0.41	39 (37)
Seroprotection Day 0 (HI titer ≥ 1:40)							
H1N1 A/California/07/2009	23 (79)	15 (40)	0.002*	8 (44)	14 (64)	0.22	60 (57)
H3N2 A/Texas/50/2012	25 (86)	23 (62)	0.03*	9 (50)	12 (55)	0.77	69 (65)
B-Yamagata lineage B/Massachusetts/2/2012	26 (90)	25 (68)	0.04*	12 (67)	17 (77)	0.50	80 (75)
Immunological response measure	Frail (N = 29) Mean (95% CI)	Non-frail (N = 37) Mean (95% CI)	*P* value[a]	Frail (N = 18) Mean (95% CI)	Non-frail (N = 22) Mean (95% CI)	*P* value[a]	Overall (N = 106) Mean (95% CI)
Geometric Mean Titers D0							
H1N1 A/California/07/2009	4.3 (2.8–6.6)	3.2 (2.2–4.6)	0.29	2.1 (1.4–3.1)	2.9 (1.8–4.6)	0.28	3.2 (2.6–3.9)
H3N2 A/Texas/50/2012	3.4 (2.5–4.6)	3.4 (2.5–4.6)	1.00	3.6 (2.4–5.4)	2.5 (1.7–3.6)	0.22	3.2 (2.7–3.8)
B-Yamagata lineage B/Massachusetts/2/2012	4.0 (2.8–5.7)	4.0 (2.7–5.9)	1.00	3.9 (2.3–6.3)	4.3 (2.8–6.5)	0.76	4.0 (3.3–4.9)
Geometric Mean Titers D21							
H1N1 A/California/07/2009	13 (8.4–19.8)	4.8 (3.6–6.6)	<.001*	5.9 (3.5–10.0)	6.4 (3.9–10.5)	0.81	6.9 (5.6–8.6)
H3N2 A/Texas/50/2012	14 (9.7–19.9)	6.8 (4.6–10.0)	0.01*	6.1 (3.7–10.0)	6.6 (4.4–9.9)	0.80	8.1 (6.5–10.0)
B-Yamagata lineage B/Massachusetts/2/2012	13 (9.1–18.2)	9.0 (6.3–12.8)	0.16	7.7 (4.5–13.3)	10.3 (7.3–14.5)	0.36	9.9 (8.2–12.1)

Seroconversion: 4-fold rise in post vaccination titer at Day 21 given Day 0 titer ≥ 10; Seroprotection: HI titer ≥ 40;
a- *P* value for tests: Chi-square/Fisher's Exact test (Seroconversion and Seroprotection D0 and D21); T-test (Geometric Mean Titers);
*significant at *P* value <0.05

age and was significant after multiple comparison adjustment for the A/H3N2 ($P = 0.002$) vaccine strain.

Although greater than half of our cohort was considered to be obese, obesity was only a nominally significant predictor of being seroprotected post vaccination for the A/H1N1 vaccine strain ($P = 0.02$) for those <65 years of age. Socioeconomic status (SES) and baseline health among frail persons <65 years of age not significantly associated with immune system outcomes to influenza vaccine, nor did they substantially change frailty estimates (data not shown). There was no evidence of an interaction between the predictor frailty and obesity or baseline $\log_2$ HAI titers for either post-vaccination seroprotection or seroconversion status.

All but one participant was known to have received the previous season's influenza vaccine. Data from the electronic medical record (EMR), indicated that 59% of frail adults <65 years of age had received at least two prior influenza vaccines and among non-frail adults <65 years, 65% had received at least two prior influenza vaccines. Among both frail and non-frail adults ≥65 years, 50% had received at least two prior influenza vaccines.

Discussion

To our knowledge, this is the first analysis conducted that assessed the effect of physical frailty on influenza vaccine immune response that includes community-dwelling persons younger than 65 years of age and the only study that stratifies these effects by age. One study which assessed vulnerability, a concept similar to frailty, among community-dwelling adults ≥50 years of age, found no consistent pattern of the effect of frailty on immunological response to the 2008–2009 influenza vaccine.[13] Vulnerability in their cohort was significantly associated with seroconversion for the A/H1N1 vaccine strain only; only 10% of the cohort had high vulnerability scores and models did not stratify by age.[13]

Frailty in adults ≥65 years of age has been shown to result in lower immunological responses to influenza vaccine compared to non-frail persons.[11] Interestingly, in our cohort, the opposite picture was seen for persons <65 years of age with frailty being a significant predictor of post-vaccination seroprotection status for the A/H1N1 vaccine strain, of seroconversion status for the A/H3N2 vaccine strain, of seroconversion status and of the A/H1N1 and A/H3N2 vaccine strains for $\log_2$ post-vaccination HAI titers.

Previously denoted as infirmity, frailty is now viewed as distinct from old age, disability and co-morbidity, although there is overlap among these categories.[14–16] Frailty is a multidimensional concept that involves a number of biological systems: nervous, endocrine, immune and musculoskeletal[17] and is marked by losses in function and strength.[8] The physical frailty definition is built around declines in mobility, strength, endurance, nutrition and physical activity.[8,10] Of our 4-item frailty measure, the greatest median difference for both age groups was seen in the SF-12 physical component score (PCS) which is a summary report of broad physical health status. Lower PCS scores indicate greater limitations in physical functioning and role participation caused by physical problems, poor general health and higher levels of bodily pain.[18]

Table 3. Multivariable Regression: Association of frailty (ref = non-frail) to 2013–2014 vaccine strains stratified by age group (adjusted for BMI and baseline log_2 titers)

Variable (frail vs. non-frail)	< 65 years (N = 66)			≥ 65 years (N = 40)			*P* value for age difference
	Odds Ratio	95% Confidence Interval	*P* value	Odds Ratio	95% Confidence Interval	*P* value	
Seroprotection at Day 21[a]							
H1N1 A/California/07/2009	8.79	1.78 – 43.31	0.008**	0.54	0.12 – 2.52	0.43	0.009*
H3N2 A/Texas/50/2012	6.00	1.25 – 28.75	0.02*	0.36	0.06 – 1.99	0.23	0.014*
B/Massachusetts/2/2012	7.79	1.15 – 52.61	0.03*	0.46	0.05 – 4.67	0.51	0.05
Seroconversion[b]							
H1N1 A/California/07/2009	6.86	1.55 – 30.37	0.011*	0.75	0.17 – 3.30	0.70	0.045*
H3N2 A/Texas/50/2012	5.85	1.86 – 18.40	0.003**	0.29	0.05 – 1.73	0.17	0.005**
B/Massachusetts/2/2012	2.68	0.81 – 8.78	0.107	0.61	0.14 – 2.68	0.51	0.221
Log_2 D21 HAI antibody titers[c]							
H1N1 A/California/07/2009	0.55	0.15	<0.001**	0.06	0.25	0.82	0.06
H3N2 A/Texas/50/2012	0.50	0.17	0.005**	−0.26	0.17	0.15	0.002**
B/Massachusetts/2/2012	0.29	0.14	0.05	−0.17	0.14	0.23	0.04*

*significant at *P* value <0.05

**significant after adjusting for multiple comparisons 0.05/6, *P* value 0.0083

[a]Logistic Regression: Event sizes for Seroprotecting at Day 21: H1N1 (N = 38 <65 years; N = 22 ≥65 years); H3N2: (N = 48 <65 years; N = 21 ≥65 years); B: (N = 51 <65 years; N = 29 ≥65 years)

[b]Logistic Regression: Event sizes for Seroconverting: H1N1 (N = 13 <65 years; N = 11 ≥65 years); H3N2: (N = 25 <65 years; N = 9 ≥65 years); B: (N = 19 <65 years; N = 12 ≥65 years)

[c]Linear regression equation: Log_2 D21 HAI titer = $B_0 + B_{1*Frail} + B_{2*BMI} + B_{3*log2\ baseline\ titer} + E$

The associations among stress, SES and health are robust; indeed, a primary explanation of the association between low SES and poor health is exposure to stress.[19] Stress is associated with worsening physical functioning, with decreased overall physical health and poor mental health.[20] Stress promotes immune dysfunction including increasing levels of inflammation and reducing the immune response to vaccines.[21–22] In turn, chronic levels of circulating pro-inflammatory cells negatively affect both innate and adaptive immune response[23] and certain pro-inflammatory cytokines (e.g., IL-6 and TNF-α) have been associated with increased risk of frailty.[23–24] Surprisingly, the significantly lower levels of self-reported SES and baseline health in our cohort among frail persons <65 years of age was not significantly associated with immune system outcomes to influenza vaccine nor did they substantially change frailty estimates (data not shown).

Higher levels of chronic inflammation have also been associated with increased adipose tissue.[25] Obesity in middle-age adults has been shown to initially result in higher fold increase of antibodies to influenza vaccine but to decline substantially with a 3–4 fold reduction in antibody response within 12 months of vaccination.[26] Since the pandemic influenza outbreak in 2009, obesity has been considered an independent risk factor for both increased influenza-related morbidity and mortality.[26] Obesity has also been linked to frailty.[9,24,27] Obesity was accounted for in each model; there was no evidence of effect modification of obesity on frailty (results not shown).

Strengths and limitations

We used a 4-item physical frailty score. It is possible that our frailty sample size would have been higher for one or both age groups had a fifth frailty item been included, as has been noted in other research.[28]

The relatively small size of the sample was a limitation as it prevented the inclusion of all potential confounders in the final analyses and may have reduced power to detect significant differences in sample characteristics between the frail and the non-frail especially in the age-stratified analyses. It is also possible that the absence of a significant moderating effect of obesity on the association of frailty with the outcomes was due to small numbers of obese and frail individuals within each age group. Sensitivity analyses including confounders of sex, race, smoking status, depression and baseline health found no evidence of effect modification of these covariates on frailty status for either age category nor were there significant changes with these included covariates on frailty estimates.

The factors that may limit the generalizability of these results are also its strengths. Although the racial distribution was dissimilar to the U.S. adult population, the large number of African-Americans in our sample demonstrated the ability to recruit and enroll minority populations in research studies and allowed us to assess racial differences in our outcomes. Furthermore, a larger proportion of the study group had diabetes or another chronic condition. While patients with diabetes were intentionally oversampled, the prevalence of diabetes and other high risk conditions among adults <65 years of age may have contributed to the relatively high prevalence of frailty observed.

Documented influenza vaccine receipt during one and two prior seasons was similar for both age categories of frail adults. Though not all older people become frail, research with larger sample sizes might allow for further examinations of frailty by age to understand why physical frailty seems to have a positive effect on immune response to influenza vaccine in adults 50–64 years of age, but not in those 65 and older, and to determine which specific factors may be driving this association.

Patients and methods

Study design and participants

This was an observational prospective study of adults ≥50 years of age who were recruited from three family practices and the University of Pittsburgh community during the 2013–2014 influenza season (September-November 2013) using

nonprobability convenience sampling. To be eligible, participants had to self-report prior season receipt of influenza vaccine, have no known egg allergies or Guillian Barré syndrome and not have already received but intended to receive the standard dose influenza vaccine for the current season. Participants were ineligible if they had an immunocompromising condition or were on immunosuppressant drugs, a history of allograft, or were cognitively impaired. Participants provided written informed consent prior to study initiation. The University of Pittsburgh Institutional Review Board approved this study.

Data collection

Baseline data were collected via interview with direct entry by the research assistants into REDCap™ (a secure, online database management system).[29] Baseline demographics included sex, race, ethnicity, self-reported age, presence (yes/no) and type (1 vs. 2) of diabetes, and smoking status. Height and weight from the EMR if available, or from self-report were used to calculate BMI. BMI was calculated as [weight (lb.) ÷ (height (in.)^2 X 703]; categorical obesity was defined as BMI $\geq$30. Questions on depression (9-item Patient Health Questionnaire), stress (4-item Perceived Stress Scale), SES (MacArthur Scale of Subjective Social Status, scored 0 low to 9 high) and overall health state (EQ-5D VAS, scored 0 low to 100 high) were also obtained at baseline.

Frailty

Physical frailty was measured at the Day 21 post-vaccination visit using a 4-item summed frailty score based on weakness, self-reported exhaustion, walking time and physical activity. Grip strength measured weakness, using a Layfayette hydraulic hand dynamometer (Model J00105, Lafayette Instrument Company, Lafayette, IN). Three measurements were taken on each hand while the participant was seated with his/her elbow flexed at 90° and shoulder adducted and neutrally rotated with the forearm and wrist held in a neutral position.[30] The average of these measurements for each side was then calculated; grip strength values for each side were age- and gender- adjusted to U.S. norms.[31]

The Short-Form Survey-12 (SF-12) (version 2, 4-week recall) was used to assess exhaustion (vitality scale), walking time (physical function scale) and physical activity (physical component summary score). Use of this instrument for these physical frailty components has been demonstrated in a systematic review of modifications to Fried et al's. (2001) frailty phenotype.[28,32] Each of the SF-12 frailty components was adjusted to U.S. population norms[33] using QualityMetric Health Outcomes™ 4.5 Scoring Software (Lincoln, RI).

The four frailty components were used as T-scores. Scores for any of the four components at or below the 25th percentile for this cohort were determined to be a deficit.[10] A 2-level categorical frailty variable was created by counting the number of deficits across the four components, with <2 deficits indicating non-frailty and $\geq$2 deficits indicating frailty. Missing values were allowed for one frailty component and were imputed with zero;[10,14] participants with two or more missing frailty components were dropped from analysis.

Biological samples and laboratory methods

Non-fasting whole blood samples were obtained on participants at baseline (pre-) and 21 days post influenza vaccination (range 19–35 days) using serum tubes with clot activator and silicone coated interior additive (BD Vacutainer, REF 367820) and held at room temperature until centrifugation to separate serum. Aliquoted serum samples were frozen at -80°C until assayed.

Prior to testing, sera were treated with receptor-destroying enzyme (RDE) (Denka Seiken, Co, Japan) adding three parts of RDE to one part of sera with overnight incubation at 37°C. RDE-treated sera were serially diluted in phosphate-buffered saline (PBS) two-fold across using V-shaped 96-well bottom microtiter plates. RDE was inactivated by incubation at 56°C for 30–45 min and then cooled to room temperature before being diluted with 1x PBS or 0.85% NaCl to a final sera concentration of 1:10.

Following Centers for Disease Control and Prevention (CDC) standardized protocols, sera were tested in HAI assays against each vaccine strain included in the 2013–2014 influenza vaccine, measuring the ability of antibodies to inhibit 100% agglutination of hemagglutinin to 0.8% turkey erythrocytes in PBS (Lampire Biologicals, Pipersville, PA, USA). 25 μl of each vaccine strain adjusted to ~8 HAI units/50 μl were added to each well. HAI titers were the reciprocal dilution of the last well that contained inhibited agglutination; all tests were conducted in duplicate. Positive and negative serum controls were included in each plate. Reference sera used came from Fluzone vaccine (Sanofi Pasteur) or the 2009 pandemic H1N1 FluMist vaccine (MedImmune).

Outcome measures were $\log_2$ GMTs, seroprotection and seroconversion. Seroprotection was defined as an HAI titer $\geq$1:40 at Day 0 and at Day 21. Seroconversion was defined as a 4-fold rise in HAI titer post-vaccination given a pre vaccination of $\geq$10.

Influenza vaccine

After the blood draw at the baseline visit, all participants received an intramuscular injection of the 2013–2014 seasonal trivalent influenza vaccine containing influenza strains A/H1N1/California/7/2009-pdm09-like virus, A/H3N2/Texas/50/2012-like virus and B/Massachusetts/2/2012-like virus. All but two participants received one 0.5 mL dose of one of five lot numbers of Fluzone influenza vaccine manufactured by Sanofi Pasteur. One participant received one 0.5 mL dose of Afluria and the other received one 0.5 mL dose of Fluvirin influenza vaccine.

Statistical analyses

All analytical procedures were performed using SAS® 9.3 (Cary, NC). Due to the skewness of the HAI titers at Day 0 and Day 21 they were transformed using the $\log_2$ method. GMTs were computed by first calculating the means and 95% CIs of the $\log_2$ HAI titers for each time point and then calculating the anti-log of those values.

Summary statistics of demographics and immunological response (seroconversion, seroprotection, GMTs) were conducted across all participants and by frailty status within age groups (<65 years and ≥65 years) using Chi-square/Fisher Exact tests for categorical variables and ANOVA/Kruskal Wallis tests for continuous variables. Proportions are reported for categorical variables and means and standard deviations or median and quartiles one and three are reported for continuous variables.

Differences in rates of seroconversion and seroprotection within age groups by frailty status were tested using Chi-square tests. Differences in GMTs within age groups by frailty status were tested using t-tests.

Logistic regression (seroconversion and seroprotection at Day 21) and linear regression (log_2 transformed Day 21 antibody titers) models run separately by age group for each vaccine strain and by each outcome assessed the association of frailty with immunological response to influenza vaccination. Adjustment covariates were added to models based upon their univariate relationship to the outcomes, their effect on frailty estimates, and those noted to be associated with frailty. Initial models adjusted for sex, race, smoking status, obesity, depression, baseline health status (characteristics noted to be associated with frailty), SES and baseline HAI titers.

Due to small event sizes for the outcomes, covariates that were non-significant ($P > 0.05$) and showed no evidence of effect modification on frailty, no substantial change to frailty P-value estimates and no substantial change in overall model fit statistics, were removed from the final models. Emphasis was put on creating parsimonious models that were consistent across strains and outcomes. Final models were adjusted for BMI and baseline HAI titers.

Statistical significance of two-sided tests was set at type I error (alpha) equal to 0.0083 (0.05/6) after adjusting for multiple comparisons using Bonferroni correction. Nominal P-values of <0.05 are also reported.

Disclosure of potential conflicts of interest

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Author contributions

Study concept and design: MPN, RKZ, TMR. Acquisition of participants and data: KKM, MS, SS, CEC, TMR. Data analysis and interpretation: KKM, CJL, MB, MPN, RKZ. Preparation and critical review of manuscript: KKM, MPN, RKZ, MB, CJL, JB, AMK, TMR.

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