Frailty Is Associated With Increased Hemagglutination-Inhibition Titers in a 4-Year Randomized Trial Comparing Standard- and High-Dose Influenza Vaccination

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Background. Although high-dose (HD) vaccines have been reported to stimulate higher antibody responses compared with standard-dose (SD) influenza vaccines, there have been limited studies on the impact of frailty on such responses.

Methods. We conducted a randomized, double-blind trial (2014/2015 to 2017/2018) of SD versus HD trivalent split-virus vaccine (Fluzone) in 612 study participants aged 65+ over 4 influenza seasons. Hemagglutination inhibition antibody titers for influenza H1N1, H3N2, and B vaccine subtypes were measured at baseline and at 4, 10, and 20 weeks postvaccination and frailty was measured using a validated frailty index.

Results. Geometric mean antibody titers were significantly higher in HD compared with SD vaccine recipients for all influenza subtypes at all time points postvaccination. However, frailty was positively correlated with 4-week titers and was associated with increased odds of being a vaccine responder. For influenza A subtypes, this was mostly limited to HD recipients.

Conclusions. Frailty was associated with higher titers and increased antibody responses at 4 weeks after influenza vaccination, which was partially dependent on vaccine dosage. Chronic inflammation or dysregulated immunity, both of which are commonly observed with frailty, may be responsible, but it requires further investigation.

Keywords. frailty; antibody; influenza; aging; vaccination.

Influenza is an important threat to the health of older adults. In the United States and Canada, the majority of hospitalizations and deaths due to influenza occur in adults aged 65 years and older [1]. Vaccination is often described as the cornerstone for prevention of influenza, and systematic reviews suggest that the protective efficacy of influenza vaccination is approximately 60%; however, estimates vary depending on the subtype [2, 3] and decline to approximately 30% in older adults [4]. High-dose (HD) influenza vaccine has been shown to reduce influenza illness rates by 24% [5], but there have been limited studies on the effect of frailty on antibody responses to vaccination as a correlate of protection in the older population.

People aged 65 years and over are by no means a homogeneous group, varying by functional status, number of chronic conditions, and degree of frailty. Frailty has been defined as a state of increased vulnerability to adverse health outcomes due to a decline in reserve and function across multiple physiologic systems; hence, the ability to cope with acute or chronic stressors is compromised [6]. It has been estimated that 15% of community- and residential care-dwelling adults 65 and older in the United States are frail, and 45% are prefrail according to Fried's frailty phenotype model; of those who are frail, approximately half were hospitalized in the previous year [7]. In Canada, 24% of community-dwelling older adults aged 65 and older are considered frail as measured using a Frailty Index [8].

Although influenza vaccine immunogenicity has been reported in older adults [9–12], less is known regarding the impact of frailty on vaccine-induced antibody production in older adults. Studies are varied, with some reporting no significant impact of frailty on influenza vaccine antibody response [13–15], whereas others suggest that antibody response is increased [16] or decreased [17] with frailty. Given the relationship between frailty and age-associated immune decline [18], precipitated in part by age-related chronic inflammation [19, 20], improving our understanding of the impact of frailty on the antibody response to influenza vaccination would inform both clinical care and underlying pathophysiological mechanisms.

In order to assess the impact of frailty on antibody production after vaccination, we used data from a randomized trial comparing HD to standard-dose (SD) influenza vaccine in

older adults conducted over 4 influenza seasons; preliminary results from the first year have previously been reported. [21]. To our knowledge, only 2 other studies comparing SD and HD influenza vaccine have reported on the impact of frailty on antibody responses: one in the community [14] and the other in a residential care setting [22].

METHODS

Study Design

This study was conducted to compare the immunogenicity of an HD versus SD formulation of trivalent split-virus influenza vaccine in community-dwelling older adults using hemagglutination inhibition antibody (HAI) titers. A double-blind, rerandomization design (ie, participants enrolled in previous years were eligible for enrollment in subsequent years) was used, in which antibody titers to each of the vaccine subtypes prevaccination and at 4, 10, and 20 weeks postvaccination were measured over 4 influenza seasons (October 2014–April 2015, October 2015–April 2016, October 2016–April 2017, and October 2017–April 2018). Hence, a pool of 246 unique participants were reenrolled and rerandomized to SD or HD each season (for years 1–4: 106, 175, 174, and 157, respectively) (Table 1) for a total of 612 study participants over the 4 seasons (Figure 1). Not all participants took part in the trial every season, and new participants were recruited for years 2–4. The study protocol was approved by the Institutional Review Board of the University of Connecticut Health Center (UCHC) and the Health Sciences North Research Ethics Board (Sudbury, ON,

Canada) and registered at ClinicalTrials.gov (NCT02297542). All study participants provided written informed consent to participate in the study.

Sites and Study Participants

Older adults (age 65 years and older) were recruited through the UConn Center on Aging Recruitment Core from the communities belonging to and surrounding Hartford, Connecticut, and through the Health Sciences North Research Institute (HSNRI) from the community of Greater Sudbury, Ontario, Canada. Inclusion criteria included the following: at least 65 years old and vaccinated in the previous influenza season. Exclusion criteria included the following: known immunosuppressive disorders or medications including prednisone in doses >10 mg/day, a previous severe reaction to the vaccine, egg, latex, or thimerosol allergies, or refusal of vaccination. Research coordinators ensured that vaccinations were scheduled at least 2 weeks after any acute respiratory illness.

Randomization and Blinding

Study participants were randomized to the HD (60 µg of subtype-specific hemagglutinin [HA]; ie, 180 µg total) or SD (15 µg of subtype-specific HA; ie, 45 µg total) vaccination group in the fall of each year with rerandomization of those who had participated in the previous year. Randomization was computer generated as a 1:1 allocation to the 2 vaccine groups at each of the 2 study sites. The vaccine was administered by a nurse not involved in the study. Study staff including research coordinators and laboratory staff, investigators, and participants

Table 1.   Characteristics of Participants Randomized to Standard-Dose (SD) or High-Dose (HD) Vaccine[a]

Study factors			SD	HD	P Value
Age			77 ± 7.3 (65–96)	77 ± 7.7 (65–97)	.53
Body mass index (BMI)			28 ± 5.1 (15–48)	28 ± 4.6 (17–40)	.24
Sex	Female		204 (64.6%)	206 (69.6%)	.22
	Male		112 (35.4%)	90 (30.4%)	
Year	1 (2014–2015)		53 (16.8%)	53 (17.9%)	.95
	2 (2015–2016)		90 (28.5%)	85 (28.7%)	
	3 (2016–2017)		89 (28.2%)	85 (28.7%)	
	4 (2017–2018)		84 (26.6%)	73 (24.7%)	
Site	HSNRI		187 (59.2%)	169 (57.1%)	.66
	UCHC		129 (40.8%)	127 (42.9%)	
CMV serostatus	Negative		166 (52.5%)	121 (40.9%)	.005
	Positive		150 (47.5%)	175 (59.1%)	
Laboratory-confirmed flu	Negative		296 (93.7%)	286 (96.6%)	.13
	Positive		20 (6.3%)	10 (3.4%)	
Frailty index	Continuous variable		0.10 ± 0.07 (0–0.41)	0.11 ± 0.07 (0–0.39)	.12
	Categorical	Robust	166 (52.5%)	140 (47.3%)	.32
		Prefrail	120 (38%)	130 (43.9%)	
		Frail	29 (9.2%)	25 (8.4%)	
		Missing	1 (0.3%)	1 (0.3%)	

Abbreviations: CMV, cytomegalovirus; HSNRI, Health Sciences North Research Institute; UCHC, University of Connecticut Health Center.

[a]Age, BMI, and frailty index (continuous) are presented as mean ± standard deviation (minimum-maximum), and differences between SD and HD are estimated by t test. The remaining categorical variables are presented as count (frequency), and differences between SD and HD are estimated by χ^2 test.
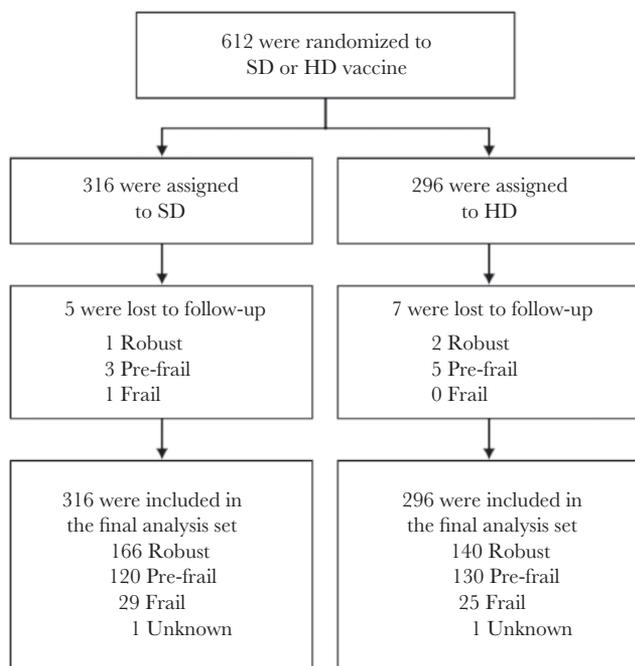
Figure 1. Consolidated Standards of Reporting Trials (CONSORT) diagram describing the enrollment of participants, allocation to treatment (standard dose [SD] or high dose [HD]), and loss to follow-up. Frailty groups were defined by a frailty index.

remained blinded in the study until all data entry for the study was completed and the database for each study year was locked.

Study Interventions

After informed consent, study participants were characterized according to demographic data (age, sex, and ethnicity), chronic medical conditions including risk factors for influenza illness (pulmonary, cardiac, metabolic, renal, or neoplastic disorders), health attitudes, symptoms, and functional impairments. A frailty index (FI) was calculated based on 40 items previously validated in outcomes of influenza [23–25], and using published cutoffs, participants were defined as frail (FI > 0.21), prefrail (0.1 < FI ≤ 0.21), and robust (FI ≤ 0.1) [26]. Blood samples were collected at the prevaccination and 4, 10, and 20 weeks postvaccination visits.

Influenza Surveillance

Influenza surveillance included weekly contact with study subjects to assess flu-like symptoms or acute respiratory infection (ARI), and it included nasopharyngeal swabs (within 5 days of onset of symptoms) for polymerase chain reaction (PCR) detection of influenza virus and postinfluenza season detection of an antibody response to influenza infection. Routine screening for symptoms of ARI also occurred at the 4-, 10-, and 20-week visits when blood samples were collected. Influenza illness was documented by PCR detection of influenza during an ARI or seroconversion (4-fold rise in antibody titers) in association

with an ARI. This included upper (coryza or sore throat) or lower (cough or shortness of breath) respiratory tract symptoms, headache, malaise, myalgia, or fever (>99°F or 37.3°C orally or 100°F rectally) [27]. Hospitalizations and deaths attributed to acute cardiopulmonary illness were tracked through the influenza season.

Hemagglutination Inhibition Antibody Titers

Hemagglutination inhibition antibody titers were performed using previously described standard methods [28, 29]. Influenza subtypes used for HAI testing were as follows: Year 1, A/Texas/50/2012 (H3N2), A/California/7/2009 (H1N1), and B/Massachusetts/2/2012; Year 2, A/Switzerland/9715292-2013, A/California/7/2009 (H1N1), and B/Phuket/3073/2013; Year 3, A/Hong Kong/4801-2014 (H3N2), A/California/7/2009 NYNC X-179A (H1N1), and B/Brisbane/60/2008; and Year 4, A/HongKong/4801/2014 (H3N2), A/Michigan/45/2015 (H1N1), and B/Brisbane/60/2008. Laboratory testing was conducted after each study year, and participant serum was randomized before plating. Antibody responses were expressed as the 4-week postvaccination titer relative to prevaccination, and participants were categorized as responders if they exhibited a 4-fold difference.

Cytomegalovirus Serostatus

Cytomegalovirus (CMV) serostatus was determined in serum using the CMV IgG ELISA Kit (Genesis Diagnostics Inc., Cambridgeshire, UK) according to the manufacturer's instructions.

Statistical Analysis

To estimate the effect of baseline frailty on natural log-transformed HAI titers at 4 weeks postvaccination or the odds of a participant exhibiting at least a 4-fold rise in titers, we used generalized estimating equations, accounting for repeated participants across years; the regression coefficient or odds ratios (ORs) and 95% confidence intervals (CIs) were reported. Frailty was investigated both as a standardized continuous variable (ie, transformed to mean = 0, standard deviation [sd] = 1), and categorically, with robust used as the reference (ref). Analyses using minimal models (ie, log baseline titer adjusted for analyses involving log 4-week titers, univariate for analyses of the odds of a 4-fold response) were first conducted for frailty, age (per decile), sex (ref = female), study site (ref = HSNRI), dose (ref = standard), study year (ref = year 1), and CMV serostatus (ref = negative) for each influenza subtype. Multivariable analyses were then conducted by adjusting for all variables included in minimal model analyses (ie, for log 4-week titers, all covariates and log baseline titers; for the odds of a 4-fold response, all covariates); the decision to include all variables in the multivariable model was based on minimization of the quasi-likelihood under the independence model criterion (QIC).

A similar approach was used to estimate the effect of frailty on the difference in log-transformed titers between weeks 4 and 20. All analyses were conducted using R version 3.6.

RESULTS

Participants

A total of 612 study participants were recruited over 4 influenza seasons (106, 175, 174, and 157 participants, respectively) and randomized to HD or SD each year; they are described in Table 1. These participants were between 65 and 97 years old (mean, 77), 410 (67%) were female, 296 (48%) received the HD vaccine, and 356 (58%) were enrolled at the HSNRI, with the remainder enrolled at the UCHC. Laboratory-confirmed influenza was observed in 30 participants during the 4 years of surveillance (7, 6, 1, and 16, respectively), 20 of whom had received SD in the current season, and 10 who had received HD ($P = .13$). The mean FI across years was 0.11 ± 0.07 (range, 0–0.41), 54 (9%) participants were categorized as frail, and 325 (53%) participants were CMV positive.

High-Dose Vaccine Induced Significantly Higher Antibody Titers Over the Course of the Study

Geometric mean antibody titers (GMTs) were significantly higher in HD compared with SD vaccine recipients for all influenza subtypes, across all visits postvaccination, with exception to influenza B at week 20 ($P = .063$) (Table 2; Figure 2). Specifically, the GMT levels in HD and SD recipients at 4 weeks postvaccination (excluding those that later developed laboratory-confirmed influenza in that study year), respectively, were as follows: H1N1, 111 (95% CI, 98–125) and 68 (95% CI, 62–75); H3N2, 202 (95% CI, 177–232) and 123 (95% CI, 108–139); and influenza B, 92 (95% CI, 83–102) and 67 (95% CI, 60–73). At 20 weeks postvaccination, GMTs for HD recipients remained higher than SD recipients, and both SD and HD recipients were higher than their prevaccination levels (Table 2; Figure 2). Similarly, the proportion of participants who exhibited a 4-fold increase in antibody titers at 4 weeks was significantly higher in the HD group, regardless of subtype: H1N1, 35% vs 12%; H3N2, 49% vs 35%; and B, 30% vs 12% (Table 2).

Increased Frailty Is Associated With Enhanced Antibody Responses to Influenza Vaccination

Results from regression analyses with the outcomes of log antibody titers 4 week postvaccination and participants having at least a 4-fold increase in antibody titers are shown in Figure 3. In general, older age, male sex, UCHC study site, and CMV positivity were associated with reduced odds of a 4-fold response, although statistical significance varied by subtype; there

Table 2. Antibody Responses Against Influenza A (H1N1, H3N2) and B for Standard-Dose (SD) and High-Dose (HD) Recipients After Vaccination[a]

Viral type	Measure	Time point	SD	HD	P Value
H1N1	GMTs	Prevaccination	43 [39–47]	41 [36–45]	.46
		Week 4	68 [62–75]	111 [98–125]	<.001
		Week 10	55 [50–60]	81 [72–90]	<.001
		Week 20	55 [50–60]	69 [62–77]	.002
	4-fold change (0 to 4 weeks)				
		Yes	38 (12%)	103 (34.8%)	<.001
		No	274 (86.7%)	188 (63.5%)	
		Missing	4 (1.3%)	5 (1.7%)	
H3N2	GMTs	Prevaccination	45 [40–51]	51 [45–58]	.18
		Week 4	123 [108–139]	202 [177–232]	<.001
		Week 10	95 [84–107]	145 [126–167]	<.001
		Week 20	80 [70–90]	117 [103–133]	<.001
	4-fold change (0 to 4 weeks)				
		Yes	110 (34.8%)	145 (49%)	<.001
		No	202 (63.9%)	146 (49.3%)	
		Missing	4 (1.3%)	5 (1.7%)	
B	GMTs	Prevaccination	40 [36–44]	36 [33–39]	.13
		Week 4	67 [60–73]	92 [83–102]	<.001
		Week 10	54 [49–59]	67 [60–74]	.003
		Week 20	51 [46–56]	58 [52–65]	.063
	4-fold change (0 to 4 weeks)				
		Yes	39 (12.3%)	89 (30.1%)	<.001
		No	273 (86.4%)	202 (68.2%)	
		Missing	4 (1.3%)	5 (1.7%)	

Abbreviations: GMTs, geometric mean titers.

[a]For the calculation and comparison of GMTs, participants that developed influenza were removed; GMTs are reported as mean [95% confidence interval], and significance (P) was determined by t test. For 4-fold change, the count (frequency) of participants that exhibited a 4-fold or more increase in antibody titers from prevaccination (week 0) to 4 weeks postvaccination is reported; significance was determined by χ² test.
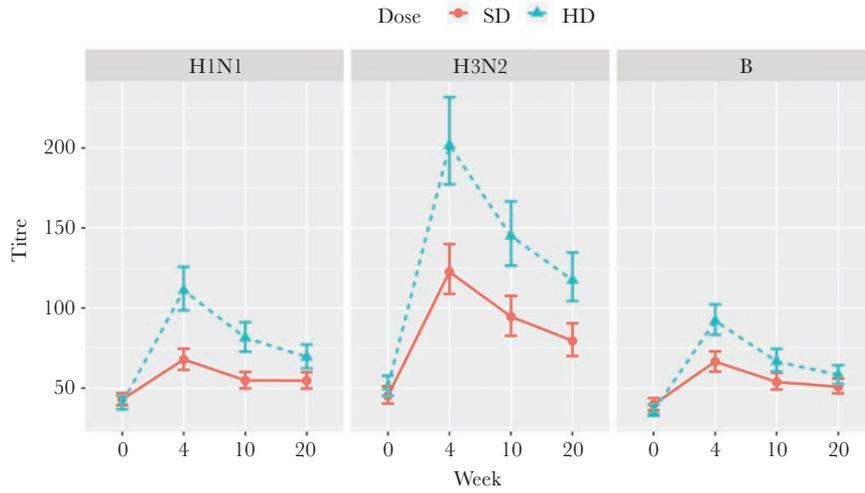
Figure 2. Comparison of influenza A (H1N1, H3N2) and B hemagglutination inhibition antibody titers prevaccination (week 0) and 4, 10, and 20 weeks postvaccination for participants randomized to either the standard-dose (SD) or high-dose (HD) vaccine. Participants who developed laboratory-confirmed influenza were not included.

was little difference in associations to 4-week titers (Figure 3A) or the odds of a 4-fold response (Figure 3C). Associations with antibody responses varied significantly between years, which was especially dependent on subtype, whereas HD vaccine

was associated with significantly increased 4-week titers (adjusted natural log titer: H1N1 = 0.52 [95% CI, 0.42–0.63], H3N2 = 0.39 [95% CI, 0.24–0.53], B = 0.38 [95% CI, 0.28–0.48]) (Figure 3A) and the odds of having a 4-fold response
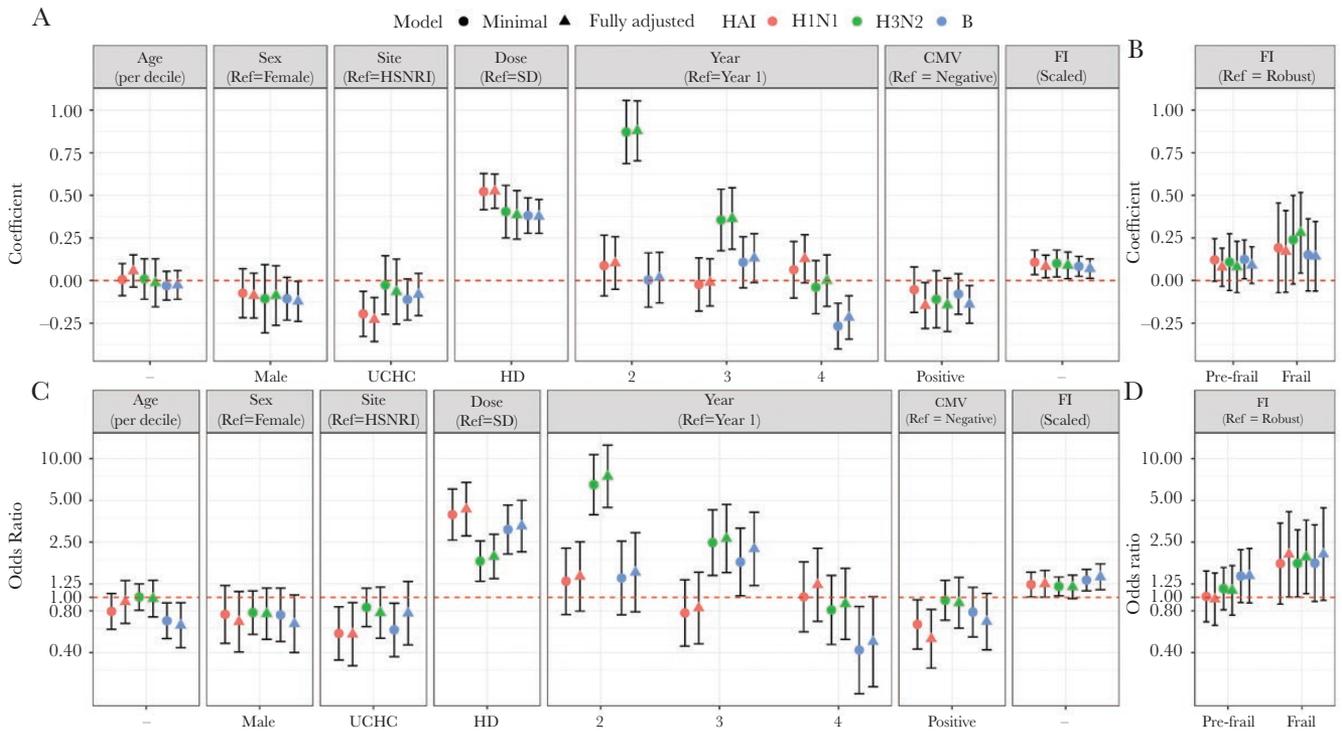
Figure 3. Regression analyses to estimate the effect of frailty (FI) and other factors on natural log-transformed in hemagglutination inhibition antibody (HAI) titers at 4 weeks postvaccination (A and B) and the odds of a 4-fold increase in titers (C and D). Specifically, generalized estimating equations were used to estimate the effect participant factors and frailty as a continuous variable (A and C) and frailty as a categorical variable (B and D). Circles denote the estimate from minimal models (for A/B, baseline log titer adjusted only; C/D, univariate analysis) and triangles denote the estimate in multivariable models, adjusting for age (by decile), sex, site, dose, year, cytomegalovirus (CMV) serostatus, and frailty, and for A and B, baseline log titer amounts as well. Influenza subtypes are denoted by color. Points and error bars represent the regression coefficient (A and B) or odds ratio (C and D) and 95% confidence interval. Reference categories are listed in the header for each variable, with remaining levels listed on the x-axis. HD, high dose; HSNRI, Health Sciences North Research Institute; SD, standard dose; UCHC, University of Connecticut Health Center.

(adjusted OR: H1N1 = 4.3 [95% CI, 2.8–6.7], H3N2 = 2.0 [95% CI, 1.4–2.8], B = 3.3 [95% CI, 2.1–5.0]) (Figure 3C), regardless of subtype.

When considered as a continuous variable, a 1-sd increase in frailty was associated with increased 4-week titers (adjusted natural log titer: H1N1 = 0.083 [95% CI, 0.017–0.149], H3N2 = 0.089 [95% CI, 0.011–0.168], B = 0.070 [95% CI, 0.013–0.127]) (Figure 3A) and a higher odds of a 4-fold response, regardless of subtype (adjusted OR: H1N1 = 1.25 [95% CI, 1.00–1.56], H3N2 = 1.19 [95% CI, 0.98–1.44], B = 1.41 [95% CI, 1.13–1.74]) (Figure 3C). This trend was similarly observed when frailty was categorized; however, statistically significant differences were only apparent when comparing robust and frail individuals, and this was not uniform across subtypes for either 4-week titers (adjusted natural log titer: H1N1 = 0.17 [95% CI, −0.069 to 0.410], H3N2 = 0.28 [95% CI, 0.044–0.517], B = 0.14 [95% CI, −0.062 to 0.347]) (Figure 3B) or the odds of a 4-fold response (adjusted OR: H1N1 = 2.05 [95% CI, 1.01–4.15], H3N2 = 1.96 [95% CI, 1.06–3.60], B = 2.05 [95% CI, 0.95–4.41]) (Figure 3D).

Although frailty was related to an increased fold-change response at week 4 relative to baseline, the decline in antibody levels from week 4 to week 20 was also greater. In a fully adjusted multivariable model, for every 1-sd increase in frailty, the natural log difference in HAI titers from week 4 to week 20 decreased, regardless of subtype: H1N1, 0.038 ± 0.020 (P = .053); H3N2, 0.055 ± 0.028 (P = .055); and B, 0.042 ± 0.20 (P = .035). To give these findings context, the average decline in antibody levels for H1N1, H3N2, and B was 0.33, 0.41, and 0.34 natural log units.

Associations With Frailty Appear to Be Limited to High-Dose Vaccine Recipients

Given the substantial impact that vaccine dosage has on antibody levels, we performed a stratified analysis to ascertain whether the effect of frailty differed between SD and HD recipients (Figure 4). For influenza A, the effect of a 1-sd increase in frailty on antibody responses was only apparent in HD recipients, more so when 4-week titers were considered (adjusted natural log 4-week titers: H1N1 = 0.16 [95% CI, 0.053–0.270], H3N2 = 0.13 [95% CI, 0.021–0.243]) (Figure 4A) than the odds of a 4-fold response (adjusted OR: H1N1 = 1.29 [95% CI, 0.98–1.69], H3N2 = 1.33 [95% CI, 1.00–1.78]) (Figure 4B); for SD recipients, this effect was negligible. However, for influenza B, the positive effect of frailty on the odds of a 4-fold response was not appreciably different between SD and HD recipients (adjusted OR: SD = 1.47 [95% CI, 1.01–2.14], HD = 1.39 [95% CI, 1.05–1.85]) (Figure 4B), whereas for the 4-week titer analysis, stratification resulted in the frailty effect being nonsignificant for both SD and HD recipients (Figure 4A).

DISCUSSION

In this analysis of data from a randomized trial of influenza vaccination in older adults, we found that HD vaccination resulted in significantly higher antibody titers and a greater number of participants exhibiting a 4-fold increase in titers at 4 weeks postvaccination, compared with SD vaccination. Furthermore, in contrast to our hypothesis, higher frailty was associated with increased antibody responses to influenza vaccination, although this depended on whether frailty was treated as a continuous or categorical variable during analysis. It is interesting to note that the relationship between frailty and antibody responses was only apparent in HD recipients for H1N1 and H3N2, whereas for influenza B, antibody responses increased with frailty for both SD and HD recipients. A recent study confirms the enhanced immunogenicity of HD vaccine over SD vaccine in the 2017–2018 influenza season, which was not demonstrated for influenza B strains. However, approximately one half of study participants were age 65–70 years old, and there were no measures of frailty included in this randomized trial [30].

With regards to the association between frailty and influenza vaccine antibody titers, previous studies have shown varied results. For example, a study of the 2014–2015 influenza season in Germany [13] found no difference between prefrail and frail participants in the HAI response to H1N1, H3N2, or B, as did another study of the 2011–2012 and 2012–2013 influenza seasons in the United States and Canada [14]; for both of these studies, frailty was considered categorically using Fried's
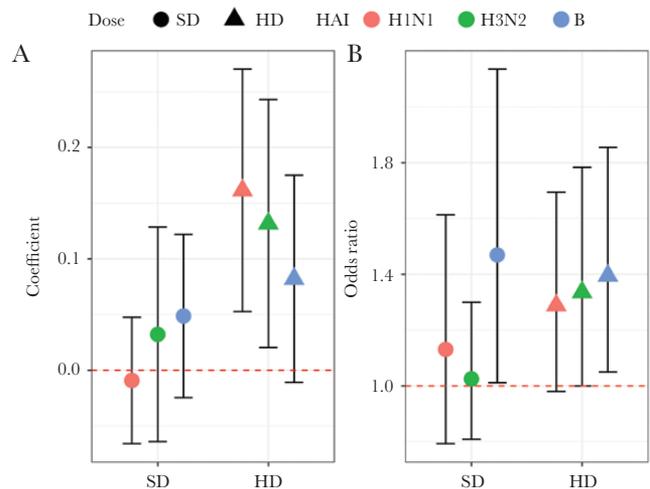
Figure 4. Comparison of the effect of frailty on the antibody response of standard and high dose recipients. Using generalized estimating equations, the effect of frailty as a continuous variable on natural log-transformed hemagglutination inhibition antibody (HAI) titers at 4 weeks postvaccination (A) and the odds of a 4-fold increase titers (B) was estimated for standard-dose ([SD] circles) and high-dose ([HD] triangles) recipients in separate models, adjusting for age, sex, site, dose, year, and cytomegalovirus serostatus, and for A, baseline log titer amounts as well. Influenza subtypes are denoted by color and points, and error bars represent the regression coefficient (A) or odds ratio (B) and 95% confidence interval.

phenotype model [31]. Likewise, a study of the 2006 to 2012 influenza seasons (excluding 2009–2010) in the United States found no significant difference among frail, prefrail, or robust participants, as determined using an FI [15]. In contrast, a study of community-dwelling seniors during the 2007–2008 influenza season in the United States found that frailty (measured using Fried's phenotype model) was associated with a reduction in the antibody response to vaccination [17]. Moehling et al [16], who studied the 2013–2014 influenza season in United States, found that no differences in the antibody response to vaccination were apparent across categories of a 4-item frailty score (ie, weakness, self-reported exhaustion, walking time, and physical activity) when all older adults were considered. However, after stratifying their cohort, they found that frail participants under age 65 were more likely to be seroprotected against influenza and seroconvert after vaccination, compared with nonfrail participants [16]. To our knowledge, this is the only study supporting a protective role of frailty in the generation of antibody titers against influenza after vaccination.

Our data suggest that the effect of frailty on influenza vaccine responses is distinct from the effect of advanced age; that is, frailty was found to be associated with increased antibody responses, regardless of subtype, whereas increasing age was more likely to be associated with reduced responses, as has been previously recognized [32]. In many ways, this is counterintuitive, because one would expect frailty to accelerate the effects of aging (ie, via immunosenescence) rather than contradict them; this is supported by recent work indicating that frailty correlates with reduced B-cell diversity [33]. However, one of the major pathophysiological components of frailty is chronic inflammation, one of the most prominent mediators being interleukin (IL)-6 [34]. We have shown previously that the addition of IL-6 to peripheral blood mononuclear cell cultures leads to enhanced T-cell responses after stimulation with live virus [35]. Frailty is also known to be associated with increased numbers of monocytes [36], and the chemokine MCP1 [37], both of which have been hypothesized to support antibody responses to influenza vaccination [38, 39]. Hence, a possible mechanism for our observations may be that the chronic inflammation that accompanies frailty induces a sort of adjuvant-type effect, resulting in increased antibody titers after vaccination. Why this occurs only in (1) HD recipients for influenza A subtypes and (2) both SD and HD for influenza B is unknown, but, clearly, further study into these phenomena are warranted.

Strengths of this study include the following: a multiyear, multisite, randomized trial platform; use of a validated and standardized approach to measuring frailty, expressed both as a continuous and categorical variable; and inclusion of multiple influenza subtypes. Our associations with frailty were statistically significant regardless of subtype, but the most robust associations were identified when frailty was considered as a continuous measure. This is a major difference from much of

the available literature, which tends to divide frailty into binary or ternary categories. Although a discretization or "binning" approach simplifies interpretation, it also reduces statistical power, and it may explain why the majority of studies that have investigated the relationship between frailty and vaccine antibody responses did not observe significant differences [40].

CONCLUSIONS

In summary, we found that frailty as measured using an FI was associated with an increased antibody response to influenza vaccination regardless of subtype, but this depended on vaccine dose. This interesting, yet somewhat counterintuitive, finding may be due to the altered immune and inflammatory profiles commonly observed with frailty, although this requires further investigation.

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