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 (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 thimerosal 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) Vaccinea |
|----------------------------------|---------|---------|-------------|
| Study factors                   | SD      | HD      | PValue      |
| 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         |
| Year                            | Male   | 112 (35.4%) | 90 (30.4%)  |             |
| 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.
aAge, 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 χ² test.
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/Phuket/3073/2013; Year 4, A/Hong Kong/4801/2014 (H3N2), A/Michigan/45/2015 (H1N1), and B/Brussels/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 $0.11 \pm 0.07$ (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).

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

| 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.

*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 $\chi^2$ test.
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 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.

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.
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.

Acknowledgments

We thank the clinical research teams at University of Connecticut Health Center and Health Sciences North Research Institute for their dedication to the study participants and diligence in coordinating the multiple components of this study protocol.

Financial support. This work was funded by the National Institute on Aging, National Institutes of Health (RO1 AG048023; to G. A. K., J. E. M., and coinvestigators). J. E. M. is supported by the Health Sciences North Volunteer Association Chair in Healthy Aging and G. A. K. is supported by the Travelers Chair in Geriatrics and Gerontology.

Potential conflicts of interest. J. E. M. reports grants from US National Institutes of Health, honoraria for participation in advisory boards from Sanofi, travel support from Sanofi, during the conduct of the study: participation in data monitoring board for GSK, from Pfizer, Merck, ResTORbio, and Medicago, and other from VBI and Janssen for clinical trial work, outside the submitted work. M. K. A. reports grants from Sanofi, GSK, and Pfizer, travel support from Sanofi and GSK, and honoraria from Sanofi and Pfizer, outside the submitted work. G. A. K. reports grants from the US National Institutes of Health and honoraria for participation in advisory boards from ResTORbio and Janssen. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Reed C, Chaves SS, Daily Kirley P, et al. Estimating influenza disease burden from population-based surveillance data in the United States. Plos One 2013; 10:e0118369.
2. Belongia EA, Simpson MD, King JP, et al. Variable influenza vaccine effectiveness by subtype: a systematic review and meta-analysis of test-negative design studies. Lancet Infect Dis 2016; 16:942–51.
3. Osterholm MT, Kelley NS, Sommer A, Belongia EA. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. Lancet Infect Dis 2012; 12:36–44.
4. Beyer WE, McElhaney J, Smith DJ, et al. Cochrane re-arranged: support for policies to vaccinate elderly people against influenza. Vaccine 2013; 31:6303–8.
5. Diazgranados CA, Dunning AJ, Kimmel M, et al. Efficacy of high-dose versus standard-dose influenza vaccine in older adults. N Engl J Med 2014; 371:635–45.
6. Dent E, Kowel P, Hoogendijk EO. Frailty measurement in research and clinical practice: a review. Eur J Intern Med 2016; 31:3–10.
7. Bangsbo-Roche K, Sephaki CL, Huang J, et al. Frailty in older adults: a nationally representative profile in the United States. J Gerontol A Biol Sci Med Sci 2015; 70:1427–34.
8. Hoover M, Rotermann M, Sammartin C, Bernier J. Validation of an index to estimate the prevalence of frailty among community-dwelling seniors. Health Rep 2013; 24:10–7.
9. Montomoli E, Torelli A, Manini I, Gianchecchi E. Immunogenicity and safety of the new inactivated quadrivalent influenza vaccine vaxigrip tetra: preliminary results in children ≥6 months and older adults. Vaccines 2018; 6:14.
10. Merani S, Pawelec G, Kuchel GA, McElhaney JE. Impact of aging and cytomegalovirus on immunological response to influenza vaccination and infection. Front Immunol 2017; 8:784.

11. Weinberger B, Grubeck-Loebenstein B. Vaccines for the elderly. Clin Microbiol Infect 2012; 18(Suppl 5):100–8.

12. Wilkinson K, Wei Y, Szwajcer A, et al. Efficacy and safety of high-dose influenza vaccine in elderly adults: a systematic review and meta-analysis. Vaccine 2017; 35:2775–80.

13. Bauer JM, De Castro A, Bosco N, et al. Influenza vaccine response in community-dwelling German prefrail and frail individuals. Immun Ageing 2017; 14:17.

14. DiazGranados CA, Dunning AJ, Robertson CA, et al. Efficacy and immunogenicity of high-dose influenza vaccine in older adults by age, comorbidities, and frailty. Vaccine 2015; 33:4865–71.

15. Talbot HK, Nian H, Chen Q, et al. Evaluating the case-positive, control-test-negative study design for influenza vaccine effectiveness for the frailty bias. Vaccine 2016; 34:1806–9.

16. Moehling KK, Nowalk MP, Lin CJ, et al. The effect of frailty on HAI response to influenza vaccine among community-dwelling adults ≥ 50 years of age. Hum Vaccin Immunother 2018; 14:361–7.

17. Yao X, Hamilton RG, Weng NP, et al. Frailty is associated with impairment of vaccine-induced antibody response and increase in post-vaccination influenza infection in community-dwelling older adults. Vaccine 2011; 29:5015–21.

18. Fulop T, McElhaney J, Pawelec G, et al. Frailty, inflammation and immunosenescence. Interdiscip Top Gerontol Geriatr 2015; 41:26–43.

19. Müller L, Di Benedetto S, Pawelec G. The immune system and its dysregulation of immune changes with aging. Semin Immunol 2018; 40:83–94.

20. Merani S, Kuchel GA, Kleppinger A, McElhaney JE. Influenza vaccine-mediated protection in older adults: impact of influenza infection, cytomegalovirus serostatus and vaccine dosage. Exp Gerontol 2018; 107:116–25.

21. Nace DA, Lin CJ, Ross TM, et al. Randomized, controlled trial of high-dose influenza vaccine among frail residents of long-term care facilities. J Infect Dis 2015; 211:1915–24.

22. McNeil S, Johnstone J, Rockwood M, et al. Impact of Hospitalization due to Influenza on Frailty in Older Adults: Toward a Better Understanding of Burden of Disease. San Diego, CA; 17–21 October.

23. Mitnitski AB, Mogilner AJ, Rockwood K. Accumulation of deficits as a proxy measure of aging. ScientificWorldJournal 2001; 1:323–36.

24. Searle SD, Mitnitski A, Gahbauer EA, et al. A standard procedure for creating a frailty index. BMC Geriatr 2008; 8:24.

25. Hooper M, Rotermann M, Sannmartin C, Bernier J. Government of Canada SC. Validation of an index to estimate the prevalence of frailty among community-dwelling seniors. 2013. Available at: https://www150.statcan.gc.ca/n1/pub/82-003-x/2013009/article/11864-eng.htm. Accessed 11 May 2020.

26. Gravenstein S, Miller B, Ershler W, et al. Low sensitivity of CDC case definition for H3N2 influenza in elderly nursing-home subjects. Clin Res 1990; 38:A47.

27. World Health Organization. WHO Manual on Animal Influenza Diagnosis and Surveillance. Geneva, Switzerland: World Health Organization; 2002.

28. Lancaster GI, Febbraio MA. The immunomodulating role of exercise in metabolic disease. Trends Immunol 2014; 35:262–9.

29. Cowling BJ, Perera RAPM, Valkenburg SA, et al. Comparative immunogenicity of several enhanced influenza vaccine options for older adults: a randomized, controlled trial. Clin Infect Dis 2019;cia1034. doi: 10.1093/cid/cia1034.

30. Frasca D, Blomberg BB. Effects of aging on B cell function. Curr Opin Immunol 2009; 21:425–30.

31. Gibson KL, Wu YC, Barnett Y, et al. B-cell diversity decreases in old age and is correlated with poor health status. Aging Cell 2009; 8:18–25.

32. Soysal P, Stubbs B, Lucato P, et al. Inflammation and frailty in the elderly: a systematic review and meta-analysis. Ageing Res Rev 2016; 31:1–8.

33. Zhou X, Hopkins JW, Wang C, et al. IL-2 and IL-6 cooperate to enhance the generation of influenza-specific CD8 T cells responding to live influenza virus in aged mice and humans. Oncotarget 2016; 7:9171–83.

34. Samson LD, Boots AMH, Verschure WMM, et al. Frailty is associated with elevated CRP trajectories and higher numbers of neutrophils and monocytes. Exp Gerontol 2019; 125:110674.

35. Youssefzadeh MJ, Schafer MJ, Noren Hooten N, et al. Circulating levels of monocytic chemotactant protein-1 as a potential measure of biological age in mice and frailty in humans. Aging Cell 2018; 17:e12706.

36. Mcdonald JU, Zhong Z, Groves HT, Tregoning JS. Inflammatory responses to influenza on immunological response to influenza vaccination and infection. Front Immunol 2017; 35:262–9.

37. Yousefzadeh MJ, Schafer MJ, Noren Hooten N, et al. Circulating levels of monocytic chemotactant protein-1 as a potential measure of biological age in mice and frailty in humans. Aging Cell 2018; 17:e12706.