Cytomegalovirus infection and responsiveness to influenza vaccination in elderly residents of long-term care facilities

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Cytomegalovirus infection and responsiveness to influenza vaccination in elderly residents of long-term care facilities

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ABSTRACT

Ample evidence suggests that infection with cytomegalovirus (CMV) leads to accelerated aging of the immune system and may contribute to poor responsiveness to influenza vaccination in older persons. The objective of this study was to investigate whether CMV infection, acquired earlier in life, affects the response to influenza vaccination in a randomized controlled trial among older persons in long-term care facilities.

During the 1997–1998 influenza season, 731 residents (median age 83 [interquartile range 78–88], 75.4% female) in 14 long-term care facilities in the Netherlands were randomly assigned to receive 15 or 30 μg of inactivated influenza vaccine, followed by a 15 μg booster vaccine or a placebo vaccine at day 84. Blood samples were collected at day 0, day 25, day 84 and day 109. Seroresponses to influenza vaccination were measured by hemagglutination-inhibition tests to the A/H3N2 strain at all time points. Subsequently, baseline levels of IgG anti-CMV antibodies were measured using an automated chemiluminescent microparticle immunoassay. Participants with CMV antibody level ≥6 AU/mL were considered to harbor CMV infection.

At baseline, no differences in pre-vaccination geometric mean antibody titers (GMT) were observed between participants with (n = 571, 78.1%) or without CMV infection (n = 160, 21.9%). During follow-up, participants with and without CMV infection had similar responses to influenza vaccination as measured with changes in GMT (linear mixed model, adjusted for gender, age, pre-vaccination GMT and vaccination strategy, p = 0.46). Analogously, no association was found between CMV infection and a more than 4-fold increase in antibody titer (Generalized Estimating Equations, adjusted OR 1.14 [95%CI 0.80;1.64]) or an antibody titer ≥40 (adjusted OR 1.24 [95%CI 0.86;1.80]).

In conclusion, CMV infection did not explain poor responsiveness to influenza vaccination in residents of long-term care facilities.

1. Introduction

Aging is associated with an increased susceptibility to infections and a reduced response to vaccination [1–4]. The age-related decline in the protective immune response can mostly be explained by replacement of naïve T cells by memory T cells and a decrease in the diversity and function of the T cell population [3–5].

Accumulating evidence suggests that infection with cytomegalovirus (CMV) contributes to the age-associated changes in immunity [5–8]. CMV seroprevalence varies between countries but in general rises with advancing age from 60% in persons aged 40–49 to >90% in persons aged 80 and over [9–11]. Once infected with CMV, the immune system is not able to eliminate the virus [5], resulting in latent CMV infection. Most infected persons remain free of clinical symptoms because of efficient CMV immunosurveillance [12]. The presence of CMV infection is considered to be the driving force behind the oligoclonal expansions of T cells observed in older persons [5,12,13].

Although this immunological imprint of CMV infection on the T cell population is widely recognized, the clinical effect of CMV infection in older persons is largely unknown. Interestingly, Trzonkowski and colleagues observed higher concentrations of anti-CMV antibodies in nursing home residents and staff that did not show a serological response to influenza vaccination [14]. It

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may therefore be hypothesized that the response to influenza vaccination will be lower in older persons with CMV infection due to a functional impairment of immune effector mechanisms. The demonstration of such an effect would offer opportunities for optimizing vaccination strategies by prior determination of the CMV status in this population. Therefore, we investigated whether the presence of CMV infection affects the response to influenza vaccination in a large randomized controlled trial among older persons in long-term care facilities in the Netherlands.

2. Material and methods

2.1. Study population and design

The current study is embedded in a randomized controlled multicenter trial of influenza vaccination strategies in long-term care facilities that was conducted in the Netherlands during the 1997–1998 influenza season. The trial has been described previously in detail [15,16]. In short, 2444 residents of 14 Dutch long-term care facilities were invited by mail to participate. Informed consent was obtained from 815 residents or their legal representatives. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center. Participants were randomly assigned to one of four treatment arms by stratified block randomization using random number tables for each long-term care facility. The four treatment arms were: (1) a 15 μg dose of vaccine on day 0 and a placebo vaccine on day 84, (2) a 15 μg dose of vaccine on day 0 and a 15 μg dose of booster vaccine on day 84, (3) a 30 μg dose of vaccine on day 0 and a placebo vaccine on day 84, and (4) a 30 μg dose of vaccine on day 0 and a 15 μg dose of booster vaccine on day 84. The initial vaccine dose (15 or 30 μg) was given as one or two 0.5 mL intramuscular injections in the same arm.

2.2. Vaccines

The trivalent split virus vaccine for the 1997–1998 influenza season (Pasteur Mérieux, Connaught, France) contained an amount of split virus equivalent to 15 mg hemagglutinin of A/Nanchang/933/95 (H3N2), A/Johannesburg/82/96 (H1N1), and B/Harbin/7/94. An identical lot number was used for all vaccinations. The placebo vaccine existed of phosphate buffered saline (PBS).

2.3. Laboratory measurements

Blood samples were collected at day 0, day 25, day 84 and day 109 and stored at −20 °C. In 2007, pre- and post-vaccination titers were measured. All tests were performed in triplicate. Because influenza A/H3N2 was the predominant virus subtype since 1968 until 2009, we performed the hemagglutination inhibition (HAI) test to the A/H3N2 strain. The assay conditions included using turkey erythrocytes for agglutination, a filtrate of Vibrio cholerae fimbriae as source of receptor destroying enzyme and four hemagglutinating units of the vaccine strain A/Nanchang/933/95 (H3N2), which was propagated on Madine Darby canine kidney cells [17].

Pre-vaccination antibody titers and serological responses to vaccination are presented and analyzed in three ways: (1) geometric mean antibody titers (GMT) with 95% confidence intervals, (2) HAI antibody titer ≥4-fold compared to day 0, and (3) seroprotection defined as HAI antibody titer ≥40 [18].

In 2009, IgG anti-CMV antibodies were measured using an automated chemiluminescent microparticle immunoassay (Architect, Abbott Laboratories, Abbott Park, IL). Participants with a CMV antibody level ≥6 AU/mL were considered to harbor CMV infection. Participants with CMV infection were further divided in a group of participants with a CMV antibody level of 6.0–249.9 AU/mL and a group of participants with a CMV antibody level ≥250 AU/mL.

2.4. Other clinical parameters at baseline

Demographic and medical data were collected at day 0 from multidisciplinary patient files. The six-item list by Katz et al. was used to assess disability in activities in daily living (ADL) [19].

2.5. Statistical analysis

Baseline differences in continuous data between participants in the four treatment arms were tested with Jonkheere-Terpstra tests. Differences in categorical data including CMV infection were tested with Chi square tests. Differences in pre-vaccination GMT were tested with One Way ANOVA. Differences between participants with and without CMV infection were analyzed with Mann-Whitney U tests for continuous data, Chi square tests for categorical data and independent t-tests for differences in pre-vaccination GMT.

Within each treatment arm, seroresponses to influenza vaccination at day 25, day 84 and day 109 were compared between participants with and without CMV infection. Differences in GMT were tested with independent t-tests. Differences in the percentage of participants with an antibody titer ≥4-fold compared to day 0 and differences in seroprotection rate (percentage with an antibody titer ≥40) were tested with Chi square tests.

The effect of CMV infection on GMT prior or after vaccination during follow-up was investigated with linear mixed model analysis. Predicted means and 95% CI were adjusted for gender, age, pre-vaccination GMT and vaccination strategy. A Generalized Estimating Equations approach for binary data was used to investigate the effect of CMV infection on an antibody titer ≥4-fold and to investigate the effect on seroprotection rate (antibody titer ≥40). Again, these analyses were adjusted for gender, age, pre-vaccination GMT and vaccination strategy.

Data were analyzed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL).

3. Results

The numbers of participants in each treatment group at each time point during follow-up are presented in Fig. 1. At day 0, blood samples were collected for 731 out of 815 participants that gave informed consent. The median age of the total study population was 83 years (interquartile range [IQR] 78–88) and 75.4% of the population was female. CMV infection (CMV antibody level ≥6.0 AU/mL) was observed in 571 participants (78.1%). Table 1 shows the characteristics of the study population for each of the four treatment arms. There were no differences in socio-demographic characteristics, clinical characteristics, ADL disability score, pre-vaccination GMT and CMV infection between the groups.

Table 2 describes the characteristics of the study population depending on the presence of CMV infection. Characteristics of participants with CMV infection did not differ substantially from those without CMV infection and vaccination strategies were equally distributed. There was no difference in pre-vaccination GMT between both groups ($p = 0.32$). Moreover, we observed no differences in pre-vaccination GMT between those participants with CMV antibody levels <6 AU/mL (mean 23.1 [95% CI 18.2–29.2]), those with CMV antibody levels between 6.0 and 249.9 AU/mL (mean 19.7 [95% CI 16.5–23.6]) and those with CMV antibody level ≥250 AU/mL (mean 20.6 [95% CI 17.9–23.8], One-Way ANOVA, $p = 0.54$). In addition, no difference in pre-vaccination GMT was found between those participants with CMV antibody level ≥250 AU/mL and those with CMV antibody level between 6.0 and 249.9 AU/mL ($p = 0.85$).

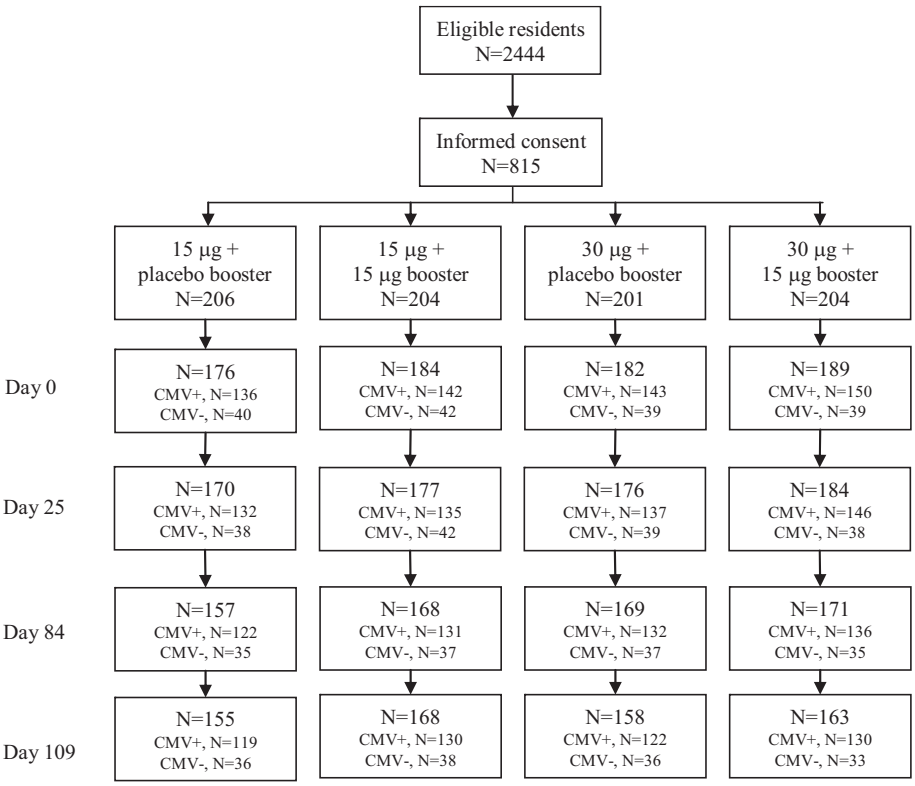

Fig. 1. Number of participants and CMV status during follow-up.

In Table 3 we compared seroresponses to influenza vaccination at day 25, day 84 and day 109 between participants with and without CMV infection, separately within each treatment arm of the study. We did not observe any differences in GMT between participants with or without CMV infection at any time point.

In addition to analyzing GMT as the outcome variable, we also analyzed seroresponse (antibody titer ≥4-fold compared to day 0) and seroprotection rate (antibody titer ≥40) as outcomes within each treatment group (Table 3). Except for those participants that had received a dose of 30 μg and placebo booster at day 109, we observed no statistically significant differences in seroresponse and seroprotection rate between participants with or without CMV infection at any time point during follow-up.

Finally, we performed additional analyses beyond the various treatment strategies. We used linear mixed model analysis to investigate the effect of CMV infection on GMT seroresponsiveness upon vaccination with influenza after adjustment for unequal distributions of gender, age, pre-vaccination GMT and vaccination strategy. No difference in GMT after vaccination during follow-up was found between participants with and without CMV infection (Fig. 2, $p = 0.46$). Analogously, no association was found between CMV infection and a more than 4-fold increase in antibody titer from baseline (adjusted OR 1.14 (95% CI 0.80;1.64, $p = 0.47$), or when an antibody titer ≥40 was taken as an endpoint (adjusted OR 1.24 (95% CI 0.86;1.80, $p = 0.25$).

4. Discussion

In the present study, we have shown that CMV infection, defined as a CMV antibody level ≥6.0 AU/mL, does not affect the response to influenza vaccination in older individuals in long-term care facilities. This lack of effect is independent of the dose, number of

Table 1
Socio-demographic, functional and clinical characteristics of the participants at baseline depending on vaccination strategy.

	Vaccination strategy				*p*-value
	Dose 15 μg + placebo (*n* = 176)	Dose 15 μg + 15 μg booster (*n* = 184)	Dose 30 μg + placebo booster (*n* = 182)	Dose 30 μg + 15 μg booster (*n* = 189)	
Age (years)	83 (78–88)	83 (77–87)	83 (77–88)	84 (78–88)	0.86
Females	125 (71.0%)	143 (77.7%)	139 (76.4%)	144 (76.2%)	0.47
Length of stay (months)	22 (8–48)	22 (10–46)	23 (10–42)	20 (9–44)	0.63
Katz-score	8 (6–10)	8 (4–9)	8 (5–9)	7 (4–9)	0.08
Number of medicaments	5 (3–6)	4 (3–6)	4 (2–6)	4 (3–6)	1.00
Diagnosis of dementia	122 (69.3%)	126 (68.5%)	122 (67.0%)	126 (66.7%)	0.94
Influenza status					
Pre-vaccination GMT	22.7 (18.1–28.4)	22.0 (18.0–27.0)	18.4 (15.2–22.4)	20.6 (17.1–24.8)	0.50
High pre-vaccination titer (≥40)	70 (39.8%)	72 (39.1%)	66 (36.3%)	75 (39.7%)	0.89
CMV infection (≥6 AU/mL)	136 (77.3%)	142 (77.2%)	143 (78.6%)	150 (79.4%)	0.95

Continuous data are presented as median with corresponding interquartile range. Differences were tested with Jonkheere Terpstra tests (*p* for trend). Pre-vaccination GMT is presented as mean with corresponding 95% confidence interval. Differences were tested with One-Way ANOVA. Categorical data are presented as number (percentage). Differences were tested with chi square tests.

Table 2
Characteristics of the study population depending on the presence of CMV infection.

	CMV infection		*p*-value
	Yes	No	
	Level ≥ 6 AU/mL (*n* = 571)	Level < 6.0 AU/mL (*n* = 160)	
Age (years)	83 (78–88)	83 (77–87)	0.39
Females	439 (76.9%)	112 (70.0%)	0.07
Length of stay (months)	23 (10–45)	19 (9–39)	0.10
Katz-score	8 (5–9)	7 (4–9)	0.28
Number of medicaments	4 (3–6)	4 (2–6)	0.02
Diagnosis of dementia	389 (68.1%)	107 (66.9%)	0.77
Influenza status			
Pre-vaccination GMT	20.3 (18.1–22.6)	23.1 (18.2–29.2)	0.32
High pre-vaccination titer (≥40)	217 (38.0%)	66 (41.2%)	0.46
Vaccination strategy			
Dose 15 μg + placebo	136 (23.8%)	40 (25.0%)	
Dose 15 μg + 15 μg booster	142 (24.9%)	42 (26.2%)	0.95
Dose 30 μg + placebo booster	143 (25.0%)	39 (24.4%)	
Dose 30 μg + 15 μg booster	150 (26.3%)	39 (24.4%)	

Continuous data are presented as median with corresponding interquartile range. Differences were tested with Mann-Whitney U tests. Pre-vaccination GMT is presented as mean with corresponding 95% confidence interval. Differences were tested with independent t-tests. Categorical data are presented as number (percentage). Differences were tested with chi square tests.

vaccinations, CMV antibody level and various parameters of comorbidity.

This lack of impact of CMV infection on the response to influenza vaccination is unexpected. Influenza vaccination is clinically effective in 70–90% of younger adults, but in only 17–53% of older persons [20]. Aging is associated with low numbers of CD8+ naïve T cells and increased numbers of memory cells [21]. CMV infection is considered a major driver of oligoclonal expansions of CD8 T cells in old age, and the CD57+ CD8 T cell pool in particular [5–7,12,13,22], which is thought to represent a highly differentiated population of late memory T cells [23–25]. Others have therefore postulated that CMV infection is causally related to the age-associated dysfunction of the immune system [5,12,13], exemplified by a lower number of circulating naïve T cells, which would imply that the decreased response to influenza vaccination in older persons could be attributed to CMV infection [4].

The hypothesis that past and/or persistent CMV infection modulates responsiveness to influenza vaccination was supported by a trial by Trzonkowski et al. in 154 nursing home residents and staff who all received influenza vaccines containing antigens of influenza strains A/Beijing/262/95 (H1N1), A/Sydney/5/97 (H3N2) and B/Beijing/184/93 [14]. Strong reciprocal correlations were observed between anti-CMV antibodies and titers of all anti-hemagglutinins ranging from −0.41 for anti-H3 to −0.74 for anti-H1. In addition, older participants who did not show a serological response to the influenza vaccine had higher concentrations of anti-CMV antibodies compared to their counterparts who did respond to the vaccine. The authors concluded from these observations that CMV infection may have had a negative impact on the effectiveness of influenza vaccination. However, our results are not in line with these earlier findings. Differences in study design, participant selection and recruitment, and health status of the participants may well have contributed to the discrepancy between our study and the study by Trzonkowski et al. In the latter, age and the presence of disease have likely affected the response to influenza vaccination as CMV seroprevalence is highest in older subjects with comorbidity. Our data presented here are free from confounding by age and disease as the randomized design achieved similar pre-vaccination titers and CMV seroprevalence between the groups. Another explanation may be that our analysis was restricted to the serological response to the influenza strain A/H3N2, because the study by Trzonkowski observed the weakest correlation between CMV antibody level and the H3N2 titer [14].

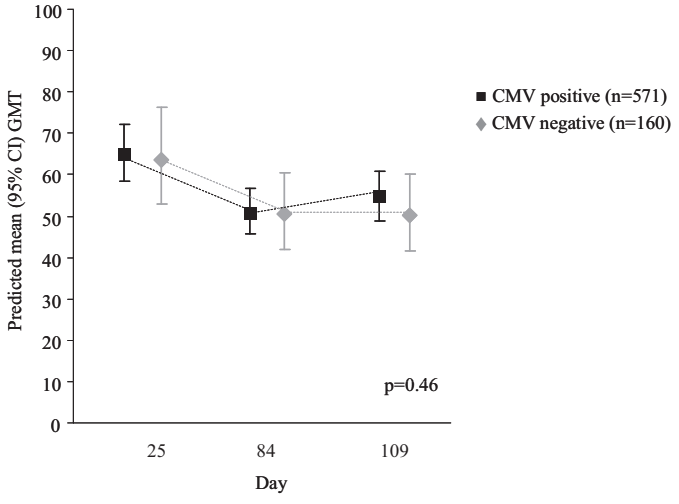

Fig. 2. Effect of CMV infection on GMT seroresponse to vaccination, adjusted for gender, age, pre-vaccination GMT and vaccination strategy. Predicted mean and 95% CI, and *p*-value were obtained by linear mixed model analysis.

This study has several strengths. First, we made use of a large randomized controlled trial on influenza vaccination among older persons in long-term care-facilities, allowing us to efficiently investigate whether CMV infection affected the outcome of influenza vaccination [16]. In addition, we used three ways to assess seroresponsiveness to vaccination (GMT, HAI antibody titer rise ≥4-fold compared to day 0, and HAI antibody titer ≥40) at multiple time points during 3 months of follow-up, which enabled us to thoroughly and extensively study the response to influenza vaccination. As the current trial had sufficient power to show improvements in protective seroresponses during the follow-up period between the four treatment groups [16], we consider it likely that it could also show an effect of CMV infection on protective seroresponses. In the group of participants that had received a dose of 30 μg and placebo booster, a single *p*-value below 0.05 was found for the percentage of participants with an antibody titer ≥40 at day 109, but this may be considered a chance finding as this association was not found in any other treatment group and the seroprotection rate was higher in the CMV positive rather than in the CMV negative group, which is unexpected. Lastly, as a result of the design

Table 3
Seroresponses to influenza vaccination at day 25, 84 and 109 depending on dose and CMV infection.

	Dose 15 μg + placebo		*p*-value	Dose 15 μg + 15 μg booster		*p*-value	Dose 30 μg + placebo booster		*p*-value	Dose 30 μg + 15 μg booster		*p*-value
	CMV infection			CMV infection			CMV infection			CMV infection		
	Yes	No		Yes	No		Yes	No		Yes	No	
n	136	40		142	42		143	39		150	39	
GMT												
Day 25	56.7 (44.4–72.4)	74.3 (42.0–131.2)	0.33	52.5 (40.4–68.3)	67.2 (40.5–111.6)	0.37	74.6 (58.9–94.4)	53.5 (31.9–89.6)	0.21	71.3 (56.7–89.6)	74.1 (45.3–121.1)	0.88
Day 84	47.2 (37.2–60.0)	64.9 (37.1–113.7)	0.24	42.5 (32.8–55.1)	63.1 (37.5–106.1)	0.16	53.2 (42.2–67.0)	37.3 (22.5–62.0)	0.17	57.4 (45.2–73.0)	59.3 (37.0–94.9)	0.91
Day 109	47.1 (36.8–60.5)	57.6 (33.4–99.2)	0.46	52.5 (40.9–67.3)	59.5 (35.3–100.4)	0.64	52.2 (40.9–66.6)	32.9 (19.2–56.6)	0.09	66.6 (52.4–84.6)	66.4 (42.1–104.7)	0.99
≥4-fold increase												
Day 25	53 (40.2%)	10 (26.3%)	0.12	40 (29.6%)	13 (31.0%)	0.87	66 (48.2%)	17 (43.6%)	0.61	63 (43.2%)	17 (44.7%)	0.86
Day 84	39 (32.0%)	6 (17.1%)	0.09	36 (27.5%)	12 (32.4%)	0.56	53 (40.2%)	11 (29.7%)	0.25	48 (35.3%)	14 (40.0%)	0.61
Day 109	35 (29.4%)	7 (19.4%)	0.24	44 (33.8%)	14 (36.8%)	0.73	45 (36.9%)	11 (30.6%)	0.49	55 (42.3%)	16 (48.5%)	0.52
Titer ≥ 40												
Day 25	87 (65.9%)	26 (68.4%)	0.77	89 (65.9%)	28 (66.7%)	0.93	106 (77.4%)	25 (64.1%)	0.09	106 (72.6%)	27 (71.1%)	0.85
Day 84	77 (63.1%)	24 (68.6%)	0.55	73 (55.7%)	25 (67.6%)	0.20	89 (67.4%)	20 (54.1%)	0.13	96 (70.6%)	25 (71.4%)	0.92
Day 109	77 (64.7%)	22 (61.1%)	0.69	80 (61.5%)	24 (63.2%)	0.86	84 (68.9%)	18 (50.0%)	0.04	96 (73.8%)	25 (75.8%)	0.82

GMT is presented as mean with corresponding 95% confidence interval. Differences were tested with independent t-tests. Categorical data are presented as number (percentage). Differences were tested with chi square tests.

of the study, we were also able to assess any potential effect of vaccination dose and booster vaccination.

A limitation of our study is that the analysis was limited to the serological response to the single influenza strain A/H3N2. The results can therefore not be generalized to other influenza virus strains or other viruses. However, the serological response to A/H3N2 has proven to be highly relevant for older persons, as this strain has, since its appearance in 1968, most often been implicated in influenza-related morbidity and mortality in older persons. Another limitation of our study is that the main outcome of our study was the humoral memory response to influenza vaccination. Primary immune responses to neo-antigens or cellular immunity were not studied and could therefore still be affected by CMV infection. In addition, our study population consisted of older persons living in long-term care facilities. Although the clinical relevance of influenza vaccination may be highest in this particular subgroup of older persons, the results of our study may not be generalized to older persons in the general population at large due to possible differences in health and immune status.

Our study population consisted of older individuals living in long-term care facilities with many comorbid conditions and impaired immune response. Our study therefore provides an excellent opportunity to investigate the effect of CMV status on responsiveness to influenza vaccination. Although our results do not exclude a role of CMV infection in the development of age-related changes in the immune system in older persons in the general population at large, the present data from a large randomized controlled trial do not suggest that CMV infection had any negative effect on the immune response to influenza vaccination, which is an important and clinically relevant trigger of the immune system, in older persons in long-term care facilities. For that reason, these findings also do not support a role for determining CMV status in an effort to optimize individual vaccination strategies in older persons in long-term care facilities. These findings warrant further validation in other study populations.

Contributors: Professor Gussekloo had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis (guarantor). *Study concept and design:* den Elzen, Cools, Kroes and Gussekloo. *Acquisition of data:* Vossen, Cools, Kroes and Gussekloo. *Analysis and interpretation of data:* den Elzen, Vossen, Cools, Westendorp, Kroes and Gussekloo. *Drafting of the manuscript:* den Elzen, Vossen and Gussekloo. *Critical revision of the manuscript for important intellectual content*: den Elzen, Vossen, Cools, Westendorp, Kroes and Gussekloo. *Statistical analysis:* den Elzen and Gussekloo. *Obtained funding:* Cools, Kroes and Gussekloo. *Administrative, technical, or material support*: Vossen, Cools and Gussekloo. *Study supervision:* Cools, Kroes and Gussekloo. All authors have approved the final article.

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References

[1] Webster RG. Immunity to influenza in the elderly. Vaccine 2000;18(16):1686–9.
[2] Yoshikawa TT. Epidemiology and unique aspects of aging and infectious diseases. Clin Infect Dis 2000;30(6):931–3.
[3] Miller RA. The aging immune system: primer and prospectus. Science 1996;273(5271):70–4.
[4] Kovaiou RD, Herndler-Brandstetter D, Grubeck-Loebenstein B. Age-related changes in immunity: implications for vaccination in the elderly. Expert Rev Mol Med 2007;9(3):1–17.
[5] Pawelec G, Derhovanessian E, Larbi A, Strindhall J, Wikby A. Cytomegalovirus and human immunosenescence. Rev Med Virol 2009;19(1):47–56.
[6] Khan N, Shariff N, Cobbold M, et al. Cytomegalovirus seropositivity drives the CD8 T cell repertoire toward greater clonality in healthy elderly individuals. J Immunol 2002;169(4):1984–92.
[7] Looney RJ, Falsey A, Campbell D, et al. Role of cytomegalovirus in the T cell changes seen in elderly individuals. Clin Immunol 1999;90(2):213–9.
[8] Pawelec G, Akbar A, Caruso C, Solana R, Grubeck-Loebenstein B, Wikby A. Human immunosenescence: is it infectious? Immunol Rev 2005:205257–68.
[9] Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988–2004. Clin Infect Dis 2010;50(11):1439–47.
[10] Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol 2010;20(4):202–13.
[11] Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. Seroprevalence of cytomegalovirus infection in the United States, 1988–1994. Clin Infect Dis 2006;43(9):1143–51.
[12] Koch S, Larbi A, Ozcelik D, et al. Cytomegalovirus infection: a driving force in human T cell immunosenescence. Ann NY Acad Sci 2007:111423–35.
[13] Olsson J, Wikby A, Johansson B, Lofgren S, Nilsson BO, Ferguson FG. Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study. Mech Ageing Dev 2000;121(1–3):187–201.
[14] Trzonkowski P, Mysliwska J, Szmit E, et al. Association between cytomegalovirus infection, enhanced proinflammatory response and low level of anti-hemagglutinins during the anti-influenza vaccination—an impact of immunosenescence. Vaccine 2003;21(25–26):3826–36.
[15] Roos-Van Eijndhoven DG, Cools HJ, Westendorp RG, Ten Cate-Hoek AJ, Knook DL, Remarque EJ. Randomized controlled trial of seroresponses to double dose and booster influenza vaccination in frail elderly subjects. J Med Virol 2001;63(4):293–8.
[16] Cools HJ, Gussekloo J, Remmerswaal JE, Remarque EJ, Kroes AC. Benefits of increasing the dose of influenza vaccine in residents of long-term care facilities: a randomized placebo-controlled trial. J Med Virol 2009;81(5):908–14.
[17] Kelly H, Attia J, Andrews R, Heller RF. The number needed to vaccinate (NNV) and population extensions of the NNV: comparison of influenza and pneumococcal vaccine programmes for people aged 65 years and over. Vaccine 2004;22(17–18):2192–8.
[18] Beyer WE, Palache AM, Luchters G, Nauta J, Osterhaus AD. Seroprotection rate, mean fold increase, seroconversion rate: which parameter adequately expresses seroresponse to influenza vaccination? Virus Res 2004;103(1–2):125–32.
[19] Katz S, Downs TD, Cash HR, Grotz RC. Progress in development of the index of ADL. Gerontologist 1970;10(1):20–30.
[20] Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine 2006;24(8):1159–69.
[21] Goronzy JJ, Lee WW, Weyand CM. Aging and T-cell diversity. Exp Gerontol 2007;42(5):400–6.
[22] Derhovanessian E, Maier AB, Beck R, et al. Hallmark features of immunosenescence are absent in familial longevity. J Immunol 2010;185(8):4618–24.
[23] Gratama JW, Kardol M, Naipal AM, et al. The influence of cytomegalovirus carrier status on lymphocyte subsets and natural immunity. Clin Exp Immunol 1987;69(1):16–24.
[24] Wang EC, Taylor-Wiedeman J, Perera P, Fisher J, Borysiewicz LK. Subsets of CD8+, CD57+ cells in normal, healthy individuals: correlations with human cytomegalovirus (HCMV) carrier status, phenotypic and functional analyses. Clin Exp Immunol 1993;94(2):297–305.
[25] Wang EC, Moss PA, Frodsham P, Lehner PJ, Bell JI, Borysiewicz LK. CD8highCD57+ T lymphocytes in normal, healthy individuals are oligoclonal and respond to human cytomegalovirus. J Immunol 1995;155(10):5046–56.