The effect of cytomegalovirus infection on the immune response to influenza vaccination: a meta-analysis

Silke Coopman, Katholieke Universiteit Leuven

Promotor: Prof. Catharina Matheï, Katholieke Universiteit Leuven

Master of Family Medicine

Masterproef Huisartsgeneeskunde
Academiejaar: 2017 – 2018

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Het effect van CMV-infectie op de influenza vaccinatie immuunrespons: een meta-analyse

Huisarts-in-opleiding: Silke Coopman, Katholieke Universiteit Leuven
Academiejaar: 2017-2018
Promotor: Catharina Matheï, Katholieke Universiteit Leuven
Praktijkopleider: Fonteyn Dirk

Context: Influenza is voornamelijk in de oudere populatie geassocieerd met een aanzienlijk risico op ernstige morbiditeit en mortaliteit, wat voorkomen kan worden door influenza vaccinatie. Ouderen hebben echter een verminderde influenza vaccinatierespons. Dit is gerelateerd aan leeftijdsgebonden veranderingen in het immuunsysteem, waarnaar verwezen wordt als immunosenescentie. Het is bewezen dat cytomegalovirus (CMV) een rol speelt in dit proces.

Onderzoeksvraag: Wat is het effect van CMV-infectie op de humorale immuunrespons na influenza vaccinatie?

Methode: Een systematische literatuurreview werd uitgevoerd in MEDLINE en EMBASE om alle studies te identificeren over het effect van een positieve CMV serostatus of de hoogte van de CMV titer op elke vorm van humorale immuunrespons na influenza vaccinatie bij immuuncompetente personen. Een gemodificeerde versie van Newcastle-Ottawa Quality Assessment Scale werd toegepast om de kwaliteit van de studies te evalueren. Indien mogelijk werden meta-analyses uitgevoerd met Review Manager 5.

Resultaten: Veertien studies voldeden aan de inclusiecriteria. Twee studies toonden een positief effect van CMV seropositiviteit op de antistofrespons na influenza vaccinatie. Vijf studies konden geen effect aantonen. Vier studies besloten tot een negatief effect. Er lijkt een negatieve correlatie te zijn tussen de hoogte van de CMV titer en de post-vaccinatie influenza antistoftiter.

Conclusies: Heterogene studies toonden verschillende resultaten. Tot nu toe blijft het effect van CMV-infectie op de humorale immuunrespons na influenza vaccinatie onduidelijk.

Contact: silke_coopman@hotmail.com

The effect of cytomegalovirus infection on the immune response to influenza vaccination: a meta-analysis

Silke Coopman, Catharina Matheï, Frederik Vanstraelen

Abstract

Background Influenza is associated with a considerable risk of severe morbidity and mortality - especially in the older population - which can be prevented by influenza vaccination. However, older persons have a decreased influenza vaccination response. This has been related to the age-associated changes in the immune system referred to as immunosenescence. Cytomegalovirus (CMV) has been shown to play a role in this process. In this review we focus on the effect of CMV infection on the humoral immune response to influenza vaccination.

Methods A systematic review of literature was performed in MEDLINE and EMBASE to identify all studies concerning the effect of a positive CMV serostatus or magnitude of CMV titer on any form of humoral immune response to influenza vaccination in immunocompetent individuals. A modified version of the Newcastle-Ottawa Quality Assessment Scale was applied for literature quality evaluation. When possible, meta-analyses were performed by using Review Manager 5.

Results Fourteen studies met the inclusion criteria. Two studies showed a positive effect of CMV seropositivity on the influenza vaccination antibody response. Five studies revealed no effect. Four studies concluded with a negative effect. There seems to be a negative correlation between the magnitude of the CMV titer and the post-vaccination influenza antibody titer.

Conclusion Heterogeneous studies showed different results. Until now, the overall effect of CMV infection on the influenza vaccination response remains questionable.

1. Introduction

Every year one billion people suffer from influenza infection. Three to five million of these cases are severe. Moreover, influenza infections annually cost the lives of 300,000 to 650,000 people. In developed countries more than 90% of influenza-related deaths are within elderly (1). Influenza vaccination prevents hospitalizations and deaths in older adults (2). However, these vaccines are not protective in an important proportion of old individuals (3). The immune response to influenza vaccination declines with age and the rate of seroprotection is only 20 to 70% in the elderly (4–6). This poor humoral response to influenza vaccination has been attributed to a phenomenon called immunosenescence, characterized by changes in the T-cell compartment and a chronic low-grade state of inflammation sometimes referred to as inflamm-aging. There is evidence that the cytomegalovirus is a driving force in this so-called concept of immunosenescence. (7,8).

CMV is an epidemic and universally common beta-herpesvirus with a seroprevalence increasing with age. Numbers range from 40% in young adults to more than 70% in people over 60 years and up to 90% in 80-plus-year-olds in the USA (9–11). In severely immunocompromised patients, infection with CMV may lead to severe morbidity and mortality. In immunocompetent hosts CMV rarely causes an acute symptomatic infection. But once infected with CMV, the immune system is unable to eliminate the virus, resulting in a latent infection. A

hypothesis states that the impact of CMV on the immune system is due to episodes of subclinical reactivation. Another theory states that CMV infection is accompanied by a higher level of a low-grade chronic inflammation that in turn provides an ongoing stimulation of the immune system in older adults (12). CMV infection is associated with oligoclonal expansions of highly differentiated T-cells of which many are CMV specific, suggesting that the containment of CMV and its recurrent reactivation requires considerable immunological resources which eventually get exhausted. This may cause immune responses to other challenges to be reduced (13).

The importance of influenza vaccination has been highlighted. Potential negative influences on influenza vaccination need to be investigated. In this review we will examine the potential effect of CMV infection on influenza vaccination, in which we will focus on the humoral immune response.

2. Methods

The protocol, which can be assessed through Appendix A, was consistent with the PRISMA criteria.

2.1 Search strategy

We searched through two electronic databases (MEDLINE and EMBASE) to identify articles published in English between 1 January 2003 and 31 December 2017. The search string consisted of the general keywords cytomegalovirus and influenza.

2.2 Selection of studies

Two review authors (SC and FV) independently screened the search results for relevance on the basis of title and abstract. In case of doubt articles were retained. Duplicates between the two databases were removed. The remaining articles were evaluated on the basis of the full article text using agreed selection criteria (Appendix A).

Except for case reports, all types of studies were eligible for inclusion. Only studies assessing the impact of CMV on the humoral influenza vaccine response in immunocompetent individuals were included; studies conducted in immunocompromised patients such as patients infected with human immunodeficiency virus or transplant patients were excluded.

The exposure of interest was CMV serostatus. We accepted the definition as applied in the studies to determine a positive CMV serostatus.

The primary endpoint of interest was the humoral response to influenza vaccination three to five weeks after administration, which is appointed as the peak antibody response. In particular, we were interested in articles in which the immune response was expressed in terms of seroprotection, seroconversion or difference in geometric mean titer (GMT). Reaching a hemagglutination-inhibition (HI) antibody titer of at least 40 is defined as seroprotection. Seroconversion is stated as an increase in HI antibody titer of at least fourfold. However, other definitions to ascertain the humoral response to influenza vaccination were accepted.

Besides the antibody response after three to five weeks, we were also interested in the persistence of the antibody response beyond the peak antibody response.

Discrepancies were discussed and, if required, a third review author (CM) was consulted.

2.3 Data extraction

We extracted the following data using a standardized data-collection form: last name of the first author, publication year, study region and setting, number of cases and controls, age and sex distribution, method of exposure and endpoint assessment, type of influenza vaccination, the way of administering the vaccine, CMV status and humoral response to influenza

vaccination. Data extraction was carried out by one review author (SC), and was independently checked by a second review author (CM).

2.4 Quality appraisal

A modified version of the Newcastle-Ottawa Quality Assessment Scale adapted to cross-sectional studies was applied for quality evaluation (14). On the basis of seven questions (see Appendix B) studies were assessed with regard to appropriateness of research design, recruitment strategy, response rate, representativeness of sample, objectivity/reliability of outcome determination, power calculation provided, and appropriate statistical analyses. A maximum score of 8 could be obtained.

2.5 Data analysis

The heterogeneity between studies was evaluated by the chi-square-based *Q* statistical test. In case of statistical study heterogeneity a random-effect model (the DerSimonian and Laird method) was applied. If statistical study heterogeneity was not observed a fixed-effects model (the Mantel-Haenszel method) was used. Subgroup analyses were carried out to further find heterogeneity source by age. All analyses were performed by using Review Manager 5.

3. Results

A total of 2,145 references to publications were initially retrieved. After screening on the basis of title and abstract, and after removal of duplicates between the two databases, 34 articles were selected. Eventually, on the basis of the full article text, 14 articles were included in our review (15–28). The selection and inclusion of the studies is schematically displayed in a flowchart in Figure 1.
The median score on the Ottowa-Newcastle Quality Assessment scale was 6 (range 4-8) (Table 1). Lower scores were mainly due to risk of selection bias.

3.1 Characteristics of the included studies

Tables 2 and 3 give an overview of the characteristics and main findings of the 14 included studies. In total, 2,226 subjects were investigated. Sample sizes varied from 54 to 815 participants (15,16). The selection method of the population was not always clear. Study settings varied from universities to academic hospitals to residential care centers. Not all studies described study settings clearly.

Study	Selection				Comparability		Outcome		Score
McElhaney 2015	*			*	*	*	*	*	6
Furman 2015	*	*		*	*		*	*	6
den Elzen 2011	*			*	*	*	*	*	6
Strindhall 2016				*	*	*	*	*	5
Haq 2016	*	*	*	*	*	*	*	*	8
Goldeck 2017	*			*	*	*	*	*	6
Merani 2017				*	*	*	*	*	5
Derhovanessian 2012				*	*	*	*	*	5
Wald 2013				*	*		*	*	4
Frasca 2015				*	*	*	*	*	5
Reed 2017				*	*	*	*	*	5
Trzonkowski 2003	*			*	*	*	*	*	6
Moro-García 2011	*			*	*	*	*	*	6
Turner 2013	*			*	*	*	*	*	6

Table 1 Quality assessment of studies based on a modified version of the Newcastle-Ottowa Quality Assessment Scale.

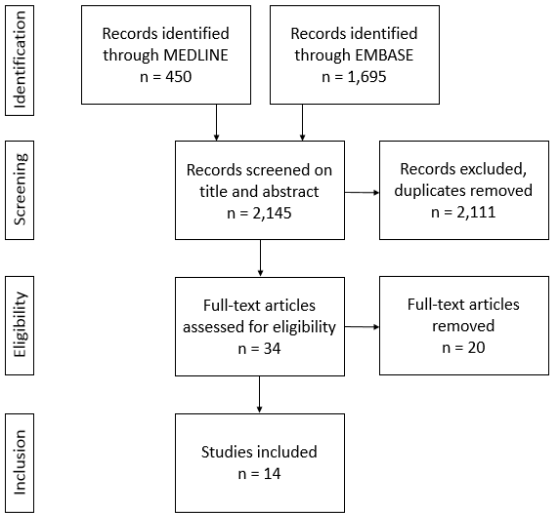


Figure 1 Flow diagram of the study selection.

All but two studies (21,26) classified their population according to gender, and 64.5% or 1,193 out of 1,851 participants were female.

Some studies (16–18,22,26,28) included young people as well as elder people. Six studies made a comparison between a younger and older group of adults (16–18,22,26,28). Half of the studies limited their population to an older population, with a lower limit of 60 years old (15,19–21,23–25). One study assessed an exclusively young population (27). Mean age was reported in 12 studies and ranged between 66 and 88, and 21 and 44 for the older and younger populations respectively. Median age was reported for one study (18).

Variability was observed with regard to the administered influenza vaccine types. A trivalent vaccine consisting of the influenza A subtypes H1N1 and H3N2, and the influenza type B, was administered in all but two studies. One study only administered the H1N1 subtype (28). There was a great heterogeneity in specific vaccine strains. In one study the authors did not mention the influenza vaccine type (23).

There was also a difference in the route of administration of the vaccines. In at least six studies an intramuscular vaccine was given to the subjects (15,18,19,21,25,27). Derhovanessian et al. applied an intradermal vaccine (16). Seven studies did not mention the route of administration (17,20,22–24,26,28).

3.2 Humoral immune response in relation to CMV serostatus

Eleven studies investigated the effect of a positive CMV serostatus on the humoral immune response to influenza vaccination (Table 2). Seroconversion was chosen as outcome measure in seven studies. It was described in function of age in five studies (16,17,22,26,28). Humoral immunity was defined in terms of seroprotection in three studies (15,22,28), of which two studies described seroprotection in function of age (22,28). Difference in GMT was used as an outcome measure in five studies (15,18,20,22,28). Three of them specified the outcome according to age (18,22,28). In none of these articles the effect of CMV on seroconversion, seroprotection or difference in GMT was described in function of sex.

Other outcome measures were described in various ways. Strindhall et al. defined responders to influenza vaccination as participants who reached a HI titer of 40 in combination with a fourfold titer increase (25). Goldeck et al. on the other hand demanded a HI response to at least 2 vaccine strains with a ≥ fourfold HI titer rise, provided that the post-vaccination titer was at least 10, or a HI titer ≥ 40 in initially seronegative (HI titer < 10) samples (19). Reed et al. investigated the effect on peak antibody response and antibody persistence (24).

Four studies concluded to a negative effect of CMV seropositivity on the humoral immune response to influenza vaccination (16,17,24,28). Three studies revealed a variable effect of CMV in different age groups (16,18,28). One of these studies only showed a negative effect in the older group, while it found no effect in the younger group (16). Another study concluded to a negative effect only in the younger group, with no effect in the older group (28). A positive effect was shown in two studies (18,21). One of them concluded to a positive effect only in a group of young adults, while there was no effect seen from CMV on the influenza immune response in older adults (18). Five other studies revealed no effect (15,19,20,22,25) (Table 2).

Strindhall et al. were the only ones who described the effect of CMV seropositivity on the humoral immune response in function of sex. They could not find a difference in HI titers according to sex (25).

3.3 Vaccination response in CMV positives in function of anti-CMV titer

Table 3 frames the five studies that researched the influence of the magnitude of the CMV titer on the humoral immune response to influenza vaccination (15,22,23,26,27). Three studies showed a negative correlation between CMV titer and post-vaccination influenza antibody titer (23,26,27). Den Elzen et al. could not find an effect of the CMV antibody level (15). One study showed a weak positive effect (22) (Table 3).

3.4 Antibody persistence

Four studies measured the effect of CMV status on antibody persistence, three to six months after vaccination (15,22,24,26). Merani et al. found a weak positive correlation between CMV titer and influenza antibody titer after four weeks, but no effect of CMV was found after 10 and 20 weeks (22). Den Elzen et al. found a seroprotection on day 109, but only in the group that received a high dose vaccine with a placebo booster (15). Reed et al. concluded to a negative effect on antibody persistence (24), whereas Trzonkowski et al. found a general negative effect (26).

3.5 Meta-analyses

In a first meta-analysis data of two studies that investigated the effect of CMV seropositivity on the antibody response to H1N1 by HAI in terms of seroconversion, were pooled (16,17) (Figure 2). Overall, a negative association (odds ratio 0.20, 95% CI 0.08, 0.54) was observed between a positive CMV serostatus and humoral response to influenza vaccination. A subgroup analysis according to age showed a negative association in the group younger than 60 (odds ratio 0.15, 95% CI 0.04, 0.63), but not in the older age group (odds ratio 0.27, 95% CI 0.70, 1.00). A second meta-analysis of two studies that investigated the effect of a positive CMV serostatus on the H3N2 antibody response in terms of seroconversion, showed no evidence of an association between CMV serostatus and humoral response to influenza vaccination (odds ratio 0.53, 95% CI 0.06, 5.05) (15,16) (Figure 3).

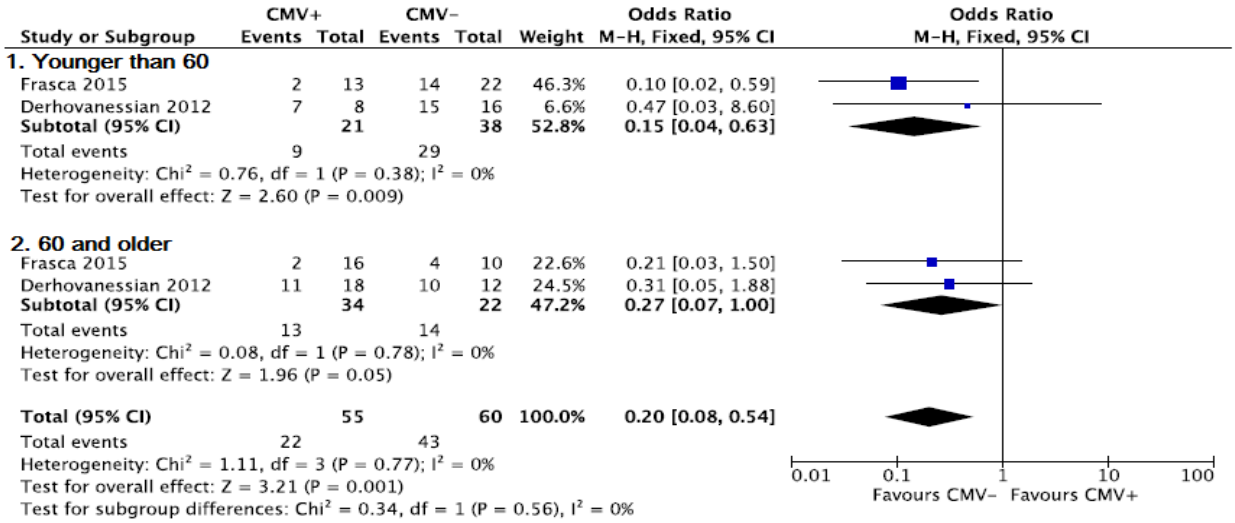


Figure 2 Forest plot of CMV serostatus and antibody response to H1N1 by HAI in terms of seroconversion with subgroup analysis according to age.

Year	1st author	Population			Exposure	Humoral immune response to influenza vaccination				
		Characteristics	N	Age: mean years	CMV IgG+ N (%)	**Seroprotection** HAI antibody titer ≥ 40	**Seroconversion** ≥ 4-fold increase in HAI antibody titer	**Difference in GMT**	Other	Comment
2015	McElhaney J.	Cross-sectional study (Canada)	221	all ≥ 65	No data		🟢			CMV+ subjects had a higher HIA ratio (d28/d0 or post-/prevaccination titer) (p < 0.0251) and there was a better HIA response in both groups (healthy and T2DM) (no data).
		healthy	119	75.74±6.64 (SD)						
		vs. diabetes type 2	102	74.57±6.45 (SD)						
2015	Furman D.	Stanford-Ellison cohorts (USA)	91	median age				🟢		CMV+ young adults exhibited enhanced antibody responses (HAI assay at day 0 and 28±7) compared to young CMV- and old subjects (no data). Older subjects showed no difference in GMT according to CMV serostatus (no data). Older subjects had an overall down-regulation of immune components and a decreased vaccination response regardless of their CMV status.
		2008	91 (89 completed)							
		young (yYear1)		24,5 (20-30)	yY1 57%					
		old (oY1)		78 (61-89)	oY1 59%					
		2009	77 returned							
		young (yY2)		26 (22-32)	yY2 55%					
		old (oY2)		77 (62-89)	oY2 60%					
		2010-2011								
		independent young cohort	37	27 (19-44)	51%					
2011	den Elzen W.	RCT in residential care center	815	83	571 (78.1%)	🟡	🟡	🟡		CMV seropositivity (≥ 6 AU/ml) had no effect on influenza vaccination response on day 25, 84 and 109, independent of vaccine dose, number of vaccines and CMV antibody level, except on d109 in group that got 30 µg vaccine with placebo booster: 68.9% of CMV+ reached seroprotection vs. 50% in CMV- (p=0.04) (data for meta-analysis). Seroconversion rates in CMV+ were 39% on d25 and 31% on d84 -109 vs. 44% on d25, 27% on d84 and 30% on d109 in CMV-. Seroprotection in CMV+ was reached in 68% on d25 and 59% on d84-109, vs. 66% on d25, 59% on d84 and 56% on d109 in CMV-.
		Mailing to 2444 residents								
		in 1997 (The Netherlands)								
2016	Strindhall J.	Jönköping (Sweden), 2011	88	all 69	73 (83%)				🟡	Definition of response: titer > 40 and 4-fold increase at d30. For influenza B, repeated vaccinations and an inverse CD4/CD8 ratio had a negative impact on vaccine response (graph, no data).
2016	Haq K.	Community in Canada	70	all 65+	44 (62.8%)			🟡		The serum antibody response to A/H3N2 (GMT) at week 4 did not differ with CMV serostatus (graph, no data).
2017	Goldeck D.	Antwerp hospital (Belgium)	56	all 65+	29 (52%)				🟡	Definition of responder: HI response to ≥ 2 of 3 vaccine strains; a ≥ 4-fold HI titer rise from day 0 to 21 provided that the titer at d21 was at least 10; or a seroconversion to a HI titer ≥ 40 in initially seronegative (HI titer < 10) samples. No difference in CMV serostatus was found: 34% of CMV+ responded vs. 41% of CMV-.
		Responders (R)	21	M 73 F 70	10 (47.6%)					
		Non Responders (NR)	35	M 72 F 73	19 (54.3%)					
2017	Merani S.	Connecticut (USA)	≥65y: n=106	75	60	🟡	🟡	🟡		CMV seropositivity did not impact the response to influenza vaccination, but impaired the response to influenza virus-challenge. CMV- subjects had a higher influenza B prevaccination GMT, but no difference was found in GMT fold increase of seroprotection rates between CMV- and CMV+ elderly post-vaccination at week 4, 10 and 20 (graph, no data).
			High Dose n=53	75	31 (58.5%)					
			Standard Dose n=53	75	29 (54.7%)					
			20-40y: n=19							
2012	Derhovanessian E	Clinical trial Antwerp (Belgium)	54		26 (48%)					In 60+ years olds CMV seropositivity was associated with a lower response rate after 3 weeks: 44% of CMV+ responded vs. 83% of CMV- (p=0.033). No difference was found in humoral response in the younger group according to CMV serostatus: 100% of CMV+ responded vs. 93,7% of CMV- (p=0.52). Definition of response: ≥ 4-fold antibody titer rise against 2 or 3 vaccine strains between day 0 and 21. Responsiveness to single virus strains was generally lower in the CMV+ group, but only reached statistical significance for Perth (p=0.045) (data for meta-analysis).
		Young group	24	37.5 (18-59)	8 (33.3%)		🔴			
				CMV+: 43.2						
				CMV-: 34.7						
		Older group	30	68.4 (≥60)	18 (60%)					
				CMV+: 67						
				CMV-: 70.5						
2013	Wald A.	Washington (USA), 2009	97 without protective baseline sera							There was no difference by CMV serostatus in the % of participants achieving a seroprotective titer at d42. In younger participants mean HA titer was lower in CMV+ (142) compared with CMV- (385) (p=0.013). Among the older group, CMV serostatus was not associated with differential antibody titers.
		105 of 131 gave consent	Group 1: 42	44 (18-64)	17 (40%)	🟡	🟡	🔴		
			Group 2: 55	70 (≥65)	43 (62%)					
2015	Frasca D.	Recruitment unclear	62		30 (48.4%)					CMV seropositivity was associated with decreased response to pH1N1 at day 7 and 28-42 in both young and elderly individuals (data for meta-analysis): seroconversion rates in young participants were 15% in CMV+ vs. 64% in CMV-, seroconversion rates in older participants were 12.5% in CMV+ vs. 40% in CMV-. Switched memory B cells (which predict good serum antibody response) were decreased in young and elderly CMV+ individuals.
		Young group	36	(20-59)	14 (38.9%)		🔴		🔴	
				CMV+: 44						
				CMV-: 41						
		Older group	26	all ≥60	16 (61.5%)					
				CMV+: 66						
				CMV-: 67						
2017	Reed R.	Recruitment through clinics and volunteer subject pool of Sanders-Brown Center on Aging (USA)	98	74 (60-91)	68 (69%)				🔴	CMV serostatus did not predict peak antibody response, which is the higher of the 2- or 4-week post-vaccine titers. Age was associated with a lower peak antibody response, but only in CMV+ subjects taking beta blockers. The highest antibody persistence (spring titer) was found in CMV- subjects who were not taking beta blockers, so CMV+ adults had poorer antibody persistence.

Table 2 Effect of a positive CMV serostatus on the humoral immune response to influenza vaccination. CMV: cytomegalovirus, N: amount, HAI=HIA=HI: hemagglutination inhibition assay, GMT: geometric mean titer, SD: standard deviation, vs.: versus, d: day, T2DM: type 2 diabetes mellitus, y: years, yY1: young year one, oY1: old year one, yY2: young year 2, oY2: old year 2, RCT: randomized controlled trial, AU/ml: arbitrary units per milliliter, AEU/ml: arbitrary ELISA units per milliliter, p: p-value. Green sphere: positive effect, yellow sphere: no effect, red sphere: negative effect, grey area: not investigated.

Year	1st author	Population			Influence of CMV titer on humoral immune response to influenza vaccination
		Characteristics	N	Age: mean years	
2017	Merani S.	Connecticut (USA)	≥65y: 106 20-40y: 19	75	There was a weak positive correlation between CMV titer and influenza antibody titer at week 4 for the A/H1N1 (p=0.05) and B (p=0.01) strains that was not significant for the A/H3N2 strain (p=0.08).
2011	den Elzen W.	RCT in residential care center Mailing to 2444 residents in 1997 (The Netherlands)	815	83	CMV seropositivity (≥ 6 AU/ml) had no effect on influenza vaccination response (in terms of seroconversion, seroprotection or difference in GMT) on day 25, 84 and 109, independent of CMV antibody level. CMV antibody levels were divided into 3 groups: < 6 AU/mL vs. 6-249.9 AU/mL vs. ≥ 250 AU/mL.
2003	Trzonkowski P.	Residents and staff of nursing homes in Gdansk (Poland)	154 91 elderly 63 young	 65-99 19-40	Non-responders had higher levels of anti-CMV IgG (graph, no data). Values of those titres in young groups were lower than those in elderly. Influenza antibody titres were measured after 1 and 6 months.
2011	Moro-García M.	Nursing home in Oviedo (Spain) Classification according to the Barthel index as a measure of functional status	100 group 0: 24 group 1: 26 group 2: 27 group 3: 23	 85.7 (75-97) 86 (77-94) 87 (74-97) 88.2 (69-96)	A negative correlation between CMV antibody titer and influenza antibody titer was found (graph, no data). Elderly with a worse functional status had a lower antibody titer and lower functional response after influenza vaccination, but they had a gradually increased CMV response. Higher CMV antibody titres were found in elderly with worse functional status. Influenza antibody titres were divided titer by time since immunization.
2013	Turner J.	Healthy university students recruited by campus advertisement (UK)	158	21 (±SD 3)	Higher anti-CMV IgG titres were associated with weaker antibody response after 4 weeks to the A/Brisbane antigen. There was no relationship between anti-CMV IgG titer and antibody response to the other influenza strains.

Table 3 Vaccination response in CMV positives in function of anti-CMV titer. CMV: cytomegalovirus, N: amount, y: years, SD: standard deviation, p: p-value, GMT: geometric mean titer, AU/mL: arbitrary units per milliliter. Green sphere: positive effect, yellow sphere: no effect, red sphere: negative effect.

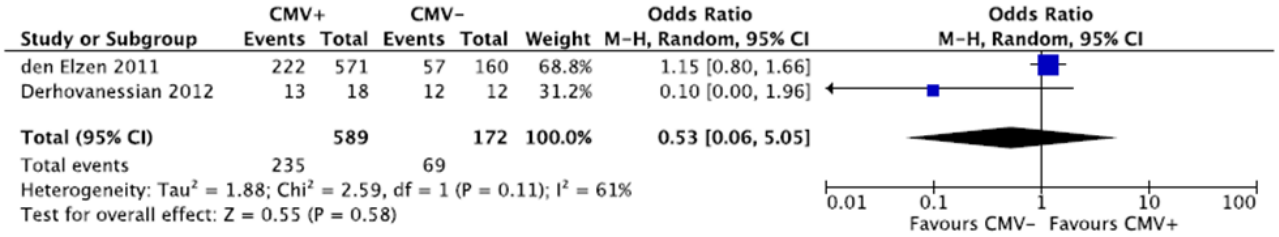


Figure 3 Forest plot of CMV serostatus and antibody response to H3N2 by HAI in terms of seroconversion.

4. Discussion

To our knowledge, this is the first systematic review investigating the impact of CMV infection on the humoral response to influenza vaccination. A main finding of this review is that the number of studies on this topic is very limited, which is surprising given on the one hand the importance of influenza prevention for public health and on the other hand the well-known effects of CMV infection on the immune system. Furthermore, the available studies showed contradictory results regarding the impact of CMV infection on the humoral immune response to influenza vaccination for which no satisfactory explanation could be provided.

Two studies concluded to an improved response to influenza vaccination in subjects with a positive CMV serostatus. The authors of these studies hypothesized that CMV infection is accompanied by a higher level of a low-grade chronic inflammation that in turn provides an ongoing stimulation to the immune system which might lead to a better vaccination response. Furman et al. observed this positive effect of CMV only in young people. Furman et al. state that the elderly have an overall down-regulation of immune components and a decreased vaccination response regardless of their CMV status (18). The five studies showing no effect of CMV infection on influenza vaccination response in an older population are in line with this theory. Four studies concluded to a negative effect. A negative relationship would not be illogical since CMV has proven to be a driving force in the concept of immunosenescence. Derhovanessian et al. hypothesized that the deleterious effect of latent CMV infection on influenza antibody response might be mediated through the accumulation of highly differentiated T-cells, which may lead to exhaustion of immunological resources (16). Frasca et al. explain the negative correlation by their finding that CMV infection is associated with increased levels of inflammation, which correlates with lower B-cell functionality, leading to poorer antibody production (17).

The results of studies assessing the post-vaccination influenza antibody response in function of CMV titer, showed slightly less variability. The majority of the studies revealed a negative correlation although the largest study failed to show any relation and one study even showed a weak positive correlation. Studies show that a poorly controlled CMV infection, expressed as a high anti-CMV IgG titer, is associated with higher circulating levels of inflammatory markers (23,27,29), which may reflect immune dysfunction and weak performance of vaccinations.

How can we explain these different observations?

Age seemed to be an important covariate, as half of the comparisons that were made between age groups resulted in a variable effect of CMV infection. Results were however again discordant, varying from a positive or negative effect only among younger participants, to a negative effect only in the older group. Not a single study concluded to a positive effect of CMV infection in an older group when compared to a younger group. As already mentioned, the immune response to influenza vaccination declines with age, apart from a possible effect of CMV infection. This decline with aging, in the antibody response to influenza vaccination and the associated loss of vaccine efficacy, has been attributed to age-related changes in B- and T-cells (30–35).

Only one study assessed the impact of CMV infection on influenza vaccination response in function of gender. However, it could be an important factor to take into account, since evidence strongly suggests immunological differences between men and women (36).

Other factors that might influence the influenza vaccination response are health status, ethnicity and medication use. Reed et al. for example did show a negative effect

of CMV infection on antibody persistence only in the subjects taking beta blockers (24).

Another source of heterogeneity could be the large variety in the administered influenza vaccines.
The HAI antibody response is strain-specific. Every year there is the possibility of a mismatch between the vaccine strains and the circulating strains. The degree of a mismatch has shown to be correlated with a significant reduction in vaccine efficacy in all age groups (37). This mismatch appeared to be the case in for example the study of Merani et al.: the vaccine and the circulating A/H3N2 strains were a poor match with only little cross-reactivity (22). Also Turner et al. made speculations that strain-specificity might explain contradictory findings. They showed that higher anti-CMV IgG titers are associated with weaker antibody responses to the A/Brisbane vaccine component, but they couldn't find any relationship between CMV and antibody response to other influenza strains (27). Strain-specific differences in the magnitude of antibody responses to influenza vaccination have been reported in different contexts (38–41). In addition, age-associated deterioration in antibody response to vaccination is not uniform between strains (4).
The antigenic sin might also contribute to the inconsistent findings when comparing vaccine response in different age cohorts. It refers to an immunological phenomenon which implies that the first influenza strain an individual encounters in life will affect the immune response to other influenza strains later in life (42). The antigenic sin theory may explain the relative protection of older adults against H1N1 strains, in contrast to H3N2 strains (43–45). To our knowledge, this phenomenon has not been explored yet in the context of CMV, but it could be important for further investigators to clearly identify the subtype(s) of influenza vaccination covered by their studies, whether or not in different age groups.

The way of administration of influenza vaccines may also affect the influence of CMV infection on vaccination response. Nowadays there is the choice between an intramuscular, intradermal or intranasal way of administration. For example, Derhovanessian et al. chose for an intradermally administered high-dose, split non-adjuvanted trivalent vaccine (Intanza, Sanofi Pasteur) (16). Intradermal immunization leads to a higher activation of dermal antigen-presenting cells, resulting in potent T-cell activation (46). Just like the intramuscularly administered subunit vaccine containing the MF59C.1 adjuvant (Fluad, Novartis), it seems that these vaccine types can induce higher antibody responses in elderly compared to other conventional vaccines (47).
Similarly the vaccine dosage is a factor that should be taken into account when comparing (future) studies on this topic. It has been proven that high-dose vaccines lead to higher antibody titers and deliver better clinical protection in the elderly (48–50).

A further remark is that the manner in which the change in antibody response after vaccination is assessed may critically influence the assignation of responder or non-responder status (51). Some participants could be incorrectly classified as non-responders, because of high pre-vaccination antibody titers. An antibody titer already high due to earlier vaccinations against the same strain does not need to increase further (WHO-criterium). Strindhall et al. concluded that their results were impaired by pre-existing protective titers (25). Because of possible erroneous classification, immunosenescence could have been over-estimated in some studies (51). Some studies did hold vaccination history into account. Trzonkowski et al. for example only selected participants without a history of influenza vaccination (26).

Most studies did not examine the effect of CMV on antibody persistence. However, antibody persistence is important since it

may contribute to clinical protection for over 6 to 18 months post vaccination (36,37). Peak antibody titers after vaccination depend mainly on short-lived plasma B-cells, whereas antibody persistence depends on memory B-cells and long-lived plasma cells (24). Antibody persistence may be a more meaningful measure of clinical protection (30).

Furthermore, whereas vaccine-specific serum antibodies are indeed a well-established correlate of protection for influenza infection, their effectiveness in the elderly has been questioned (52). There is evidence that antibody responses are not the best predictor of clinical efficacy in older adults (47). While lower antibody titers may translate to poorer clinical outcomes upon exposure to influenza (53), humoral responses are often deficient and no guarantee for immunity (35,54). T-cell responses to internal proteins of influenza virus may be better correlates of protection to influenza in older people than influenza antibodies (55–57). It has been reported that in the elderly, humoral and cellular responses against influenza vaccination do not necessarily correlate (58).

Although studies on the effect of CMV on influenza vaccination response show contradictory results, the potential severe effects of CMV infection in certain other fields are well known. Progress towards a clinically efficient CMV vaccine has been made in recent clinical trials, which show promising results for an adjuvanted glycoprotein B vaccine and DNA vaccines targeting glycoprotein B and pp65 (59–61). Currently two target populations are selected. The first main target population consists of pregnant women, to prevent congenital transmission of CMV which can cause severe neurological injury in newborns. A second goal is prevention of viremia and end-organ disease in solid organ and hematopoietic stem cell transplant patients (61). In the future a CMV vaccine could be applied to increase influenza vaccination response in the elderly. But now evidence is clearly still insufficient to vaccinate for this reason.

5. Conclusion

Studies on the effect of CMV infection on the humoral immune response to influenza vaccination showed contradictory results and were limited in number. The included studies selected heterogeneous populations of different age with different vaccination histories, to which various seasonal vaccines were administered at different times. This made it difficult to directly compare the current literature, and to draw general clear conclusions. Integration of these studies together with (hopefully) future ones, should eventually lead to a better understanding of this subject. Until now, the overall effect of CMV infection on the influenza vaccine response remains questionable.

References

1. World Health Organization. Seasonal influenza fact sheet. December 2017. Available via: www.who.int/mediacentre/factsheets/fs211/en/. Consulted 6 January 2018.
2. Darvishian M, et al. After adjusting for bias in meta-analysis seasonal influenza vaccine remains effective in community-dwelling elderly. J Clin Epidemiol 2014; 67: 734-744.
3. Nichol K, Nordin J, Nelson D. Effectiveness of influenza vaccine in the community-dwelling elderly. N Engl J Med 2007; 357(14): 1373-1381.
4. Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine 2006; 24(8): 1159-1169.
5. Mahnke Y, Saqr A, Hazenfeld S, et al. Age-related changes in durability and function of vaccine-elicited influenza-specific CD4(+) T-cell responses. Vaccine 2011; 29: 8606-8614.

6. Gross P, Hermogenes A, Sacks H, et al. The efficacy of influenza vaccine in elderly persons. A meta-analysis and review of the literature. Ann Intern Med 1995; 123: 518-527.

7. Pawelec G, McElhaney J, Aiello A. The impact of CMV infection on survival in older humans. Curr Opin Immunol 2012; 24(4): 507-511.

8. Derhovanessian E, Larbi A, Pawelec G. Biomarkers of human immunosenescence: impact of cytomegalovirus infection. Curr Opin Immunol 2009; 21(4): 440-445.

9. Badami K, McQuilkan-Bickerstaffe S, Wells J. Cytomegalovirus seroprevalence and 'cytomegalovirus-safe' seropositive blood donors. Epidemiol Infect 2009; 137: 1776-1780.

10. Cannon M, Schmid D, Hyde T. Review of CMV seroprevalence and demographic characteristics associated with infection. Rev Med Virol 2010; 20: 202-213.

11. Staras S, Dollard S, Radford K. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. Clin Infect Dis 2006; 43(9): 1143-1151.

12. Collerton J, Martin-Ruiz C, Davies K, et al. Frailty and the role of inflammation, immunosenescence and cellular ageing in the very old: Cross-sectional findings from the Newcastle 85+ Study. Mech Ageing Dev 2012; 133(6): 456-466.

13. Wills M, Poole E, Lau B. The immunology of human cytomegalovirus latency: could latent infection be cleared by novel immunotherapeutic strategies? Cell Mol Immunol 2015; 12(2): 128-138.

14. Modesti P, Reboldi G, Cappuccio F, et al. Panethnic differences in blood pressure in Europe: a systematic review and meta-analysis. PLoS ONE 2016; 11(1): e0147601.

15. den Elzen W, Vossen A, Cools H. Cytomegalovirus infection and responsiveness to influenza vaccination in elderly residents of long-term care facilities. Vaccine 2011; 29(29-30): 4869-4874.

16. Derhovanessian E, Theeten H, Hähnel K. Cytomegalovirus-associated accumulation of late-differentiated CD4 T-cells correlates with poor humoral response to influenza vaccination. Vaccine 2013; 31(4): 685-690.

17. Frasca D, Diaz A, Romero M. Cytomegalovirus (CMV) seropositivity decreases B cell responses to the influenza vaccine. Vaccine 2015; 33(12): 1433-1439.

18. Furman D, Jojic V, Sharma S, et al. Cytomegalovirus infection enhances the immune response to influenza. Sci Transl Med 2015; 7(281): 281ra43.

19. Goldeck D, Theeten H, Hassouneh F, et al. Frequencies of peripheral immune cells in older adults following seasonal influenza vaccination with an adjuvanted vaccine. Vaccine 2017; 35(34): 4330-4338.

20. Haq K, Fulop T, Tedder G, et al. Cytomegalovirus seropositivity predicts a decline in the T cell but not the antibody response to influenza in vaccinated older adults independent of type 2 diabetes status. Gerontol A Biol Sci Med Sci 2016; 72(9): 1163-1170.

21. McElhaney J, Garneau H, Camous X, et al. Predictors of the antibody response to influenza vaccination in older adults with type 2 diabetes. BMJ Open Diabetes Research and Care 2015; 3(1): e000140.

22. Merani S, Kuchel G, Kleppinger A. Influenza vaccine-mediated protection in older adults: Impact of influenza infection, cytomegalovirus serostatus and vaccine dosage. Exp Gerontol 2017; pii: S0531-5565(17)30456-4. doi: 10.1016/j.exger.2017.09.015. [Epub ahead of print].

23. Moro-García M, Alonso-Arias R, López-Vázquez A, et al. Relationship between functional ability in older people, immune system status, and intensity of response to CMV. Age 2012; 34: 479-495.

24. Reed R, Greenberg R, Segerstrom S. Cytomegalovirus serostatus, inflammation, and antibody response to influenza vaccination in older adults: The moderating effect of beta blockade. Brain, Behavior, and Immunity 2017; 61: 14-20.

25. Strindhall J, Ernerudh J, Mörner A, et al.

Humoral response to influenza vaccination in relation to pre-vaccination antibody titres, vaccination history, cytomegalovirus serostatus and CD4/CD8 ratio. Infect Dis 2016; 48(6): 436-442.

26. 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): 3826-3836.

27. Turner J, Campbell J, Edwards K, et al. Rudimentary signs of immunosenescence in Cytomegalovirus-seropositive healthy young adults. Age 2014; 36(1): 287-297.

28. Wald A, Selke S, Magaret A. Impact of human cytomegalovirus (CMV) infection on immune response to pandemic 2009 H1N1 influenza vaccine in healthy adults. J Med Virol 2013; 85(9): 1557-1560.

29. Trzonkowski P, Mysliwska J, Godlewska B, et al. Immune consequences of the spontaneous pro-inflammatory status in depressed elderly patients. Brain, Behavior, and Immunity 2004; 18(2): 135-148.

30. Merani S, Pawelec G, Kuchel G. Impact of aging and cytomegalovirus on immunological response to influenza vaccination and infection. Front Immunol 2017; 8: 784. doi: 10.3389/fimmu.

31. Eto D, Lao C, DiToro D, et al. IL-21 and IL-6 are critical for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4 T cell (Tfh) differentiation. PLoS One 2011; 6(3): e17739.

32. Frasca D, Diaz A, Romero M, et al. Intrinsic defects in B cell response to seasonal influenza vaccination in elderly humans. Vaccine 2010; 28(51): 8077-8084.

33. Frasca D, Diaz A, Romero M, et al. Unique biomarkers for B-cell function predict the serum response to pandemic H1N1 influenza vaccine. Int Immunol 2012; 24(3): 175-182.

34. Goronzy J, Fulbright J, Crowson C, et al. Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals. J Virol 2001; 75: 12182-12187.

35. Saurwein-Teissl M, Lung T, Marx F, et al. Lack of antibody production following immunization in old age: association with CD8(+)CD28(-) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. J Immunol 2002; 168: 5893-5899.

36. Zhu J, Shearer G, Norman J, et al. Host response to cytomegalovirus infection as a determinant of susceptibility to coronary artery disease: sex-based differences in inflammation and type of immune response. Circulation 2000; 102(20): 2491-2496.

37. McElhaney J, Kuchel G, Zhou X. T-cell immunity to influenza in older adults: a pathophysiological framework for development of more effective vaccines. Front Immunol 2016; 7: 41.

38. Sheridan P, Paich H, Handy J, et al. Obesity is associated with impaired immune response to influenza vaccination in humans. Int J Obes 2012; 36(8): 1072-1077.

39. Talbot H, Coleman L, Crimin K, et al. Association between obesity and vulnerability and serologic response to influenza vaccination in older adults. Vaccine 2012; 30(26): 3937-3943.

40. Phillips A, Gallagher S, Carroll D. Preliminary evidence that morning vaccination is associated with an enhanced antibody response in men. Psychophysiology 2008; 45(4): 663-666.

41. Langlois P, Smolensky M, Glezen W. Diurnal variation in responses to influenza vaccine. Chronobiol Int 1995; 12(1): 28-36.

42. Kim J, Skountzou I, Compans R. Original antigenic sin responses to influenza viruses. J Immunol 2009; 183: 3294-3301.

43. Boon A, deBeauchamp J, Krauss S, et al. Cross-reactive neutralizing antibodies directed against pandemic H1N1 2009 virus are protective in a highly sensitive DBA/2 mouse influenza model. J Virol 2010; 84(15): 7662-7667.

44. Remschmidt C, Rieck T, Bödeker B. Application of the screening method to

monitor influenza vaccine effectiveness among the elderly in Germany. BMC Infect Dis 2015; 15:137.

45. Bragstad K, Emborg H, Fischer T, et al. Low vaccine effectiveness against influenza A(H3N2) virus among elderly people in Denmark in 2012/13 – a rapid epidemiological and virological assessment. Euro Surveill 2013; 18(6): 11-17.

46. Arnou R, Icardi G, De Decker M, et al. Intradermal influenza vaccine for older adults: a randomized controlled multicenter phase III study. Vaccine 2009; 27(52): 7304-7312.

47. Camilloni B, Basileo M, Valente S. Immunogenicity of intramuscular MF59-adjuvanted and intradermal administered influenza enhanced vaccines in subjects aged over 60: a literature review. Human Vaccines Immunother 2015; 11: 553-563.

48. DiazGranados C, Dunning A, Jordanov E. High-dose trivalent influenza vaccine compared to standard dose vaccine in elderly adults: safety, immunogenicity and relative efficacy during the 2009-2010 season. Vaccine 2013; 31(6): 861-866.

49. Falsey A, Treanor J, Tornieporth N. Randomized, double-blind controlled phase 3 trial comparing the immunogenicity of high-dose and standard-dose influenza vaccine in adults 65 years of age and older. J Infect Dis 2009; 200(2): 172-180.

50. DiazGranados C, Dunning A, Kimmel M, et al. Efficacy of high-dose versus standard-dose influenza vaccine in older adults. N Engl J Med 2014; 371(7): 635-645.

51. Mosterín Höpping A, McElhaney J, Fonville J. The confounded effects of age and exposure history in response to influenza vaccination. Vaccine 2016; 34(4): 540-546.

52. McElhaney J, Effros R. Immunosenescence: what does it mean to health outcomes in older adults? Curr Opin Immunol 2009; 21: 418-424.

53. Ohmit S, Petrie J, Cross R. Influenza hemagglutination-inhibition antibody titer as a correlate of vaccine-induced protection. J Infect Dis 2011; 204: 1879-1885.

54. Haq K, McElhaney J. Immunosenescence: influenza vaccination and the elderly. Curr Opin Immunol 2014; 29: 38-42.

55. Sridhar S, Begom S, Bermingham A, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. Nat Med 2013; 19(10): 1305-1312.

56. Wilkinson T, Li C, Chui C, et al. Preexisting influenza specific CD4+ T cells correlate with disease protection against influenza challenge in humans. Nat Med 2012; 18: 274-280.

57. Zens K, Farber D. Memory CD4 T cells in influenza. Curr Top Microbiol Immunol 2015; 386: 399-421.

58. Hsu J, Phoon M, Koh G, et al. Comparison of neutralizing antibody and cell-mediated immune responses to pandemic H1N1 2009 influenza virus before and after H1N1 2009 influenza vaccination of elderly subjects and healthcare workers. Int J Infect Dis 2012; 16(8): 621-627.

59. Schleiss M, Permar S, Plotkin S. Progress toward development of a vaccine against congenital cytomegalovirus infection. Clin Vaccine Immunol 2017; 24(12): e00268-17.

60. Xia L, Su R, An Z. Human cytomegalovirus vaccine development: Immune responses to look into vaccine strategy. Hum Vaccin Immunother 2018; 14(2): 292-303.

61. Anderholm K, Bierle C, Schleiss M. Cytomegalovirus Vaccines: Current Status and Future Prospects. Drugs 2016; 76(17): 1625-1645.

Appendix A

Protocol

approved

What is the effect of cytomegalovirus infection
on the immune response to influenza vaccination?

1) Background

Cytomegalovirus (CMV) is a common herpesvirus with a prevalence increasing with age. In immunocompromised patients, for example after transplantation, this virus can cause serious morbidity and mortality. In immunocompetent individuals on the other hand, CMV infection is often unnoticed. Despite the possibility of our immune system to conquer a primary infection, the virus remains present in our bodies (latency). CMV reactivation occurs intermittently in our lives, which leads to age-associated changes in our immune system; an incompletely clarified process called immunosenescence.
The functioning of our immune system declines as we age, which makes the elderly more vulnerable for infectious diseases, and which leads to a decreased immune response to vaccinations. Annual influenza vaccination is recommended for every healthy 50-/65-plus year old in Belgium.
What is already known about the effect of CMV on the humoral immune response to influenza vaccination in immunocompetent individuals?

2) Objectives

To identify, obtain and investigate all studies that evaluate the effect of CMV on the humoral response to influenza vaccination in immunocompetent patients.

3) Methods

a. Eligibility criteria
   i. **P**opulation: immunocompetent patients, no HIV, no history of transplant
      Covariate: age
   ii. **I**ntervention: CMV seropositivity, according to the definition of the included studies
      Covariates: CMV titer, according to the definition of the included studies; other measures for CMV activity
   iii. **C**omparison: CMV seronegativity, according to the definition of the included studies
   iv. **O**utcome: all forms of humoral immune response to influenza vaccination, with particular interest in immune response defined in terms of seroprotection, seroconversion or difference in geometric mean titer, in a three to five week period after vaccination
      Covariates: type of influenza vaccine, way of administration, antibody persistence

b. How to conduct the literature study?
   We will conduct a search in MEDLINE and EMBASE.
   Search strategies will be named in the Appendix.
   i. We will select studies with a publication date between 01/01/2003 and 31/12/2017.
   ii. The selected studies will be written in English.

iii. Type of studies: we will include all types of studies, except for case reports.

c. How to collect data?

i. Study selection

Two review authors, Silke Coopman (SC) and Frederik Vanstraelen (FV), will independently screen the search results for relevance on the basis of title and abstract. In case of doubt articles will be retained. Duplicates between the two databases will be removed. The remaining articles will be evaluated on the basis of the full article text using in- and exclusion criteria. Except for case reports, all types of studies are eligible for inclusions. Only studies assessing the impact of CMV on the humoral influenza vaccination response in immunocompetent individuals will be included. Immunocompromised patients such as patients with human immunodeficiency virus or transplant patients will be excluded. CMV serostatus, according to the definition of the particular studies, is the exposure of interest. The primary endpoint of interest is the humoral response to influenza vaccination three to five weeks after administration, which is appointed as the peak antibody response. In particular, interest goes out to articles in which the immune response is expressed in terms of seroprotection, seroconversion or difference in geometric mean titer (GMT). Reaching a hemagglutination-inhibition (HI) antibody titer of at least 40 is defined as seroprotection. Seroconversion is stated as an increase in HI antibody titer of at least fourfold. However, other definitions to ascertain the humoral response to influenza vaccination will be accepted. Besides the antibody response after, we are also interested in the persistence of the antibody response beyond the peak antibody response.

Discrepancies will be discussed and, if required, a third review author, Catharina Matheï (CM), will be consulted.

ii. Data extraction and management

Data extraction will be carried out by one review author (SC), and will independently be checked by a second review author (CM), using a standardized data-collection form. The extracted data will consist of last name of the first author, publication year, study region and setting, number of cases and controls, age and sex distribution, method of exposure and endpoint assessment, type of influenza vaccination, the way of administering the vaccine, CMV status and humoral response to influenza vaccination.

d. How to deal with study bias?

A modified version of the Newcastle-Ottawa Quality Assessment Scale adapted to cross-sectional studies will be applied for quality evaluation. On the basis of seven questions studies will be assessed with regard to appropriateness of research design, recruitment strategy, response rate, representativeness of sample, objectivity/reliability of outcome determination, power calculation provided, and appropriate statistical analyses.

e. How to analyze the results?

Analyses will be performed by using Review Manager 5.

Heterogeneity between studies will be evaluated by the chi-square-based *Q* statistical test. In case of statistical study heterogeneity a random-effect

model (the DerSimonian and Laird method) will be applied. A fixed-effects model (the Mantel-Haenszel method) will be used if statistical study heterogeneity is not observed. If possible, subgroup analyses will be carried out to further find heterogeneity source by age.

f. Additional information
    a. Acknowledgements: none
    b. Contributions of author: Silke Coopman, Catharina Matheï, Frederik Vanstraelen
    c. Declarations of interest: none
    d. Sources of support: none

Appendix B

NEWCASTLE-OTTAWA QUALITY ASSESSMENT SCALE

(adapted for cross sectional studies)

Based on Modesti P, Reboldi G, Cappuccio F, et al. Panethnic differences in blood pressure in Europe: a systematic review and meta-analysis. PLoS ONE 2016; 11(1): e0147601.

Selection: (Maximum 4 stars)

1) Representativeness of the sample:
    a) Truly representative of the average in the target population. * (all subjects or random sampling)
    b) Somewhat representative of the average in the target population. * (nonrandom sampling)
    c) Selected group of users.
    d) No description of the sampling strategy.
2) Sample size:
    a) Justified and satisfactory. *
    b) Not justified.
3) Non-respondents:
    a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory. *
    b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory.
    c) No description of the response rate or the characteristics of the responders and the non-responders.
4) Ascertainment of the exposure (risk factor):
    a) Validated measurement tool. *
    b) Non-validated measurement tool, but the tool is available or described.*
    c) No description of the measurement tool.

Comparability: (Maximum 2 stars)

1) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled.
    a) The study controls for the most important factor (select one). *
    b) The study control for any additional factor. *

Outcome: (Maximum 2 stars)

1) Assessment of the outcome:
    a) Independent blind assessment. *
    b) Record linkage. *
    c) Self report. *
    d) No description.
2) Statistical test:
    a) The statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). *
    b) The statistical test is not appropriate, not described or incomplete.