The effect of cytomegalovirus infection on frailty in immunocompetent elderly: a systematic review

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Het effect van cytomegalovirusinfectie (CMV) op kwetsbaarheid bij ouderen: een systematische review

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Context: Het frailty ofwel kwetsbare-oudere syndroom is een veelvoorkomend probleem dat zowel in het heden als in de toekomst samen met de toenemende vergrijzing een groot probleem zal vormen. Een verhoogde kwetsbaarheid is een risicofactor voor een verhoogd valrisico, verminderde mobiliteit, een verminderde autonomiteit, een toename in ziekenhuisopnames en sterfte. Deze gevolgen zijn gerelateerd aan leeftijdsgebonden immunologische veranderingen, geduid in de literatuur als immunosenescentie. Het is bewezen dat het cytomegalovirus (CMV) een rol speelt in dit complex proces. Aangezien het grootste deel van de ouderen positief is voor het cytomegalovirus, zou een mogelijke link tussen frailty en CMV een aanzet kunnen zijn tot verder onderzoek naar preventie of behandeling van kwetsbaarheid bij ouderen. Er kan gedacht worden aan vaccinatie tegen CMV of het ontwikkelen van antivirale therapie om immunologische veroudering tegen te gaan.

Onderzoeksvraag: Wat is het effect van cytomegalovirusinfectie op het ontstaan van frailty bij ouderen?

Methode: Een systematisch literatuuronderzoek werd uitgevoerd in MEDLINE om alle studies te identificeren over het effect van CMV seropositiviteit op het ontwikkelen van kwetsbaarheid bij immunocompetente ouderen. De gevonden resultaten werden gepresenteerd in een tabel.

Resultaten: Acht studies werden geïncludeerd. Vijf studies maakten gebruik van de criteria volgens Fried, waarvan drie studies een positieve associatie aantoonden tussen CMV infectie en kwetsbaarheid bij ouderen. Twee studies toonden geen of een negatief effect van CMV op kwetsbaarheid volgens de Fried criteria. Drie studies die gebruik maakten van enkele componenten van de Fried criteria werden onderzocht. Deze studies toonden geen associatie tussen deze componenten (wandelsnelheid en handknijpkracht) en CMV infectie.

Conclusies: Een laag aantal studies in verband met CMV en kwetsbaarheid volgens Fried criteria werd gevonden. Heterogeniciteit tussen deze studies was groot en de resultaten waren tegenstrijdig. Algemeen kon geen duidelijk effect van CMV infectie op kwetsbaarheid volgens de Fried criteria aangetoond worden. Verder onderzoek is noodzakelijk.

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The effect of chronic cytomegalovirus infection on frailty in the elderly population: a systematic review

Frederik Vanstraelen, Catharina Matheï, Silke Coopman

Abstract

Background Frailty syndrome in the elderly population is associated with a considerable risk for morbidity and mortality. As we age the functionality of our immune system declines, what makes the elderly population vulnerable and frail. Frailty has major health implications for the elderly population and acts as an independent risk factor for poor outcomes like: increased falls, a decline in mobility, disability, increased hospitalization and even death.
The prevalence of cytomegalovirus (CMV) increases with age. CMV plays a role in this process due to age-associated changes in our immune system; called immunosenescence. In this review we focus on the effect of CMV infection on prevalent and incident frailty.

Methods A systematic review of literature was performed using MEDLINE to identify all studies concerning the effect of positive CMV serostatus or titer on frailty measured by the Fried criteria or separate Fried components. Data were collected and presented in an overview in this article.

Results Eight studies met the inclusion criteria. Five studies used Fried criteria of which three studies showed a positive association of CMV infection on frailty. Two studies showed none or negative effects of CMV on Frailty. Three studies used components of Fried criteria, no significant associations were found between these components and CMV infection.

Authors' conclusions A low number of studies on CMV and frailty was found. Heterogenicity between studies showed discrepant results. The overall effect of CMV infection on frailty remains questionable. Further research is needed.

1. Introduction

Physical frailty is an important medical syndrome defined as a clinical state of increased vulnerability when exposed to a stressor (1, 2). This increased vulnerability includes developing disability, dependency, falls, need for long-term care, and mortality (2).
It is stated that the overall prevalence of frailty is 7% in community dwelling elderly aged 65 and older which increases as high as 30-40% of adults aged 80 years and older. It is also estimated that the majority of elderly nursing home residents in the United States are frail (2, 3). Frailty is a complex and multifactorial syndrome which is often defined differently in articles. A standardized and valid method for screening was developed by Fried et al (3). According to Fried et al. frailty is defined as a biologic syndrome which decreases reserve and resistance to stressors, resulting in cumulative declines causing vulnerability and loss of homeostasis in elderly (3). Fried et al. developed the Fried criteria which consists of 5 components. Frail is classified as the presence of ≥ 3 of 5

components, pre-frail as 1-2 of 5 components and non-frail as 0 of 5 components. The components are the following: unintentional weight loss, weak grip strength, exhaustion, slow walking speed, and low level of activity.
Apart from the use of frailty according to Fried criteria, studies who investigated separate components of the Fried criteria are also in our interest. Previous studies linked elevated inflammatory markers such as Interleukin-6 (IL-6) and C-reactive protein (CRP) to frailty, which means a pro-inflammatory state of the body is thought to play a central role leading frailty (4-6). There is evidence cytomegalovirus (CMV) infection could be a significant factor/stressor influencing frailty in elderly, due to its influence on immunosenescence (7-10).

Cytomegalovirus (CMV) is a common beta-herpesvirus that resides in epidemic proportion with a seroprevalence increasing with age. Numbers range from 40% in younger individuals to more than 70-83% in people over 60 years old and up to 90% in 80-plus-year-olds in the USA. Also, CMV prevalence seems to be higher in non-whites, women and persons of lower socio-economic status (SES) (11-13).
In immunocompromised patients, infection with CMV may lead to severe morbidity and mortality. In immunocompetent individuals CMV is often seen unnoticed. Despite the possibility of our immune system to conquer the primary infection, the immune system is unable to eliminate the virus, resulting in a latent infection.
Several studies hypothesize the impact of CMV on the immune system is due to subclinical reactivation throughout our lives. Other theories include that CMV infection is accompanied by a higher level of low-grade chronic inflammation that provides an ongoing stimulation of the immune system in older adults, called 'inflammaging' (7, 14-19). This ongoing pro-inflammatory state is associated with oligoclonal expansions of highly differentiated CMV specific T-cells, filling up the immunological space. It is suggested that the containment of CMV and its recurring reactivation requires considerable immunological resources which eventually get exhausted. This may cause a chronic pro-inflammatory state of the body which is linked to other comorbidities like frailty, cardiovascular disease and reduced influenza vaccination response (20, 21).

Why is it important to do this review?

The importance of frailty has been highlighted. A potential negative effect of CMV infection on frailty has to be investigated. The elderly population is the fastest growing segment in our society. A clear association between CMV infection and frailty could potentially open the door to the prevention of frailty by for example developing vaccines or antiviral therapy against CMV.

2. Methods

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

2.1 Search strategy

We searched through MEDLINE to identify articles published in English between 1 January 2002 and 31 December 2017. Keywords used in our search were cytomegalovirus and frailty.

2.2 Selection of studies

Two review authors (FV and SC) independently screened the search results for relevance based on title and abstract. In case of doubt articles were retained. Duplicates between the two databases were removed. The chosen articles were evaluated on the basis of full article text.
All types of studies were eligible for inclusion, except for case reports. Only studies that assessed the impact of CMV-infection on frailty according to Fried criteria were included; studies conducted with immunocompromised populations such as patients infected with human immunodeficiency virus or transplant patients were excluded.
The exposure of interest was CMV serostatus. The definition of positive CMV serostatus as applied in the selected articles was accepted.

The primary endpoint of interest was the significance of CMV serostatus on frailty measured by Fried criteria.
In particular, we were interested in articles who give clear note of age, immune responses to CMV infection like CMV serostatus, CMV titer and a clear definition of frailty.
Next to frailty according to Fried criteria, studies who investigated separate components of the Fried criteria are also in our interest. The five components are described above.
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, study characteristics including Fried criteria components, CMV serostatus and CMV-antibodies. Co-inflammatory markers influencing CMV response and frailty outcome were also noted. Data extraction was carried out by one review author (FV), and was independently checked by a second review author (CM).

2.4 Data analysis

Articles selected for analysis are presented in this article. A clear overview of the results are presented.

3. Results

A total of 516 references to publications were initially retrieved. After screening on the basis of title and abstract, and after removal of duplicates, 43 articles were selected. After reading the full article text, 8 articles were included in our review (6, 20, 22-30). The selection and inclusion of studies is displayed in a flowchart in Figure 1.

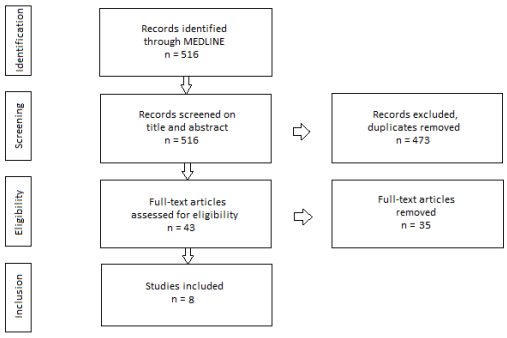

Figure 1 flow diagram of the study selection.

3.1 Characteristics of the included studies

Table 1 gives an overview of the characteristics and main findings of the 8 chosen articles. In total, 6364 subjects were investigated. Of which 2564 subjects were evaluated according to the Fried criteria and 3800 according to separate Fried outcomes. Sample sizes varied from 75 to 1490 participants(22, 24).

The method of selection of the population was not always clear. There was a variation in study settings from medical care centers based on zip codes to a group of general practitioner centers including institutionalized and home residents to academic hospitals. Not all studies made a clear note on study settings.
Three out of five studies who used the Fried criteria had a female population only(6, 22, 29). Two out of five studies who used the Fried criteria classified their population according to gender(20, 28). All studies who used components of Fried criteria (Handgrip strength and Walking pace) classified their population according to gender(24, 26, 30). In total, 64.9% or 4136 out of 6364 participants were female.
Two studies (22, 24) included young as well as elderly people.
Six out of eight selected articles limited their study to an older population, with a lower limit of 60 years old(6, 20, 26, 28-30). Mean age was reported in 7 studies, ranging between 68 and 86 for the elderly populations and 18 and 37 for the younger populations.

Variability was observed regarding measurement of frailty. Five out of eight studies (6, 20, 22, 28, 29) used the Fried criteria. Three out of eight studies (24, 26, 30) measured Handgrip strength or Walking speed. Two more studies (23, 27), not included in our analysis, used other frailty measurements like Frailty Index or Frailty Risk Score.

3.2 Frailty according to Fried in relation to CMV serostatus

Five studies investigated the effect of CMV seropositivity on frailty as measured by the Fried criteria (20, 22, 28-30) (Table 1, upper part). The chosen outcome was frailty (≥3 of 5 components) compared with robust or non-frail (0 out of 5 components). Associations were noted in Odds ratio's. One study investigated the incidence frailty, noted in hazard ratio's, in relation to serostatus in a 3-year follow-up(29). Adjusted odds ratio's were used to correct for confounding cofactors such as age, smoking, body mass index (BMI), diabetes mellitus (DMT2), congestive heart failure (CHF), education and socioeconomic status (SES) in 4 out of 5 studies (6, 20, 28, 29). One study showed no adjusted odds ratio's (22).
The effect of CMV serostatus and Interleukin-6 (IL-6), a pro-inflammatory biomarker, on frailty was measured in 4 out of 5 studies(6, 20, 28, 29). CMV serostatus in function of age was measured in 4 out of 5 studies(6, 22, 28, 29).

Three out of five studies concluded to a negative effect of CMV seropositivity on frailty measured by Fried criteria (6, 22, 29). One study revealed no effect on handgrip strength(6). One of these studies showed a negative effect of CMV in the older group, compared to a younger group(22).

One study showed a positive association between CMV seropositivity and frailty(28), another study showed no association(20).

IL-6 a biomarker for inflammation was associated with higher prevalent frailty(6). CMV seropositive subjects with high IL-6 were significant more frail(6).

In the studies with female and male populations, no clear association of frailty with sex was found(20, 28).

3.3 Frailty according to Fried in function of anti-CMV titer

In table 1, two studies that measured the association between CMV antibodies and frailty according to Fried criteria are noted(20, 28, 29).
In one study (29), CMV subjects with the highest quartile of antibody concentration had greater incidence of frailty. Incidence of frailty was significantly increased with higher CMV IgG concentrations vs seronegative individuals(29). The highest tertile of IL-6 was associated with the highest quartile of CMV IgG antibody concentration and frailty. In a 3-year longitudinal follow-up in the same study, comparison of seronegativity in women vs highest quartile of CMV antibody had a greater incidence of frailty. The same analysis was done without cardiovascular diseases (CVD): the highest quartile of CMV antibody concentrations vs CMV seronegativity still had higher incident frailty and mortality rates in women. In this study prefrailty was also associated with the highest CMV antibody concentrations on incident frailty in 3 years follow-up (29).

In the other study(28), CMV seropositivity was negatively associated with prevalent frailty in unadjusted and adjusted models. In the same study no association was found between prevalent frailty and high anti-CMV IgG concentrations. No association between prevalent frailty and high anti-CMV IgG titers was found.

3.4 Frailty components in relation to anti-CMV titer

Three studies (24, 26, 30) investigated the effect of CMV seropositivity on frailty measured by separate fried components (Table 1, lower part). Two studies researched Handgrip strength, one study researched walking pace. In these studies, no data of CMV antibody concentrations on frailty components are found.

In one study(30), the highest tertile of CMV antibody levels were associated with a below average outdoor walking pace. After adjusting for covariates (age, gender, income, education) this significance disappeared.

A study(26) on handgrip strength (HGS) and CMV serostatus/titer revealed no significant associations in either hand (no data). Furthermore, only in women, and not in men, an impact of CMV serostatus on HGS was found.
A significant negative correlation of CMV antibody titres with HGS was seen after calculating spearman's rho tests.

In another study(24), handgrip strength (HGS) was measured in relation to age, gender and CMV serostatus.
No impact of CMV serostatus on HGS, either in young or old individuals could be discerned. (no data)
Also there was no significant difference found between CMV- and CMV+ on serum cytokine levels. This suggests cytokine levels seem to have no effect on inflammatory response induced by CMV.

Year	1st Author	Population				Exposure		Frailty status				
		Characteristics	N	Age: Mean years	Sex	CMV IgG+, N%	CMV titer, N%	According to fried criter	According to Handgripstreng	According to walking spee	Other	comments
Including FRIED												
2005	Schmaltz H.	Prospective observational study of women health and aging studies (WHAS) I and II	735	74	Female	86						CMV subjects had significant higher frailty status (p=0.04) High IL-6 was significantly associated with higher prevalent frailty (p=0.05) Walking speed (p<0.01) is significant associated with CMV subjects Grip strength was not associated with CMV subjects (n=21%, p=0.08) In the unadjusted model CMV subjects were associated with Frailty (OR 3.2, 1.2-8.9 p=0.02) In the adjusted model (age, smoking, BMI, CHF, DM) CMV subjects were associated with frailty (OR 3.2, 1.1-9.2 p=0.03) In the adjusted model (model 2 + Socioeconomic status) there was no association found (OR 1.8, 0.6-5.1 p=0.27) CMV subjects with high IL-6 were significant more frail: unadjusted frail (OR 2.9, p<0.01), prefrail (OR 1.7 p=0.06). Adjusted frail (OR 2.1, p=0.01), adjusted incl. SES (OR 2, P=0.07)
2010	Wang G.	Prospective observational cohort study of women health and aging studies (WHAS) I and II	635	73.9	Female	85	lowest quartile: 22% second quartile: 20% third quartile: 20% highest quartile: 22.6%					CMV subjects in the highest quartile antibody concentrations had greater incidence of frailty (HR 3.81, 1.64-8.83) Frailty incidence increased with higher CMV IgG concentrations (18.4% in highest quartile) vs seronegative (5.6%) individuals (p=0.03) The highest tertile of IL-6 conc was associated with the highest quartile of CMV antibody concentration and frailty (adj OR= 5.18, 0.99, 27.05) In 3-year followup, women seronegative vs highest quartile of CMV antibody had greater incidence of frailty (91.8 vs 29.6 events per 1000pers/years: HR = 3.46, 95% CI 1.45,8.27) CVD free older women at highest quartile of CMV antibody vs CMV seronegative still had higher incident frailty and mortality rates (HR 2.37, 0.58-9.58)
2011	Mathel C.	Prospective observational cohort study of the BELFRAIL cohort study	567	84.7	Male and female	74	Low titer: 61% High titer: 39%					CMV subjects were negatively associated with prevalent frailty in unadjusted and adjusted models (Unadj. OR 0.52, 0.27-1.03; Adj. OR 0.45, 0.22-0.92; Adj 2. OR 0.37, 0.15-0.91). No association between prevalent frailty and high anti-CMV IgG titers. (Unadj. OR 1.51, 0.67-3.43; Adj. OR 1.55, 0.63-3.61; Adj. 2 OR 1.24, 0.39-3.87)
2012	Collerton J.	Cross-sectional data from newcastle 85+ study	552	85	Male and female	No data	No data					Fried measure: 21.6% (119/552) were classified frail, 60.3% (333/552) as pre-frail, and 18.1% (100/552) as robust No association between CMV serostatus and frailty (model 1 unadj. OR 0.94, 0.57-1.55 p=0.796; Model 2 OR 0.90, 0.54-1.49 p=0.680; adj. Model 3 OR 0.74, 0.42-1.28 p=0.276)
2017	Thomassini R.	Cross- sectional study Department of Physiotherapy Federal University of Minas Gerais in Brazil	75	60-80 (no mean age found)	Female	94.6						In the elderly group (n=75), 71 individuals (94.6%) were CMV IgG+/IgM- The 71 elderly women were classified as non-frail (n=26, 36.6%), pre-frail (n=23, 32.4%), and frail (n=22, 31%) 42/71 (59%) presented CMV DNA in their plasma (indicating CMV reactivation), in contrast with only 8/73 (10%) of the young women (p<0.0001) Elderly women classified as frail, pre-frail, and non-frail had CMV DNA in 18/22 (82%), 13/23 (56%), and 11/26 (42%) of cases, respectively. CMV DNA was detected significantly more frequently in frail elderly than in other elderly subgroups (p=0.007) The average CMV viral load in the elderly group was 2,887 copies/mL, whereas it was 273 copies/mL in the young group. The viral load were significantly higher in the elderly group than the young group (P<0.001) and higher in the frail elderly subgroup than in other subgroups (p=0.03) CMV subjects were associated with Aging (OR 11.77, RR 5.39, p<0.0001) CMV subjects were associated with Frailty (OR 6.13, RR 1.93, p=0.007) No significance was found with pre frailty
Seperate FRIED components												
2008	Aielo A.	Prospective cohort study of predominantly mexican americans	1444	70	Male and female	96	Low tertile: No data Middle tertile: No data High tertile: No data					In the unadjusted analysis, the highest tertile of CMV antibody levels were significantly associated with a below average outdoor walking pace (OR 1.418, 1.071-1.876 p=0.0144) After adjusting for covariates (age, gender, income, education) this significance disappeared (1.190, 0.888-1.595 p=0.244)
2013	Kilgour A.	Cross-sectional birth cohort of healty older men and women	866	72.5	Male and female	64	Mean titre Men 60.56 (0.47-214 IQR) Women 132.11 (2.40-277.78 IQR)					Handgripstrength was measured with Jamar Hydraulic hand dynamometer. The models using CMV status and antibody titre alone were not significantly associated with grip strength in either hand. (no data) A significant impact of CMV serostatus on HGS in women, but not in men (Spearmun's rho correlation: -2.81, p0.01) A significant negative correlation of CMV antibody titres with HGS (spearmun's rho correlation: -0.1, p=0.04)
2016	Goldeck D.	Berline BASE II cohort	1490	68	Male and female	59.7						Handgripstrength was measured in relation to age, gender and CMV serostatus. HGS was measured with Smedly Dynamometer (best of 6 results). No impact of CMV status on HGS, either in young or old individuals could be discerned. (no data) Also serum levels of cytokines were not significantly different in CMV- and CMV+ BASEII participants and therefore cannot account for possible health status affecting inflammatory response to CMV or induced by CMV.

Table 1: Effect of positive CMV serostatus or CMV titer on frailty according to Fried criteria or separate Fried outcomes. CMV= Cytomegalovirus, N: amount, p: p-value, IQR: InterQuartileRange, Green sphere: positive effect, yellow sphere: no effect, red sphere: negative effect, gray area: not investigated.

4. Discussion

To our knowledge, this seems to be the first systematic review investigating the impact of cytomegalovirus infection on frailty.
Despite the well-known effects of CMV infection on the immune system and the effects of frailty in the elderly population, it is notable that the number of studies researching this subject is limited. Not much prospective and longitudinal studies on this topic are conducted. Furthermore, the included studies showed contradictory results regarding the impact of CMV infection on frailty. No real explanation for these findings could be found. Although between these studies there were differences in study characteristics, a heterogenicity in the CMV seropositive older population can be a possible explanation. In earlier studies the possibility of a survival effect was suggested(28).

Findings for the Fried model are conflicting. Two studies concluded a positive finding for CMV seropositivity and prevalent frailty(6, 22). The authors of these studies hypothesize that prevalent frailty has a significant interaction with high levels of IL-6. The synergy between IL-6 and CMV can be seen as prove of a heightened pro-inflammatory immune response (4). It is assumed inflammatory cytokines such as IL-6, TNF-α, and IL-1 can reactivate CMV from latency to active viral replication and shedding (31, 32). The CMV-infected women with elevated IL-6, may have had chronic inflammation caused by CMV, which could explain higher prevalent frailty. However, it remains unclear how CMV infection acquired in early adulthood, contributes to functional decline later in life. It is also unclear how inflammation affects this process and whether a certain “dose” of inflammation is required(6).
Wang et al. (29) confirmed these findings and also found a positive association for high anti-CMV IgG titres and incident frailty(29). They hypothesize that more frequent viral reactivations or a longer infection cause higher CMV antibody concentrations. The higher cumulative frequency of viral reactivation could in turn represent a higher inflammatory burden. The effect of this accumulated burden could result in the manifestation of frailty. Also, in this study IL-6 is found to have a potential modifying effect on CMV infection and frailty. The synergy between IL-6 and CMV infection on frailty suggest inflammatory activation of non-CMV origin or CMV itself(2, 33). The requirement for high IL-6 concentrations could explain why not a one-to-one correspondence between CMV infection and older women was found. A different hypothesis suggested by the author would be that CMV reactivation, measured in higher antibody concentrations, is a by-product of non-CMV-related pathophysiologic changes in frail older women. These data should be confirmed in men, and a longitudinal follow up >3 years Is needed to capture the long-term effect of CMV infection on the development of frailty(29).
A smaller study(22), compared old and young women in relation to CMV infection and frailty. This study showed a positive association between CMV and aging as well as CMV and frailty. The Viral load was significantly higher in elder women compared to the younger group. CMV reactivation was significant higher in the elder group compared to the young. There was no adjustment for confounders in this study. qPCR was used to validate CMV reactivation, a biomarker for immunosenescence.

In contrary, according to Matheï et al. an inverse association between CMV seropositivity and prevalent frailty was found. No associations were found with anti-CMV IgG titres(28). The findings of this study are not consistent with previous results. The author suggests that the BELFRAIL population was considerably older than previous studies. And a survival effect would be susceptible to the long-term effects of CMV exposure. From this perspective CMV would predispose frailty instead of causing it. A survival effect may have resulted in an overrepresentation of elderly individuals who are CMV IgG seropositive but do not have persistent CMV infection, which indicates they are not susceptible to long term deleterious effects of persistent CMV infection(28).

Findings for separate components of the Fried model show no great associations between CMV infection and Hand grip strength or slow walking speed.
Two studies investigated handgrip strength (HGS) in relation to CMV serostatus(24, 26). In the study of Kilgour et al. CMV seropositivity and antibody IgG concentrations was not significant associated with hand grip strength in either hand (no data presented). However, a negative correlation between CMV seropositivity and HGS was found in women, but not in men.
Goldeck et al. found no impact of CMV status on HGS, either in young or old individuals. (no data) Also serum levels of cytokines were not significantly different in CMV- and CMV+. Therefore cytokine levels are not associated affecting inflammatory response to CMV(26). The authors of these studies hypothesized that no association between CMV and HGS could indicate that latent CMV infection could lead to increased muscle loss over an extended period of time acquired from childhood and frequent reactivations of CMV throughout life, and that the titre reflects only the current situation, which may have less impact on muscle bulk. Latent CMV infection may contribute to immunosenescence and inflammaging, which may contribute to mechanisms for sarcopenia. When studying sarcopenia, it is important to consider rate of decline rather than solely cross-sectional measures. Therefore, future longitudinal studies are crucial to develop understanding in these mechanisms. Current results are consistent with those from the BELFRAIL study where CMV serostatus was not associated with frailty.

The study of Aiello et al.(30), was used to determine if CMV was associated with walking pace. Only the unadjusted model with the highest tertile for CMV antibody concentrations showed a significant association with below average outdoor walking pace. After adjustment for covariates (age, gender, income, education) this significance disappeared. This study did point out a consistent association between high CRP levels and walking pace. However, CRP levels were weakly correlated with CMV. According to the author it is possible that chronic health conditions associated with aging may concurrently lead to higher levels of CMV antibodies and functional impairments in aging population(30). A longitudinal study is needed to clarify the relationships among CMV antibody levels and physical decline. CMV infection may be an important indicator of physical functioning, this is consistent with other findings(6).

How do we explain these different observations?

A possible reason may be the complexity and heterogenicity of studies in this research topic. There is a great difficulty in comparing data between different aging studies. One must differentiate between old and very old. A great number of confounding factors, for example CMV and gender, need to be accounted for. In addition, a lot of technical difficulties need to be solved as well. Given differences in demographic and infection measures between studies, a direct comparison of results is often not possible.
In future studies it is necessary to analyze different pro-inflammatory mediators like IL-6, CRP, TNF-α to measure the effects on frailty(24). It is possible one or more mediators play a significant role in CMV-induced immunosenescence and frailty.

Not all articles measure frailty the same, and studies investigating frailty vary widely on the criteria used for diagnosis(26). Frailty is a complex syndrome, what makes it difficult to interpret. Fried et al. developed a validated instrument to measure frailty(2, 3). By using the Fried criteria, we tried to eliminate different frailty outcomes. The Fried components could be used separately to find articles useful to demonstrate a link between CMV infection and frailty.

Measurement of CMV seropositivity and viral load in elderly is not always comparable. Ideally, a standardized measurement for chronic CMV infection and reactivation is developed. Most studies correlate IgG antibodies concentrations to CMV with the grade of immunosenescence,

an indirect method to estimate viral replication. It is suggested by Thomasini et al. (22) to test paired samples to determine a significant rise in IgG titer. Other ways to directly measure viral reactivation is to use qPCR for IgG antibodies to CMV(22)or CMV DNA in the urine(30). CMV infection causes complex changes, in which the impact on pro-inflammatory mediators still needs to be researched. CMV has the ability to infect multiple types of cells (T lymphocytes, monocytes/macrophages, dendritic cells, epithelial cells, fibroblasts, endothelial cells, and muscle cells). Therefore, further direct or indirect methods need to be developed indicating CMV reactivation.

Interleukin-6 was associated with a pro-inflammatory state of the subjects. Increasing age is associated with an increase of IL-6 levels, particular after andropause and menopause(34, 35). The study of Schmaltz et al. and Wang et al. (6, 29) suggest a link between high IL-6 concentrations, CMV and frailty. As mentioned before analysis of different pro-inflammatory mediators is necessary in the future.

As pointed out before, study demographics were highly different between the included studies. Some studies only researched women other used other infection parameters. Studies based on the Fried criteria only including women showed a positive association between CMV infection and frailty. A difference when compared with studies that included both male and females, which show no association or a negative association between CMV and frailty. Further research with both men and women is therefore needed. Also, the mean age of studies with a positive association between CMV and frailty was lower than the studies that showed no or a negative association, possibly caused by a survival effect.

In the studies who measured separate Fried outcomes, no direct associations between CMV and these outcomes were found. All studies had high study populations, had both male or female participants and roughly the same age. Explanations, according to the author, why these components are not associated with CMV infection are written above.

Although studies on the effect of CMV on prevalent or incident frailty are contradictory, it remains important to investigate the role of CMV on immunosenescence and inflammaging. The complex nature of this virus makes a one-to-one association difficult. By understanding the effects of CMV on other fields than frailty, possible new associations can be made in the future. Further cross-sectional studies with validated instruments and demographic corresponding populations can possibly further confirm the link to CMV infection and frailty. Other studies researching CMV vaccination could potentially evaluate the effect in frailty in longitudinal studies. However, a clinically efficient CMV vaccine is still in development(31, 36, 37).

5. Conclusion

The hypothesis that CMV reactivation indirectly contributes to frailty needs to be further tested. The included studies showed contradictory results and were limited in number. The results of articles according to the Fried criteria showed high heterogenicity in populations and infection parameters. This made it difficult to compare the current literature. Based on current findings we cannot draw any clear conclusions. Current studies should be integrated with future ones, to get a more homogenous population. Than hopefully we get a better understanding of CMV infection associated with frailty. Until now, the overall effect of CMV infection on frailty remains questionable. A possible link is still present.

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Appendix A

Protocol
Approved

What is the effect of cytomegalovirus infection on frailty in immunocompetent elderly?

1) Background

Cytomegalovirus (CMV) is a common herpesvirus with prevalence increasing with age. In immunocompromised patients, for example after transplantation or individuals with HIV, the virus can cause serious morbidity and mortality. In immunocompetent individuals however, CMV infection is often seen unnoticed. Despite the possibility of our immune system to conquer the primary infection, CMV has the ability to become chronically present (latent) in our body.

During our lives CMV reactivation, which occurs asymptomatic in our body, leads to age-associated changes in our immune system; an incompletely clarified process called immunosenescence based on 'inflamm-aging'. A chronic pro-inflammatory state of the body influencing the immune response which is linked to other comorbidities like frailty, cardiovascular disease and reduced influenza vaccination response.

As we age, the functionality of our immune system declines, what makes the elderly population vulnerable and frail. Frailty has major health implications for the elderly population and acts as an independent risk factor for poor outcomes like: increased falls, a decline in mobility, disability, increased hospitalization and even death. Due to the increase in aging population, with an eye on the future, it is necessary to investigate the frailty syndrome and its possible association with CMV. Nowadays not much countermeasures are available to prevent upcoming frailty in the aging elderly population. Findings of significant associations between CMV and the frailty syndrome could potentially open the door for further research in preventing frailty (for example vaccines, antiviral treatment).

2) Objectives

To identify, obtain and investigate all studies that evaluate the effect of CMV on frailty in immunocompetent elderly.

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 (high vs low), 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: effects on frailty, according to the Fried criteria for frailty syndrome

Covariates: type of frailty criteria, other sorts of frailty measurements, sex, components of Fried criteria as separate outcome

b. How to conduct the literature study?
   We will conduct a search in MEDLINE.
   Search strategies will be named in the Appendix.
   i. We will select studies with a publication date between 01/01/2002 and 31/12/2017.
   ii. The selected studies will be written in English.
   iii. Type studies: we will include all types of studies, except for case reports.
c. How to collect data?
   i. Study selection
      Two review authors Frederik Vanstraelen (FV) and Silke Coopman (SC) will independently screen the search results for relevance on basis of title and abstract. In case of doubt articles will be retained. Duplicates between the databases will be removed. The remaining articles are evaluated in full-text using in-and exclusion criteria. Except for case reports, all types of studies will be eligible for inclusion. Only studies that assess the impact of CMV-infection on frailty according to Fried criteria will be included. Immunocompromised (HIV, transplants) populations will be excluded. CMV serostatus, noted according to the definition of the particular studies, is our exposure of interest.
      The primary endpoint of interest is the significance of CMV serostatus on frailty measured by Fried criteria.
      We are interested in articles who give clear note of age, immune responses to CMV infection like CMV serostatus, CMV titer and a clear definition of frailty.
      Based on the Fried criteria which consists of 5 components, frailty is classified as the presence of $\geq$3/5 components, pre-frail as 1-2/5 components and non-frail as 0/5 components.
      Besides the use of frailty according to Fried criteria, studies who investigated separate components of the Fried criteria are also in our interest.
      In case of discrepancies, 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(FV), and will independently be checked by a second review author (CM), using a standardized data-collection form. The extracted data will be summarized as follows: last name of the first author, publication year, study region and setting, number of cases and controls, age and sex distribution, study characteristics including Fried criteria components, CMV serostatus and CMV-antibodies. Co-inflammatory markers influencing CMV response and frailty outcome are also noted.

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?
   Articles selected for analysis will be presented in the article. A clear overview of the results will be presented. If possible, a meta-analysis will be conducted using Review manager 5.

f. Additional information
   i. Acknowledgements: none
   ii. Contributions of author: Frederik Vanstraelen, Cathy Matheï, Silke Coopman
   iii. Declarations of interest: none
   iv. Sources of support: none