No relation between CMV infection and mortality in the oldest old: results from the Belfrail study

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Abstract

Objective: previous studies have demonstrated an association between cytomegalovirus (CMV) infection and mortality in adults. In this prospective study, it was investigated whether these findings could be confirmed in the oldest old.


Results: seventy-six percent were anti-CMV seropositive of whom 37.5% had an anti-CMV IgG titre in the highest tertile (>250 IU/ml). After a median time of follow-up of 1,049 days, 127 deaths occurred. Cox proportional hazard models failed to show an association between CMV serostatus and all-cause mortality. Among persons seropositive for CMV, after adjusting for multiple confounders an anti-CMV in the highest tertile was statistically significantly associated with all-cause mortality (hazard ratio: 1.64, 95% confidence interval: 1.08, 2.48).

Conclusion: in contrast to previous findings, a positive CMV serostatus was not associated with an increased risk for all-cause mortality in this cohort of very old people. This is probably the result of a survival effect. CMV seropositive subjects with high anti-CMV titres were at higher risk for all-cause mortality compared with other individuals. This may reflect CMV infection reactivation to be more common in the end stages of life.

Keywords: aged, 80 and over, cytomegalovirus, mortality, older people

Introduction

Human cytomegalovirus (CMV) belongs to the human herpesviruses and is characterised by the ability to establish persistent infection using a variety of immune evasion mechanisms [1]. Worldwide high prevalence rates of CMV infection are observed, varying between 40 and 100%, depending on sociodemographic factors, e.g. level of education, socio-economic status, gender and age [2].

The routine diagnosis of CMV infection is based on the detection of anti-CMV immunoglobuline G (IgG) and/or anti-CMV immunoglobuline M (IgM). The presence of IgG indicates a past infection, whereas anti-CMV IgM is a marker of recent infection, current infection or reactivation. The individual anti-CMV titres are relatively constant over years. However, increases in anti-CMV IgG titre do occur in CMV-infected persons. Furthermore, an association between ageing, anti-CMV IgM seropositivity and height of anti-CMV IgG titre has been demonstrated. A probable explanation for these observations is that a latent CMV infection frequently reactivates and that the rate of reactivation increases with age [3].

Persistent CMV infection has long been considered to be relatively harmless that had to be feared only as a complication of conditions characterised by impaired immune functioning. In recent years, however, likewise many other infectious agents, CMV has emerged as an important determinant in the development of many chronic diseases.
A recent meta-analysis of 55 studies involving 9,000 cases and 8,608 controls indicates that CMV infection is associated with an increased risk of coronary heart disease [4]. Furthermore, CMV has been implied in the development of inflammatory bowel diseases, immunosenescence, frailty, physical and cognitive impairment in elderly [5–8]. The mechanisms behind these associations are not fully understood, but it is believed that CMV may contribute to the chronic inflammatory state underlying most chronic diseases as a result of periodic re-activations [9].

A first indication that CMV infection might be associated with survival came from the longitudinal OCTO/ NONA marker in the so-called immune risk profile (IRP), a cluster of immune parameters found to be a main predictor of survival in the very old.

More recently, the NHANES III and EPIC-Norfolk studies demonstrated that a positive CMV serostatus produces an increased risk for all-cause mortality, largely explained by an increase in cardiovascular deaths [11, 12]. In addition, the former study showed high levels of C-reactive protein (CRP) to strengthen this association, whereas the latter study demonstrated that levels of anti-CMV IgG correlated positively with risk of mortality.

The purposes of this study were to investigate whether the association between CMV seropositivity and mortality could be confirmed in the Belfrail cohort, a population of community-dwelling individuals aged 80 years and older in Belgium, and to study among CMV seropositives the relationship between all-cause mortality and anti-CMV IgG titres in conjunction with serum high-sensitivity C-reactive protein (hsCRP) titres.

**Patients and methods**

**Study design and population**

Data for this study were derived from the Belfrail study, a representative ongoing cohort study of community-dwelling individuals aged 80 years and older in Belgium. All participants in the study gave informed consent, and the Biomedical Ethics Committee of the Medical School of the Université Catholique de Louvain of Brussels approved the study. The study design, methods, recruitment of participants and characteristics of the cohort were previously described in detail [13]. Briefly, 29 general practitioner (GP) centres were asked to include patients ≥80 years of age. No other inclusion criteria were specified, and only three exclusion criteria were used: known severe dementia (mini-mental state examination <15), in palliative care and medical emergencies, such as acute-onset heart failure or decompensated heart failure.

Between 2 November 2008 and 15 September 2009, 567 subjects were included in the Belfrail study. Women represented 63.1% (n = 358) of the study population and had a mean age of 85.0 ± 3.9 years. Male participants had a mean age of 84.3 ± 3.3 years.

**Laboratory analyses**

A blood sample was collected in the morning after fasting, and serum samples were stored at −80°C. The UniCel® DxC 800 Synchron (Beckman-Coulter, Brea, CA, USA) was used to measure hsCRP. The CMV IgG concentrations were measured on the ARCHITECT® i2000SR (Abbott Diagnostics, Abbott Park, IL, USA) by optical density units [14]. The criteria for interpretation were as follows: positive ≥26.0 AU/ml and negative <6 AU/ml. The hsCRP and anti-CMV IgG titre were both because of their skewed distribution transformed into a binary variable as follows: a low titre was defined as a titre in the lowest or mid tertile (or <250 IU/ml for anti-CMV) and a high titre was defined as a titre in the top tertile (or 250 IU/ml or greater for anti-CMV).

**Outcomes**

Detailed follow-up questionnaires were sent to the participating GP centres 1.4 ± 0.26 and 3.0 ± 0.25 years after inclusion. The questionnaire included questions on mortality and cause of mortality. The mortality follow-up was available through September 2012.

**Covariates at baseline**

Non-cardiovascular morbidities were defined as the presence of thyroid problems, anaemia, asthma, chronic obstructive pulmonary disease, Parkinson's disease, arthritis, osteoarthritis, documented osteoporosis, malignancies, depression and renal insufficiency. Cardiovascular morbidities were defined as the presence of hypertension, diabetes mellitus, hyperlipidaemia, history of angina pectoris or myocardial infarction, known cardiomyopathy, history of transient ischaemic attack or cerebrovascular accident, peripheral arterial disease, history of decompensated heart failure, chronic atrial fibrillation or known valvular heart disease. The body mass index (BMI) was calculated based on a standardised measurement of weight and height. Smoking was dichotomised as having ever or never smoked.

**Statistical analysis**

Continuous baseline variables were summarised as means and standard deviations (SD). Between groups, comparisons were performed using t-tests for normally distributed data. Categorical variables were summarised as counts and percentages and were compared using χ²-tests or Fisher's exact test.

Cox proportional hazard models were used to estimate the confounder adjusted hazard ratio (HR) and 95% confidence intervals (CIs) for the association between baseline CMV serostatus and anti-CMV titre and time until death. Age, sex, level of education, BMI, history of smoking, cardiovascular and non-cardiovascular comorbidity and hsCRP were assessed as potential confounders because they have been demonstrated in previous studies to be related to the exposures or the outcomes. To assess whether hsCRP...
modified the relationship between CMV serostatus and anti-CMV titre, and mortality, interaction terms between hCRP and CMV serostatus and anti-CMV IgG titre were tested. Both interaction terms were not significant and therefore not included in the model.

The proportionality assumption was tested based on the Schoenfeld and scaled Schoenfeld residuals for all variables and showed no violation of the assumption.

**Results**

Serological data were available for 549 individuals. Seventy-six percent (n = 406) were found to be anti-CMV seropositive and of whom 37.5% had an anti-CMV IgG titre in the highest tertile or >250 IU/ml (n = 152). A positive serostatus was statistically significantly associated with female gender and low level of education. A high anti-CMV IgG titre was statistically significantly associated with female gender, increasing age and the presence of three or more non-cardiovascular morbidities (Table 1).

After a median time of follow-up of 1,049 days (range: 48–1,413 days), 127 deaths occurred: 30 (21.0%) in individuals who were CMV seronegative and 97 (76.4%) who were seropositive. The mortality rate did not differ statistically significantly between CMV seropositives and negatives (89.3, versus 77.0 events per 1,000 person-years; unadjusted HR: 1.17, 95% CI: 0.77, 1.71).

Compared with the subjects with low anti-CMV IgG titres, the mortality rate was statistically significantly higher in among subjects with high anti-CMV IgG titres (70.4 versus 123.9 events per 1,000 person-years; unadjusted HR: 1.72, 95% CI: 1.21, 2.45).

In the multivariable Cox regression model that adjusted for age, gender, level of education, smoking status, BMI, presence of cardiovascular comorbidity, presence of non-cardiovascular morbidities and hCRP titre, a high anti-CMV IgG titre remained statistically significantly related to mortality (HR: 1.64, 95% CI: 1.08–2.48). Other factors independently associated with higher mortality were female gender (HR: 1.06, 95% CI: 1.01, 1.12), the presence of two or more non-cardiovascular diseases (HR: 1.84, 95% CI: 1.19, 2.83) and a serum hCRP titre in the highest tertile (HR: 1.65, 95% CI: 1.09, 2.51).

Figure 1 shows the predicted survival curves from Cox proportional hazard models for different permutations of anti-CMV IgG and hCRP titre.

**Discussion**

In this study of a representative sample of 549 community-dwelling Belgian individuals aged 80 years and older, we demonstrated that CMV serostatus was not associated with mortality. However, among individuals testing positive for anti-CMV, an anti-CMV IgG titre in the highest tertile or ≥250 IU/ml was correlated with mortality even after adjustment for age, gender, level of education, smoking status, BMI, comorbidity and hCRP serum level.
The results of this study seem at first sight in contrast with the findings of two previous studies. Both the EPIC-Norfolk study based on a large UK population-based cohort and the NHANES study based on a large, nationally representative US population demonstrated an independent relationship between CMV seropositivity and all-cause mortality [11, 12]. We believe these apparently contrasting findings may be the result of a survival effect. Indeed, the former study concerned a younger population compared with this study including individuals aged 40–79 years at baseline. In the latter study, CMV’s main impact on mortality was seen in individuals aged 55–75 at the time of the survey whereas CMV imposed little increased risk in the most elderly (aged 75–90).

We observed an independent association between a high anti-CMV IgG titre and all-cause mortality. This is in line with the few previous studies that have investigated this relationship in the oldest old. However, these studies all concerned very selected populations such as older Latinos, women or individuals with stable cardiovascular disease [15–17]. In these populations, CMV was highly prevalent, and hence the discriminatory power to evaluate the effect of CMV serostatus on mortality was low. Our study is the first to show that in the oldest old high CMV titres but not CMV serostatus is associated with mortality.

This correlation between CMV-specific antibody levels and differential mortality risk in older adults suggests that heightened levels of CMV-specific circulating antibodies may reflect suboptimal cytotoxic T cell-mediated immune control and that IgG titres may function as a surrogate measure of virus reactivation. This is in line with the findings of some previous studies [18, 19]. The NONA study showed the number of different CD8 T-cell clonal expansions to increase with age and a shrinkage of the CMV-specific CD8 T-cell repertoire to be associated with increased mortality, suggesting that increasing numbers of CD8 clonal expansions may reflect a compensatory mechanism to maintain essential immune protection capability against CMV [18]. Wang et al. [19] furthermore showed the number of CMV-specific CD8 clones to be inversely related to anti-CMV IgG serum titres. We hypothesise that it is not the CMV infection per se, but the way the host’s immune system interacts with the virus that will determine whether or not individuals will experience adverse effects from the infection.

In this perspective, the Belfrare population of oldest old might be over-represented by individuals capable of controlling CMV and of preventing its reactivation and hence its detrimental effects. Eventually, however, in some the immune system may become exhausted—as an isolated event or as part of cumulative dysfunctions across multiple organ systems—allowing the CMV virus to reactivate and anti-CMV IgG titres to rise.

If subclinical reactivation of CMV contributes to the development of a proinflammatory profile, we would expect high anti-CMV IgG serum levels to be associated with increased hCRP titres. Indeed, several studies of older individuals have found a relationship between positive CMV serology or high CMV IgG antibody titres and high levels of CRP [17, 20]. In the Belfrare population, high levels of CRP were not significantly associated with positive CMV serology or high CMV IgG antibody titres [21]. The current study showed the association between a high anti-CMV IgG titre
A positive CMV serostatus was not associated with an increase in mortality—and mortality to be independent of the hCRP serum level. These findings suggest that CMV reactivation—apparent from increased anti-CMV IgG titres—in the oldest old may be rather a consequence of a failing immune system or general health deterioration than that it plays an important role in the aetiology of mortality.

This study has several strengths. First, a large population-based sample designed to be representatives of community-dwelling adults aged 80 and older in Belgium was used. Furthermore, the CMV seroprevalence in the Belfrail study (76%) was lower than the seroprevalence rates in elderly populations that have been observed in other studies. The lower probability of being CMV positive increased the power to make statistical inferences. A few limitations must also be considered. In this study CMV IgG antibody titres were measured once at baseline and CMV reactivation was not directly measured. Although it is hypothesised that higher CMV IgG antibody levels represent more frequent or intense subclinical CMV reactivation from latency, this has not been conclusively proven. Residual confounding might have also affected our results. Finally, any interpretation of the results needs to take survival selection into account.

To conclude, this is the first study to investigate the association between mortality and both CMV serostatus and height of anti-CMV IgG serum titres in the oldest old. In this study, we could not confirm the results of previous studies of younger populations showing a relationship between a positive CMV serostatus and mortality. In line with previous studies in selected population of elderly, high anti-CMV IgG titres were found to be associated with mortality.

We hypothesise that many among the oldest old represent a phenotype that is less susceptible for the detrimental effects of CMV because of their capacity to strongly control the infection and thereby preventing reactivation. However, some will eventually fail to contain the virus because of exhaustion of the immune system causing the infection to reactivate and anti-CMV IgG titres to increase. From this perspective, high anti-CMV IgG titres in the oldest old could be interpreted as a measure of general deterioration but certainly further investigation is warranted.

**Key points**

- 67% of a Belgian cohort of community-dwelling individuals aged 80 and over were anti-CMV seropositive.
- A positive CMV serostatus was not associated with an increased risk for all-cause mortality in this cohort.
- CMV seropositive individuals with high anti-CMV titres were at higher risk for all-cause mortality compared with other individuals.

**Conflicts of interest**

The authors have no financial or any other kind of personal conflicts with this paper.

**Funding**

The Belfrail study (B40320084685) is funded by an unconditional grant from the Fondation Louvain, which is the support unit of the Université Catholique de Louvain and is responsible for developing educational and research projects for the university by collecting gifts from corporations, foundations and alumni.

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Received 29 July 2013; accepted in revised form 28 March 2014