Cytomegalovirus Seroprevalence and Associated Factors in a Cohort of Elderly Men

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Background: Epidemiologic associations have been observed between cytomegalovirus and chronic diseases of aging, such as functional impairment, cognitive decline, and frailty. This cross-sectional study was designed to evaluate demographic and medical history factors independently associated with CMV seropositivity in men aged ≥ 65 from 6 U.S. cities in the ongoing Osteoporotic Fractures in Men (MrOS) cohort study.

Methods: Baseline exam questions captured demographic characteristics, including age, race, education, clinic site, SES, BMI, self-rated health status, morbidities and medications, and number of children. CMV serostatus was determined in a random sample of 1586 of the men using serum samples. Prevalence ratios (PR) and 95% confidence intervals (CI) were calculated to determine associations with the variables listed above.

Results: Overall, 73.0% of the men in the cohort were CMV seropositive, an indication of chronic infection. CMV seropositivity increased from 68.9% in those subjects age 65-69 to 81.8% in those aged ≥80 years. Seroprevalence of CMV ranged by site of clinical exam, with higher levels of age-standardized CMV seroprevalence in Birmingham (84.9%), Pittsburgh (81.7%), and Palo Alto (72.6%), while Portland (69.1%), Minneapolis (65.9%), and San Diego (64.8%) had lower levels. In the final multivariable model, seroprevalence rates varied significantly based on subject age and clinic site of examination. A ten year age increase in an average-aged subject (74 years) was associated with an increased prevalence of infection ranging from 11% in Pittsburgh, PA to 29% in San Diego, CA ($p<0.0001$), and no significant increase in Birmingham, AL. Race also had a significant positive association with prevalence of latent CMV infection - subjects of non-white race had 1.24 times the prevalence as those of white race. Level of education was negatively associated with CMV seropositivity, as those who graduated from college and graduate school had 0.85 ($p=0.003$) and 0.88 ($p=0.022$) times the CMV seropositivity prevalence, respectively, of those subjects who had not graduated from high school. The number of children each subject had was also significantly positively associated with CMV positivity. Both having a single child (PR = 1.17, $p=0.017$), and having two or more children (PR s= 1.15, $p = 0.009$) were significant risk factors compared to those subjects who had no children.

Conclusions: CMV seroprevalence is high among immunocompetent elderly men in the US. Specifically, non-white men who did not graduate from high school and have children were at higher risk of CMV seropositivity than their counterparts lacking these features. Future work will focus on CMV seropositivity as a risk factor for age-related functional impairment and chronic disease outcomes. The factors found to be associated with CMV in this analysis indicate subgroups of older men at highest risk for CMV seropositivity and will be considered as potential confounders in future analyses of CMV and outcomes of aging.

BACKGROUND

Cytomegalovirus (CMV) is a ubiquitous and highly transmissible member of the Herpesviridae family. Infection is often acquired early in life and is usually asymptomatic (1). CMV is not cleared from the body, but rather persists in undifferentiated monocytes (2) and endothelial cells (3) as a combination of a chronic productive infection and latent infection with periodic subclinical reactivation (4). The vital importance of maintaining defense against this asymptomatic infection is dramatically demonstrated in immunosuppressed people, where viral escape can be potentially fatal. Although once thought to be only significant in these immunocompromised populations, CMV is gaining emerging significance as a contributing etiologic agent for numerous chronic diseases and as a marker of immune dysregulation and immunosenescence in the general, immunocompetent population.

Epidemiologic associations have increasingly been established between CMV and diseases of chronic inflammation. This can manifest as cardiovascular disease (5-8), atherosclerosis (9), and frailty (10), which itself is a major independent predictor of adverse outcomes (11). In more recent studies, CMV has also been linked to cognitive decline and impairment including vascular dementia (12, 13), functional impairment (14), prolonged hospitalization (15), and cancer (16, 17).

Animal models have repeatedly shown that CMV may cause pathological damage to cardiovascular tissue by acting as pro-inflammatory stimuli or by directly invading the cardiovascular tissue, contributing to endothelial tissue damage in the vasculature (18-21). It has been hypothesized that CMV may also be delivered to the vessel wall by infected circulating monocytes arriving at sites of cardiovascular injury or inflammation (22). CMV antigen and DNA have also been isolated in atherosclerotic vessels of the human cardiovascular system (23-25). It is thought that CMV contributes to the progression of these disease processes primarily through inflammatory mechanisms.

CMV is embedded within the host's immune system, using myeloid cells as its main reservoir. The virus exploits the host's inflammatory response to perpetuate its life cycle and avoid elimination (26-29). Set off by any number of varying stimuli, the host inflammatory response plays a critical role in reactivating CMV and stimulating viral gene expression. In turn, these resultant viral gene products upregulate production of a variety of proinflammatory mediators, such as interleukin 6 (IL-6), interleukin 1(IL-1), tumor necrosis factor alpha (TNF-alpha), interferon, C reactive protein, and a variety of additional chemokines (26-30). In turn, these agents have been independently associated with mortality from cardiovascular disease, all-cause mortality, (31-33) frailty (34, 35), and disability (36). A growing body of evidence also implicates CMV infection as a driving force in immunosenescence, the gradual accumulation of deleterious age-associated changes to immune parameters that results in a weakened ability to fight off infections and higher rates of vaccination failure (33, 37). This association is logical, as it has also been found that immunosenescence is characterized by more intense production of proinflammatory cytokines by monocytes (38), a characteristic of chronic CMV infection.

Like other herpesviridae, CMV is suppressed but not completely eliminated from the body by the immune system. However, unlike most other herpes viruses that also require constant immune maintenance, CMV has been associated with a particular age-associated expansion of effector memory CD8 T-cells. This clonal expansion occurs as a result of CMV being constantly captured, processed, and presented to T cells. It has been hypothesized that this expansion of CMV-specific clonal cells constricts the 'immunological space' of the body available to perform other tasks and explains the detrimental effects of immune-aging. Studies indicate that over time, this process leaves many CMV specific T cells present but anergic, leaving fewer naïve and undifferentiated T cells to combat novel pathogens (39). In addition, these memory effector CD8+ T cells have limited ability to proliferate and are resistant to apoptosis. (40, 41). It has also been shown that this process is not a result of deteriorating health, comorbidity, or a general loss of immune control over the virus (42, 43).

In mouse models, age-related T cell expansions within the CD8 compartment are found to impair the efficacy of antiviral T cell reactivity, and contribute to immunodeficiency by immunological senescence (44). In humans, this accumulation of CMV-specific T cells has been specifically observed to reduce T cell immunity toward influenza vaccination (33) and EBV infection (45). While 70-90% of young vaccinated individuals are protected from influenza and respond to the vaccine, in the elderly, especially those frail, the protection and responsiveness achieves only 30-50% (46-49). In elderly individuals, T cells specific to CMV may comprise up to 45% of the CD8 population, combined with low CD28 expression (45, 50). CMV seropositivity also belongs to a cluster of immune factors constituting an "immune risk profile" associated with all-cause mortality at 2, 4, and 6-year follow-up in elderly Swedes in the OCTO/NONA longitudinal studies (43, 51-53).

Associations between CMV and frailty are also troubling, considering that frailty rates have been estimated by one US cohort study to affect 7% of community-dwelling adults age 65 and older and 30% of those aged 80 and older (11). Frailty has serious health implications for the aging population as an independent risk factor for poor outcomes, including falls, worsening mobility, disability, hospitalization, and death (11). Compounding this problem, this 80 and over age group is the fastest growing segment of the American population. The number of older men is also projected to grow proportionally more than that of women. Recent projections estimate that the proportion of the population age 65 years and older will double from 7% of the total world population now to 14% in 2040. In this same period, the number of individuals in this 65 and over population is expected to increase by 160%, while the 80+ population will see growth of over 230% (54). According to the UN Population Division, 1 in 5 people in the US will be age 65 or over by 2035.

The cost of ageing is already known to be high, with about one third of health expenditures in industrialized countries spent on people aged >70 years (55). Frailty is complex in its manifestation and there is as yet no generic treatment (56). The number of hip fractures - a common consequence of frailty - in the US population is expected to double its 1990 levels by 2025, largely as a result of the increasing size of this elderly population (57). In 2005, there were predicted over 2 million fractures costing roughly $17 billion (58). In light of these statistics, robust information and epidemiology regarding any putative risk factors for frailty, such as chronic CMV infection, will be of increasing importance.

Seroprevalence

The seroprevalence of CMV has been studied quite extensively in some populations, but for other groups research is nearly non-existent. Traditionally, CMV epidemiology has focused on females in adolescence and child-bearing age, reflecting the public health significance of CMV as a prominent driver of congenital infection and disease. From these studies, many factors have been ascertained to be associated with CMV positivity. There have been very few studies focusing particular attention on CMV seroprevalence in the elderly male US population. To date, large population studies of this demographic have only been included in massive, nation-wide assessments of CMV prevalence.

Cytomegalovirus is highly ubiquitous worldwide and is spread by direct contact with bodily fluids. Both overall prevalence and age of initial viral infection varies significantly by region of the world. General trends have tended to show higher seroprevalence rates in South America, Africa, and Asia and lower in Western Europe and the United States. One study of voluntary blood donors in Delhi, India reported 95% of individuals over 18 have already been infected with CMV (59), while studies in other developing countries indicate rates approaching 100% by age 11 in hospital-visiting children of both sexes (60). Attempts to quantify CMV prevalence by rough geographic region of the United States have been rarely attempted. The NHANES III study divided the country into Northwest, Midwest, South, and West – with the associated CMV seroprevalence rates of 80%, 83%, 87%, and 77%, respectively, for men aged 60+ years.

It has long been established that CMV seropositivity increases with rising age (61-67), as each year offers a small but accumulating risk of acquiring the virus. In the US, one of the first large seroprevalence studies found CMV seroprevalence to gradually rise from 36.3% in those 6-11 years old to 54% by age 30-39 and eventually to 90.8% in those aged 80 or greater (61). This same study found that adjusting for age, the southern states had the highest CMV seroprevalence (66.2%) and the northeastern states had the lowest (50.3%). Other studies within the US have also found considerable variation by geography, differing by as much as 30 percent between states when measuring seroprevalence in women of reproductive age (68).

Race has also been a commonly identified factor associated with differences in CMV seroprevalence levels. NHANES data, a representative US survey of people age 6 and over, found that after adjusting for age, CMV seroprevalence differed substantially by race/ethnicity. Non-Hispanic white persons were positive 51.2% of the time, compared to 75.8% for non-Hispanic black and 81.7% for Mexican Americans (61). These differences became less pronounced when stratified by increasing age and adjusted for numerous demographic variables, yet they remained significant. A study of child care providers in Washington state showed an overall 2.4 times increased risk of seropositivity for subjects who were non-white race (69).

Education has also frequently been identified in connection to CMV positivity (70). A study of 8000 U.S. subjects $\geq$45 years of age using the CDC NHANES study data determined that CMV seropositivity was also highly linked to education level, in this case used as a proxy measurement of socioeconomic status. Those who had only

high school or less than high school education had a 1.71 times and 3.22 times higher risk of being positive, respectively, when compared to those with greater than high school education. This effect was seen after adjusting for age, gender, and race.

Most significantly, even in the rare studies of CMV seropositivity that focus exclusively on elderly populations, these associations are maintained. A study of community dwelling women aged 70 to 79 found that CMV-positive subjects (n = 632) were on average older and less educated than their CMV-negative counterparts (n = 92) (62). Another study using the same Women's Health and Aging Study subjects took these findings further, discovering that those who had higher CMV antibody concentrations were more likely to be older, black, and to have not completed a high school education (63). This seropositive population also had significantly higher rates of frailty (18.4% versus 5.6% in those negative).

Risk factor studies performed in Canada and the US have also repeatedly shown an increased risk of CMV infection with an increased number of children in the home. One such study estimated having two or more of your own children significantly increased the risk of CMV seropositivity (OR = 1.98) (67). Young children are an important source of CMV infection due to their inadequate hygiene behaviors and high excretion rates (71). Studies in daycare workers have increased risk of CMV infection from such behavioral practices such as caring for younger children (72) and increased frequency of diaper changing (69).

Despite the evidence accumulating around cytomegalovirus' associations with numerous chronic inflammatory conditions and vaccine failure, studies of the pathogen's prevalence in the elderly U.S. population are sparse. As the implications of CMV infection become more established, the need for accurate measures of its distribution in society becomes paramount, specifically in the elderly populations where most of these chronic conditions will manifest. This study seeks to determine the level of seroprevalence by various demographic variables, as well as determining which of these factors put elderly male individuals at risk of CMV infection. This will aid in predicting future areas of morbidity in aged populations.

This study will contribute to the understanding of the epidemiology of a ubiquitous infection that is gaining emerging significance in the public health sphere. Practically, this study will add to the existing knowledge of CMV risk factors and may help guide future efforts to prevent infection. Additionally, this study will be the first step in a program of research to evaluate CMV associations with chronic conditions such as frailty, osteoporosis, and cardiovascular disease. The high quality data from the MrOS study should be an invaluable resource to examine the virus in the setting of a seldom studied, immunocompetent population.

MATERIALS AND METHODS

Study design and population

Data for these analyses were from the Osteoporosis Fractures in Men (MrOS) study, a large prospective, longitudinal, observational cohort study of community-dwelling men aged 65 and over. The MrOS study recruited these older men from six U.S. communities from March 2000 to April 2002, with the original purpose of evaluating risk factors for fractures and related sequelae. The sites involved were Birmingham, AL, Minneapolis, MN, Palo Alto, CA, Pittsburgh, PA, Portland, OR, and San Diego, CA.

Subjects enrolled had to meet the following criteria: able to walk without assistance of another; absence of bilateral hip replacements; ability to provide self-reported data; residence near a clinical site for duration of the study; absence of a medical condition (in judgment of investigator) that would result in imminent death; ability to understand and sign an informed consent. Minimal exclusion criteria allowed for a large sample size, generalizability of results to the male population, and power to detect associations between potential risk factors and CMV seropositivity.

The study enrolled and completed the baseline exam of these participants over a 25 month period from March 2000 to April 2002. These men were recruited using multiple methods including voter and motor vehicle registrations, community-based mailings, and presentations to community groups. The strategies employed also sought to enhance the recruitment of minority groups. The baseline exam involved multiple questionnaires to capture basic physical demographic features as well as lifestyle and medical information. Fasting blood and urine draws were also obtained at the time of baseline exam to measure biochemical factors. Standard clinical evaluation measurements (height, weight, etc.) were also measured at this time. A more extensive description of recruitment methods can be found elsewhere (73).

MrOS enrolled approximately 6000 subjects and a random subcohort of 1586 men had baseline blood samples that could be used to determine CMV serostatus and were included in this study. This was a cross-sectional analysis of data collected from baseline questionnaires, a clinic visit, and CMV serostatus. There were no differences according to age, education level, race, or number of children between those subjects whose blood was sampled and those that were not (Appendix 1). This study was approved by the institutional review board at Oregon Health & Science University.

Laboratory Analyses

Nonfasting blood samples were obtained using venipuncture and stored at -70C until the time of analysis. Plasma CMV immunoglobulin G (IgG) antibodies were detected in 0.05 ml of serum according to the specifications of a qualitative enzyme-linked immunosorbent assay (ELISA) kit (CMV IgG ELISA, BD Biosciences, Franklin Lakes, New Jersey). As a lifelong infection with long periods of latency, CMV seropositivity is most consistently detected using IgG serology (26, 28). Comparable PCR-based measures of CMV instead measure active viral load, so are more suited to detecting acute viral reactivation events, rather than chronic latency.

CMV seropositivity was determined by an ELISA using 2ME-treated lysate of CMV-infected cells as antigen. 2ME treatment reduces false positive and ambiguous results. Titration of serum from 70 individuals in a previous study allowed clear discrimination of seropositive and seronegative individuals. Based on that data, a serum dilution of 1:100 was chosen to screen MrOS sera.

Each ELISA plate included duplicates of one negative, one maximal positive, and one intermediate positive sample on each plate (lab controls from normal donors). Samples were reported as seronegative if their OD was within 2SD of the seronegative serum on the plate (always less than 0.1). Samples were reported as positive if their OD was >0.2. Ambiguous samples (between 0.1 and 0.2 (N = 54)) were subject to repeat testing and serum titrated, starting with a 1:25 dilution. All were subsequently found to be negative.

Median intra-assay CV for OD was 2.8% and median inter-assay CV was 16.5%. All controls were 100% concordant for seropositivity within and between assays. All samples were run in a masked fashion by the Hill lab of OHSU and each kit contained controls and internal calibrations.

Variables

CMV seroprevalence was defined as the prevalence of CMV seropositivity in a given population. CMV seropositivity is the outcome variable and for potential risk factors, a wide range of demographic variables, medication usages, and subject comorbidities that were potentially associated with seropositivity were assessed. Race was dichotomized to white or non-white. Education was categorized as less than high school graduate, high school graduate, college graduate, and graduate school graduate. Number of children was divided into none, one, or two or more. Self-assessed socioeconomic position was divided into those with scores of 6-10 on a ladder of wellbeing, an indicator of higher position, versus those who self-scored values in the 1-5 range, or lower SEP. Self-reported health status was divided into excellent, good, fair, or poor & very poor. Morning fasting blood samples were used to measure low density lipoprotein cholesterol and high density lipoprotein cholesterol. The usage of varying medications and the presence of medical comorbidities were categorized as binary yes or no answers.

Statistical Analysis

Baseline characteristics of CMV seropositive and CMV seronegative men were first compared using Student's t-test for continuous variables, and the chi-square test for categorical variables. A generalized linear regression model was used, assuming a Poisson distribution with a log link to examine association and estimated prevalence ratios. The standard error for the model coefficients were estimated using a robust estimator. First, bivariate association with CMV positivity was tested for each risk factor. Unadjusted prevalence ratios and 95% confidence intervals were obtained. Age-adjustment of variables was performed due to age acting as a strong confounder.

Prevalence ratios are a superior method of estimating relative risk than odds ratios in this context because CMV seropositivity is known to be a fairly common event. Under these circumstances, an odds ratio will overestimate the true prevalence ratio, as it is comparing different groups. Odds are a measure of those either

with CMV and a risk factor, without CMV and without the same risk factor versus those who have one of the two factors but not the other. The odds ratio treats the variable symmetrically, but its outcome can have a very large range, from 0 to infinity. The prevalence ratio, meanwhile, is a measure of risk as a probability – comparing the probabilities of having CMV in a group with a factor versus the probability of having CMV in a group lacking that factor. These two probabilities are by definition on a range of 0 to 1 (0% to 100%), so when compared, the prevalence ratio has a much narrower range of values, which aids in ease of interpretation. Additionally, clinicians and patients in general tend to think of risk, as a probability, rather than odds. Prevalence ratios are interpreted as how large the prevalence of being CMV seropositive is in subjects with a certain characteristic relative to the seroprevalence in subjects lacking that characteristic.

A multivariate Poisson model was then built using backwards stepwise selection. Variables with significant bivariate associations with CMV positivity at a $p<0.25$ were considered for inclusion into the model. Variables were also included if they have been known to be, or implicated to be, risk factors for CMV seropositivity in prior research. The remaining variables were then assessed for the presence of quadratic relationships if they were continuous, and for the presence of interactions. A significance level of $P<0.05$ was used to determine significant associations in the final model. Stata 10.0 was used for all analyses (Stata Corporation, College Station, TX).

RESULTS

Table 1 presents CMV seroprevalence by significant demographic and clinical characteristics of our sample population. Overall, seventy-three percent of men were CMV seropositive, an indication of chronic infection. Elderly men with chronic CMV infection (n = 1157) were more likely to be older, non-white, have more children, and have less education than their CMV seronegative counterparts. Overall, the cities of Birmingham (84.9%), Pittsburgh (81.7%), and Palo Alto (72.6%) had higher levels of age-standardized CMV seroprevalence, while Portland (69.1%), Minneapolis (65.9%), and San Diego (64.8%) had lower levels (Table 3). CMV seropositivity increased from 68.9% in those subjects age 65-69 to 81.8% in those aged $\geq$80 years. All variables tested for bivariate association – including BMI, blood chemistry variables, self-assessed health status and socioeconomic position, coexisting diseases, and numerous medications, and others were provided in Appendix 1.

In the final multivariate model, multiple independent factors were found to be significantly associated with CMV seropositivity. Prevalence ratios (PRs) and 95% confidence intervals for these factors are listed in Table 4. A significant interaction was discovered between age and site of clinical examination ($p<0.0001$). Therefore, the association between every 10 years increase in age and seropositivity was stratified by site. The prevalence rate for CMV positivity was significantly increased in the following study sites for every 10 years increase in age: 11% in Pittsburgh, PA ($p= 0.022$), 19% in Portland, OR ($p = 0.004$), 28% in Minneapolis, MN ($p = 0.001$), and 29% in San Diego, CA ($p<0.001$). In Palo Alto, CA, a 10-year increase in age was associated with an 11% increase in risk of CMV seropositivity, with borderline statistical significance ($p = 0.064$). Birmingham, Alabama, showed no significantly increased risk with 10 years increase of age.

Race had a significant association with risk of CMV seropositivity. Subjects of non-white race had 1.24 times the risk as those of white race ($p<0.001$). Level of education was significantly negatively associated with prevalence, as those who graduated from college and graduate school had 0.85 ($p=0.003$) and 0.88 ($p=0.022$) times the CMV seropositivity, respectively, of those subjects who had not graduated from high school. The number of children each subject had was also positively associated with CMV positivity. Both having a single child (PR = 1.17, $p=0.017$), and having two or more children (PR = 1.15, $p = 0.009$) were significant risk factors, compared to having no children.

Table 1. Characteristics by Cytomegalovirus (CMV) Status in the MrOS Study Cohort (n = 1586)

Characteristic		Negative (n = 429, 27.0 %)	Positive (n = 1157, 73.0%)	P-value
Age (Years at Examination)	Mean ± SD	72.5 ± 5.4	74.2 ± 6.0	<0.0001*
Race	n (%)			
	White	405 (94.4)	1021 (88.3)	<0.001*
	Non-White	24 (5.6)	136 (11.8)	
Site	n (%)			
	Birmingham, AL	40 (9.3)	213 (18.4)	<0.001*
	Minneapolis, MN	82 (19.1)	157 (13.6)	
	Palo Alto, CA	73 (17.0)	200 (17.3)	
	Pittsburgh, PA	51 (11.9)	229 (19.8)	
	Portland, OR	85 (19.8)	184 (15.9)	
	San Diego, CA	98 (22.8)	174 (15.0)	
Education	Category, n (%)			
	Some high school or less	16 (3.7)	94 (8.1)	<0.001*
	Completed high school	133 (31.0)	509 (44.0)	
	Completed College	156 (36.4)	391 (26.0)	
	Completed Graduate School	124 (28.9)	253 (21.9)	
Number of Children	Category, n (%)			
	0	66 (15.4)	120 (10.4)	0.018*
	1	40 (9.3)	128 (11.1)	
	≥2	323 (75.3)	909 (78.6)	
Self-Assessed Socioeconomic Position (1-10)†	Category, n (%)			
	1-5 (Low)	79 (18.4)	273 (23.6)	0.027*
	6-10 (High)	350 (81.6)	884 (76.4)	

*Significant at p<0.05, t-tests for difference in means and Pearson chi-square tests for differences in proportions or test for trend among demographic groupings (testparm) were calculated.
† Assessed via questionnaire asking subjects to rank their own position on a ladder of US citizens by general criteria of wealth, education, and job respect.

Table 2 - Risk factors for CMV seropositivity in a cohort of elderly men

			Age-Adjusted		
		Prevalence	PR	95% CI	P-value
Age	Continuous	73.0%	1.01	1.01 - 1.02	<0.001
	65-69	68.9%	Reference		
	70-74	68.2%	0.99	0.91 - 1.08	0.829
	75-79	76.6%	1.11	1.03 - 1.21	0.01
	80+	81.8%	1.19	1.09 - 1.29	<0.001
Race					
	White	71.4%	Reference		
	Non-white	85.2%	1.22	1.13 - 1.31	<0.001
Site	Overall				<0.0001
	Birrmingham, AL	84.4%	Reference		
	Minneapolis, MN	65.6%	0.77	0.69 - 0.85	<0.001
	Palo Alto, CA	72.3%	0.83	0.76 - 0.91	<0.001
	Pittsburgh, PA	81.3%	0.94	0.87 - 1.02	0.148
	Portland, OR	68.6%	0.8	0.72 - 0.88	<0.001
	San Diego, CA	64.2%	0.75	1.01 - 1.02	<0.001
Education					
	Less than HS grad	85.6%	Reference		
	HS grad	79.0%	0.93	0.86 - 1.02	0.115
	College Grad	66.0%	0.78	0.70 - 0.86	<0.001
	Grad School Grad	67.3%	0.79	0.72 - 0.88	<0.001
Number of Children					
	0	65.1%	Reference		
	1	76.0%	1.17	1.02 - 1.33	0.027
	≥2	73.8%	1.14	1.02 - 1.28	0.019
Self-Assessed Socioeconomic Position (1-10)					
	1-5 (Low)	77.9%	Reference		
	6-10 (High)	71.3%	0.92	0.87 - 0.99	0.019

Table 3. Age-specific and age-standardized prevalence of CMV in the MrOS Sample

		Site						
Age, years	N	Birmingham, AL	Minneapolis, MN	Palo Alto, CA	Pittsburgh, PA	Portland, OR	San Diego, CA	Total
N		253	239	273	280	269	272	1586
65-69	469	0.85	0.61	0.68	0.81	0.62	0.54	0.69
70-74	434	0.80	0.52	0.72	0.71	0.69	0.60	0.68
75-79	398	0.83	0.77	0.75	0.88	0.65	0.71	0.77
80+	285	0.92	0.78	0.76	0.88	0.84	0.78	0.82
Total*	1586	0.84	0.66	0.72	0.81	0.69	0.64	0.73

*Age standardized to overall MrOS sample group by 5-year intervals

Table 4 – Risk factors for CMV seropositivity in a cohort of elderly men

N = 1586
(27% negative, 73% positive)

		PR*	95%CI	P-value
Site x Age Interaction				<0.0001
(per 10 years)				
	Birmingham, AL	1.02	0.94 - 1.12	0.608
	Minneapolis, MN	1.28	1.10 - 1.48	0.001
	Palo Alto, CA	1.11	0.99 - 1.23	0.064
	Pittsburgh, PA	1.11	1.02 - 1.21	0.022
	Portland, OR	1.19	1.06 - 1.35	0.004
	San Diego, CA	1.29	1.13 - 1.47	<0.001
Race				
	White	Reference		
	Not-White	1.24	1.14 - 1.34	<0.001
Education				
	Some high school or less	Reference		
	High School Graduate	0.98	0.90 - 1.07	0.712
	College Graduate	0.85	0.77 - 0.95	0.003
	Grad School Graduate	0.88	0.79 - 0.98	0.022
Number of Children				
	0	Reference		
	1	1.17	1.03 - 1.33	0.017
	≥2	1.15	1.04 - 1.28	0.009

*Prevalence Ratio - Fully adjusted model (age, site, race, education, number of children)

DISCUSSION

In this study of 1586 community-dwelling elderly men, we found that CMV seropositivity was both high and associated with multiple demographic and lifestyle factors, even after adjusting for age, race, education level, and number of children. This is one of the first studies specifically focusing on associations with CMV in an elderly male US population. The high level of CMV seropositivity in the US and worldwide, as well as the increasing number of links to long term chronic diseases, makes any understanding of the affected population an important public health goal.

Our overall CMV seropositivity prevalence of 73.0% is largely in agreement with the few available other studies focusing on this aged male population. Our estimates are slightly lower than a large US seroprevalence study of CMV using NHANES data (with both sexes) found 74.2% prevalence in those aged 50-59, 83.0% in those 60-69, 88.8% in those 70-79, and 90.8% in those $\geq$80 (61). The overall CMV seroprevalence estimate was 58.9%, in a cohort including ages from 6 through >80 years of age. They additionally noted that overall, CMV was roughly 10% higher in women than in the men included in the study. With this in mind, the numbers for men are actually quite close. Both studies drew their participants from the non-institutionalized/community-dwelling populations near their respective exam sites. The NHANES, however, visits 15 counties for data each year, offering them a broader pool of participants to sample from, and less likely subject to sampling variability by site location.

Numerous other studies have found risk factor results that paralleled ours. An NHANES-based study was in agreement, finding that seropositivity gradually increased with subject age and was highest in the oldest segments of the cohort. This is consistent with the known US epidemiology of the disease as each year brings a small but persistent increased risk of infection, and there is no way to remove positivity once latent infection is established (70, 74). Similar positive associations have been found between CMV prevalence and race (61, 74), geographic area (NHANES), education (70, 74), and number of children. Our finding that being of "non-white" race was associated with a roughly 25% increase in risk of seropositivity was consistent with other studies who identified a large association from race that became less pronounced with adjustment by age and other demographic factors (SES, education, etc.). Other studies interestingly also found that the largest effect size of race on seropositivity was seen in those ages 15-44, with a still significant but diminishing difference for those aged 45-74, and then an insignificant difference for those aged 75 or greater (61). Our data fits within this picture, as our aged 65+ population still maintains distinct differences in CMV positivity risk by racial group, but in a less pronounced fashion than the effect seen in studies of younger populations. We found a similar trend to other studies in terms of education, with college graduates (PR = 0.85) and graduate school graduates (PR = 0.88) receiving a protective effect against latent CMV infection compared to those who had not graduated high school. An NHANES study analysis found a protective prevalence ratio of 0.90 for high school graduates, and 0.77 for those completing some college, compared with those who had not graduated high school. Our study effects are again somewhat less pronounced. The MrOS study population was known to be of higher education than the general population, and the hardest group to recruit were those who had less than high school education who were also CMV negative.

The Staras NHANES-based study found that prevalence ratios for CMV positivity varied significantly by geographic area of the US. They found a PR of 1.32 when the South was compared to the Northeast. Similarly, they found a PR of 1.21 when the West was compared to the Northeast. Our study's PRs are not directly comparable, as they also take into account the effect of an interaction with age, so are a measure of 10 year's age impact on CMV risk at each site. No other good comparison studies are available for this age group. Of interest, our non-age-adjusted CMV seroprevalence estimates show highest levels in the South (Birmingham, AL), but also high levels in the Northeast (Pittsburgh, PA). Observed differences could be due to a multitude of factors, including that the NHANES study sampled many more sites in each geographic area (15 sites every year), had a much larger sample size (34,000), and had a much higher response rate than our cohort (49-94%). Local variation of microclimates and cultural norms makes basing estimates for an entire region on one or two sites problematic, at best.

There have been other risk factors that have been positively associated with CMV prevalence. HIV status (75), female sex (75), socioeconomic status and/or household income (61), being born outside of the US, or having a mother born outside of the US (76) have each been implicated. Unfortunately, these variables were either

unable to be examined in this study due to lack of information from the MrOS questionnaire, or due to being outside the scope of reference of this study (entirely male population). Though the self-assessed measures of socioeconomic position and self-reported health status were not significantly associated in our study, SES and household income have been implicated in numerous other studies. Education attainment has been used as a proxy measure of SES, and found to be negatively associated with CMV prevalence, similar to our education results. Lack of significance with our two measures of socioeconomic position and health status may be due to the subjective nature of these variables. The MacArthur scale of Subjective Social Status, used by the MrOS study, is a useful way to gain finer detail into the life of a subject than the normal crude measurements, such as education level and income attainment. For instance, graduating from an Ivy League college is regarded on the same level as any other university, a factor which would have serious implications on the life, health, and possibilities of the subject. The SES ladder allows for this to be factored in. However, it is still reliant on an accurate and uniform level of self-perception amongst all subjects to be meaningful. The finer details captured by the SES ladder variable may also have blurred the lines on the differences between socioeconomic groups, compared to the cruder measure of education. This may be why during model selection, education saw more pronounced differences between groups and was retained in the model, while self-assessed social status showed less pronounced differences and was dropped.

As previously mentioned, it has long been known that the majority of the US population is CMV seropositive by the time they become elderly. This indicates that many of the risk factors for CMV seropositivity are likely much more pronounced and significant in younger individuals, and thus any CMV prevention strategy would do best by targeting those populations. With that said, it is significant to note that the differences in CMV seropositivity that have been identified as arising from factors of race, education, number of children, and location in younger populations still persist in the elderly US population.

There is a high interest in a CMV vaccine, and potential biological targets have been hypothesized (77) with many current NIH grants underway in this area of study. A vaccine for CMV has been given Category 1 priority status for development by the National Vaccine Program Office of the US (78). Analysis by the same group at the US Institute of Medicine determined that a CMV vaccine would be the single most cost-effective identifiable new vaccine, based largely on savings from neonatal infections and their complications (79). The study estimated that, based on assumptions for vaccine coverage in the US, implementing a CMV vaccine (with a 100% vaccination rate and 100% uptake) would gain the US roughly 18,000-70,000 quality-adjusted life years, as well as an annual savings to the health system of $1.1 billion – 4 billion. This calculation was primarily based on the costs of known long-term sequelae in those who acquire CMV congenitally (79). Our study supports previous notions that CMV is widespread endemically in the US population, and any such vaccine would likely have a far reaching impact in terms of potential patients. Interestingly, as more long term chronic health conditions are linked with CMV, such as those that will be examined in further studies of the MrOS dataset, these cost estimates will likely be vast underestimations of the true burden of CMV on our healthcare system.

In addition to these costs, the known burden of CMV positivity on our healthcare system is also likely underestimated in its impacts on the elderly and immunosuppressed. Increased risk of flu vaccine failure has serious implications in the elderly population, with increased levels of mortality and hospitalizations in those who failed vaccination. This trend will only be further exacerbated by aging populations worldwide. Similarly, seropositive individuals remain at chronic risk of shedding infection to the established high risk groups – namely pregnant mothers and immunocompromised persons, such as those undergoing chemotherapy, HIV infection, or transplantation. As the science of transplantation improves, more access to these procedures is expected, with higher numbers of patients predicted in this at-risk group. This combination of factors indicates that CMV therapy and prevention will continue to be an active area of research with far-reaching public health significance.

This study provides a large and robust sample size of elderly men, with good geographic distribution and sampling designed to incorporate as diverse and representative a group as possible. The standardized nature of the MrOS study across multiple sites ensured high validity of sample collection, sample processing, questionnaire administration, and results reporting. In addition, the extremely thorough questionnaires and biometric assessments provided an impressive assortment of potential covariates to test for plausible associations with CMV positivity. We are confident in the reliability and validity of our CMV antibody outcome measure of viral positivity. The ELISA test is the standard for determination of CMV serostatus in clinics, hospitals, and laboratories worldwide. In rare ambiguous assays, confirmation of status was achieved by titration of the assay and repeat analysis with a higher concentration. Those samples which were truly CMV positive should have resulted in very

positive ELISA tests. The fact that all 54 ambiguous assays repeatedly tested negative is reliable confirmation of their true status.

As few studies specifically focus on CMV in this population, it provided a relatively rare chance to test for multiple suspected associations. Being a cross-sectional study, this study did not allow for any assessments of causality or temporality between CMV positivity and associated covariates. However, this is not a limitation of the study, as our primary interest was a snapshot of seroprevalence in a population at a given time, as well as identifying useful associations for further rigorous study. Temporality of associations is not an issue as the variables considered can only act in a one-directional fashion in this study, as factors like age and race are not things that could be altered by CMV status, regardless. Other measures such as education and number of children are not things that would be subject to change based on CMV status, by any known or plausible mechanism. While we cannot say that older age, non-white race, decreased education, or increased number of children causes CMV positivity, these associations will be useful in further follow-up studies where these factors will be considered as potential confounders when examining relationships between CMV positivity and poor health outcomes. These associations can also be used as helpful clinical indicators (when assessing likelihood of CMV reactivation risk when CMV serostatus is not known, for example) and for targeting populations with prevention strategies.

This is one of the first large studies measuring CMV seropositivity specifically in a cohort of elderly males. These identified risk factors will provide health care practitioners with a useful, evidence-based approach to identifying those elderly patients who are most likely to be CMV-positive. Currently, the most prominent use for this, based on available evidence, would be to identify which patients are most likely to have a decreased flu vaccination success rate, as this has been associated with CMV positivity. As the US population ages, there is a growing interest in understanding the multifactorial mechanisms that contribute to chronic inflammatory conditions in the elderly. Further studies using the MrOS data will search for associations between CMV and many of these diseases of aging, such as atherosclerosis, frailty, certain types of cancer, as well as immunosenescence. Further study of this often-overlooked chronically infected population will aid in further elucidation of likely risk factors for CMV positivity as well as plausible mechanisms for causal links to comorbid conditions and diseases.

APPENDICES

Appendix 1 – Comparison of MrOS total Cohort to Randomly Sampled Subcohort

	MrOS Cohort (N = 4996)	**Random Subcohort (N = 1586)**
Age	73.7 (mean)	73.8 (mean)
Race	89.5% white	89.9% white
Site	Birmingham 16.2% Minneapolis 16.8% Palo Alto 16.6% Pittsburgh 16.8% Portland 16.8% San Diego 16.9%	Birmingham 16.0% Minneapolis 15.1% Palo Alto 17.2% Pittsburgh 17.7% Portland 17.0% San Diego 17.2%
Education	1.71 (mean) Less than HS 6.6% Completed HS 40.3% Completed College 28.8% Completed Grad School 24.4%	1.69 (mean) Less than HS 6.9% Completed HS 40.5% Completed College 28.8% Completed Grad School 23.8%
# children	1.67 (mean) 0 = 11.2% 1 = 10.2% 2+ = 78.7%	1.66 (mean) 0 = 11.7% 1 = 10.6% 2+ = 77.7%

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