Published in final edited form as:

J Am Geriatr Soc. 2016 May ; 64(5): 998–1005. doi:10.1111/jgs.14090.

Herpesvirus Infections and the Risk of Frailty and Mortality in Older Women: The Women's Health and Aging Studies

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Abstract

Background—While persistent infection by cytomegalovirus is associated with higher mortality risk in adults, the effect of infections by other herpesviruses on long-term clinical outcomes in immunocompetent older adults is less clear. We examined the relationship between herpesvirus infections and mortality and incident frailty risks in community-dwelling older women.

Design—Nested prospective cohort study.

Setting—Women's Health and Aging Studies I and II.

Participants—Community-dwelling older women (n = 633) aged 70–79 years.

Measurements—Baseline serum antibody (immunoglobulin G) levels against four herpesviruses (herpes simplex virus types 1 [HSV-1] and 2 [HSV-2], varicella-zoster virus [VZV], and Epstein-Barr virus [EBV]), three-year incident frailty rates, and five-year mortality rates.

Results—Women seropositive for HSV-1 and HSV-2, but not VZV and EBV, had higher risks of three-year incident frailty (hazard ratio, 1.90 and 2.10; 95% confidence interval [CI], 0.96–3.74 and 1.05–4.37, respectively for HSV-1 and HSV-2) and five-year mortality (hazard ratio, 1.73 and 1.80; 95% CI, 0.93–3.20 and 0.94–3.44) than seronegative women. Incremental increases in the

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Conflict of Interest: The authors declare no financial, personal, or other potential conflicts of interest.

Author Contributions: George C. Wang designed the study, analyzed the data, and wrote the first draft of the manuscript. Christina Han performed laboratory assays and contributed to data analysis and manuscript preparation. Barbara Detrick contributed to data acquisition, study concept, and data interpretation. Vincenzo Casolaro contributed to data interpretation and manuscript preparation. David M. Levine contributed to study concept, data interpretation, and manuscript preparation. Linda P. Fried developed the WHAS cohorts and contributed to data interpretation and manuscript preparation. Jeremy D. Walston contributed to study concept, data interpretation, and manuscript preparation. All authors contributed to reviewing and editing drafts of the manuscript and approving the final version.

serum HSV-1 and HSV-2 antibody levels were associated with incrementally higher risks of incident frailty and mortality. After adjustment for potential confounders, only higher serum HSV-2 antibody levels were independently predictive of higher risks of mortality in older women (hazard ratio for each unit increase in antibody index, 1.47; 95% CI, 1.05–2.07).

Conclusion—HSV-1 and HSV-2 antibody levels are not independently associated with risks of incident frailty in older women. Only HSV-2 antibody level is independently predictive of five-year mortality risk, with each incremental increase in the antibody level adding further risk.

Keywords

Herpesvirus; frailty; mortality; herpes simplex virus; cytomegalovirus

INTRODUCTION

Herpesviruses are prevalent pathogens that establish lifelong latent and persistent infections in immunocompetent humans after an asymptomatic or self-limited primary infection.¹ These viruses persist in human host cells with minimal viral gene expression and evade host immune responses, yet still retain the capacity to reactivate and produce fully functional viral progenies to cause disease.² However, beyond the manifest diseases resulting from viral reactivations that accompany decline in or suppression of cellular immunity,^{3–5} the long-term clinical consequences of herpesvirus infections in immunocompetent humans are not well understood. We and others have previously demonstrated that infection by cytomegalovirus (CMV), a member of the herpesvirus family, increases mortality risk in adults.^{6–10}

Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2), the causative agents of recurrent orolabial and genital herpes, affect about 65 % and 25%, respectively, of adults in the U.S. by the fifth decade of life.¹¹ HSV-2 is generally a sexually transmitted disease, whereas HSV-1 is mostly acquired during childhood through nonsexual contact. Varicella-zoster virus (VZV), the agent responsible for chickenpox during primary infection and herpes zoster during reactivation, infects about 90% of humans by the time of adolescence and achieves nearly universal seroprevalence in U.S. adults 40 years of age and older.¹² Epstein-Barr virus (EBV), an oncogenic virus that infects more than 90% of most populations, usually causes an asymptomatic infection in children, infectious mononucleosis in adults, and asymptomatic reactivations in immunocompetent individuals.¹³

Despite the high worldwide prevalence of herpesvirus infections, the long-term clinical effects of persistent infections in immunocompetent individuals, beyond recent results for CMV,^{6–10} are still poorly understood. While HSV, along with CMV, has been mechanistically implicated in the pathogenesis of cardiovascular disease,^{14–19} epidemiologic studies assessing the relationship between HSV infection and cardiovascular outcomes in healthy persons have yielded inconsistent results, with higher risks for outcomes^{18,19} in some studies and no association in others.^{20,21} All-cause mortality risks due to non-CMV herpesvirus infections have not been reported. To better assess the effect of persistent herpesvirus infections on long-term clinical outcomes, we investigated in a prospective study of community-dwelling older women the relationship between baseline serum antibody

levels against four human herpesviruses, VZV, EBV, HSV-1, and HSV-2, and the risk of three-year incident frailty and five-year mortality.

METHODS

Study design

The Women's Health and Aging Studies (WHAS) I and II are two complementary, prospective observational cohort studies that enrolled community-dwelling older women in Baltimore, Maryland. WHAS I, begun in 1992, enrolled an age-stratified sample of 1,002 women aged 65 years or older who had self-reported difficulty in 2 or more of 4 domains of physical function.²² WHAS II, begun in 1994, enrolled an age-stratified sample of 436 women aged 70–79 years who had difficulty in no more than 1 physical domain.²³ Additional details on the study methods and sampling design of these studies have been published elsewhere.^{22,23} The Johns Hopkins University's institutional review board approved all research protocols. Informed consent was obtained from all participants.

Women aged 70–79 years from the combined WHAS I and II cohorts were eligible for our study. Of the 829 eligible women, 175 were excluded for not having baseline sera for antibody measurement, 19 for missing baseline covariates, and 3 for not being black or white, resulting in a sample size of 633 for the mortality analysis. For the incident frailty analysis, 77 women were further excluded for being frail at baseline, 3 for missing frailty measurement at baseline, 35 for not having a blood sample obtained within 12 months of baseline frailty assessment, and 37 for missing frailty data after baseline blood draw, resulting in a sample size of 481.

Herpesvirus immunoglobulin (IgG) and interleukin (IL)-6 measurements

IgG antibodies to VZV, EBV, HSV-1, and HSV-2 were measured in stored (–70°C) baseline sera of study participants using commercial enzyme-linked immunosorbent assay (ELISA) kits (GenWay Biotech, San Diego, California). Antibody concentration was represented by an antibody index derived by normalizing optical density values against reference standards, according to manufacturer's instructions. Seropositivity was defined as an antibody index of greater than 1.1. Plasma IL-6 concentrations were determined using a commercial ELISA kit (Quantikine Human IL-6, R&D Systems, Minneapolis, MN). All assays were performed in a masked fashion.

Outcome measurement

Frailty was defined using a validated five-component criteria,²⁴ which consisted of weight loss, exhaustion, low energy expenditure, slowness, and weakness. Participants meeting 3 or more of these criteria were classified as frail, 1 or 2 as prefrail, and 0 as nonfrail. Incident frailty was defined as the onset of frailty in a participant who was nonfrail or prefrail at baseline. Vital status was obtained through follow-up interviews with proxies, obituaries, and matching with the National Death Index. Death certificates were obtained for all but six of the women who died. Average follow-up duration was five years for mortality and three years for frailty status.

Statistical analysis

Baseline characteristics of study participants were compared across serostatus groups using the chi-square test for binary variables, and Student's *t*-test or the nonparametric equality-of-medians test for continuous variables with a normal or skewed distribution, respectively. For each of the four viruses, cox proportional hazards models were constructed to determine the relationship between baseline serostatus (as a dichotomous variable) or IgG antibody index (as a continuous variable) and time until frailty or death, controlling for the following baseline covariates: age, race, completion of high school education, coverage by private medical insurance, body mass index, pack-years of smoking, cardiovascular disease (angina, myocardial infarction, congestive heart failure, peripheral artery disease, or stroke), diabetes mellitus, and plasma IL-6 concentration. These covariates were chosen because of their association or scientific plausibility to be associated with the four persistent viral infections (HSV-1, HSV-2, VZV, EBV) and with the outcomes, frailty and mortality. Given the association between HSV infection and cognitive deficits,²⁵ we constructed an additional model that controlled for the Mini-Mental State Exam (MMSE) score, concurrently with the other covariates in the adjusted model above.

To avoid the assumption of linearity in the relationship between herpesvirus antibody indexes and mortality and incident frailty, we further categorized antibody indexes into five groups: a seronegative group and four additional groups pertaining to quartiles of antibody index in the seropositive range.

All analyses were performed using Stata 11 software (StataCorp, College Station, TX). In order to properly frame inferences derived from the study participants, study-specific probability weights, as previously described,²⁶ were used to weight each participant's data to reflect the number of persons she represented in the sampling population of community-dwelling women aged 70–79 years. To account for missingness in anti-viral antibody data, weights were further multiplied by the inverse proportion of anti-viral antibody data missingness, separately for each WHAS cohort. The survey weighting function in Stata was used to incorporate study-specific probability weights in all descriptive and regression analyses. All population percentages reported here refer to the weighted ones. Two-tailed tests were used to obtain *P* values.

RESULTS

Among the 633 participants, 79.9% were seropositive for VZV, 72.7% for EBV, 78.7% for HSV-1, and 81.7% for HSV-2 (Table 1). When baseline characteristics were compared across serostatus groups for each of the four herpesviruses, VZV and EBV seropositive women did not differ significantly from the seronegative women, except that VZV seropositive participants were more likely to have diabetes mellitus. In contrast, compared with seronegative women, HSV-1 and HSV-2 seropositive women were significantly more likely to be black, to not have completed high school education, to not have private medical insurance, to have a higher body mass index, to be smokers, to be diabetic, and to have a lower mini-mental state exam (MMSE) score. The differences we observed in sociodemographic factors between HSV-1/HSV-2 seropositive and seronegative individuals are consistent with data from the National Health and Nutrition Examination Surveys

(NHANES), nationally representative U.S. samples which found a higher seroprevalence of these viruses among those who were non-Hispanic black, had attained a lower level of education, and lived below the poverty level.^{11,27}

At baseline, 44.2% of the participants were nonfrail, 44.4% were prefrail, and 11.4% were frail. The prevalence of frailty did not differ significantly between seropositive and seronegative women for all four virus infections (Table 2).

Among community-dwelling older women who were at risk, 81 (16.8%) developed incident frailty during 3 years of follow-up and 90 (13.1%) died during 5 years of follow-up (Table 3). Compared with women who were seronegative, VZV and EBV seropositive women did not have an increased risk of developing incident frailty. In contrast, HSV-1 and HSV-2 seropositive women had greater incidence rates of frailty than seronegative counterparts (for HSV-1: 67.9 vs. 35.9 events per 1000 person-years; hazard ratio [HR], 1.90; 95% confidence interval [CI], 0.96–3.74; for HSV-2: 68.7 vs. 31.8 events per 1000 person-years; HR, 2.1; 95% CI, 1.05–4.37). After adjustment for potential confounders, differences in incident frailty risks between HSV-1 and HSV-2 seropositive and seronegative women were attenuated, with 95% CI of hazard ratios covering the null value (Table 3). Similarly, when mortality risk was considered, VZV and EBV seropositive women did not have an increased risk of dying in five years compared with seronegative women (Table 4). There was a strong suggestion that HSV-1 and HSV-2 seropositive women had greater incidence rates of mortality than seronegative women (for HSV-1: 30.7 vs. 17.8 deaths per 1000 person-years; HR, 1.73; 95% CI, 0.93–3.20; for HSV-2: 30.4 vs. 17.0 deaths per 1000 person-years; HR, 1.80; 95% CI, 0.94–3.44). After adjustment for potential confounders, hazard ratios for mortality for HSV-1 and HSV-2 seropositive women were attenuated, with 95% CI covering the null value (Table 4).

The categorization of herpesvirus IgG levels as dichotomous variables (seropositive vs. seronegative) necessarily results in loss of information. Therefore, in parallel with Cox proportional hazards models dichotomizing herpesvirus IgG values, we assessed the effect of persistent herpesvirus infections on incident frailty and mortality by modeling anti-viral IgG indices as continuous variables. As in examinations of longitudinal risks for outcome by seropositivity status, we observed that increases in VZV and EBV IgG indices were not associated with an increased risk of incident frailty. In contrast, each one unit increase in HSV-1 and HSV-2 IgG index was associated with an 81% and 57% increased risk of developing incident frailty, respectively (HR, 1.81 and 1.57; 95% CI, 1.08–3.04 and 1.21–2.04). After adjustment for potential confounders, these hazard ratios were attenuated and covered the null value (Table 5).

Similarly, increases in VZV and EBV IgG indices were not associated with an increased risk of mortality. However, each one unit increase in HSV-1 and HSV-2 IgG index was associated with a 96% and 51% increase in mortality risk, respectively (HR, 1.96 and 1.51; 95% CI, 1.10–3.48 and 1.19–1.91). After adjustment for potential confounders, the hazard ratio for HSV-1 was attenuated and covered the null value, but each one unit increase in HSV-2 independently predicted a 50% increase in mortality risk (HR, 1.47; 95% CI, 1.05–

2.07) (Table 5). The inclusion of MMSE as an additional covariate in the multivariate model did not change the effect estimates to any appreciable extent (Table 5).

To avoid the assumption of linearity in the relationship between IgG indexes and adverse outcomes, we further categorized antibody indexes into five groups in multivariate Cox proportional hazards models. We found no significantly increased frailty or mortality risk for women in each of the four seropositive groups when compared with seronegative women, for any of the four herpesviruses (data not shown).

DISCUSSION

Previous studies have suggested that CMV infection increases mortality and frailty risks in older adults,^{6–10} but such long-term effects of other persistent herpesvirus infections remain unclear. In this prospective study of community-dwelling older women aged 70–79 years, seropositive HSV-1 and HSV-2 carriers had higher risks of three-year incident frailty and five-year mortality than those who were seronegative. Further, a dose-response relationship was observed between serum HSV antibody levels and frailty and mortality risks. Incremental increases in the HSV-1 and HSV-2 antibody levels were associated with incrementally higher risks of incident frailty and mortality. After adjustment for potential confounders, only incremental increases in HSV-2 antibody level was independently predictive of incrementally higher risks of mortality, but not frailty, in older women.

All herpesviruses share a similar morphologic structure and establish persistent infections for the duration of the host's life. However, distinct herpesviruses differ in their tissue tropism and their mechanisms of pathogenesis.¹ We and others have observed that CMV seropositivity and higher CMV-specific antibody levels are associated with higher mortality risks in immunocompetent adults.^{6–10} Given the shared and unique biologic properties among the herpesviruses, it is important to determine whether this virus-related contribution to higher mortality risk is specific to CMV. While our results demonstrate that HSV-1 and HSV-2 antibody levels are associated with crude mortality risks, the adjusted analyses suggest that HSV-2, but not VZV, EBV, and HSV-1, infection predisposes community-dwelling older women to higher mortality risks. To the best of our knowledge, this is the first report showing an association between HSV antibody levels and mortality risk in older adults.

Several interpretations can be offered for these findings. The most direct is that a true underlying pathophysiologic process predisposes older women with higher HSV antibody levels to higher mortality risks. Among all herpesviruses, CMV and HSV have been most strongly and repeatedly implicated in cardiovascular disease pathogenesis in immunocompetent individuals.^{14–18,28,29} HSV has been detected in atheromatous arterial specimens^{15,16} and, when cultured with human arterial smooth muscle cells *in vitro*, induce lipid accumulation in arterial cells akin to atherosclerosis development *in vivo*.¹⁴ In cell-free preparations, HSV-1 and HSV-2 are able to initiate thrombin formation by virtue of molecular components on the viral envelope surface that enable the coagulation cascade.¹⁷ In a nested case-control study, HSV-1 seropositivity was associated with a two-fold higher risk for incident myocardial infarction and cardiovascular death,¹⁸ while in a prospective

cohort study, HSV-2 (as well as EBV and *H. pylori*) seropositivity was independently associated with the risk of cardiovascular death.¹⁹ However, this association between HSV infection and cardiovascular disease was not found in two other epidemiologic studies.^{20,21} Notably, in a pathological examination of human atheromatous coronary artery and thoracic aorta specimens where CMV and HSV DNA and antigen were detected in the vessel walls, EBV DNA and antigen were not found in the same arterial specimens.¹⁵ This discrepancy in pathological findings between CMV and HSV vs. EBV perhaps underlies a pathophysiologic distinction that explains the differences in contribution to mortality risk by CMV and HSV vs. VZV and EBV.

We conceptualized cardiovascular disease as an intermediary factor in the relationship between herpesvirus infections and mortality. Because cardiovascular disease was a covariate included in our regression analyses, the effect measures for herpesviruses estimated the contribution of herpesvirus infections to mortality beyond their effect through cardiovascular disease. However, measurement error in the cardiovascular disease variable, as well as other unmeasured cardiovascular pathologies, likely resulted in residual effects of cardiovascular disease that could not be fully adjusted for in the multivariate models.³⁰ Thus, the hazard ratios reported here likely encompass some residual contribution of cardiovascular disease to herpesvirus-related mortality risk.

Higher circulating antibody levels could reflect a higher frequency or greater magnitude of viral reactivation and antigenic stimuli, and thus a higher cumulative infectious burden over time, with concomitant pathologic damages that in the long term result in accelerated death. The observations that antibody titers increase over time in CMV shedders³¹ and rise in parallel with higher CMV viral loads measured during viral reactivation³² support the hypothesis that higher anti-viral antibody titers are consistent with poorer viral control and thus greater infectious burden. Indeed, higher VZV antibody titer was associated with higher zoster severity and occurrence of postherpetic neuralgia;³³ higher antibody titer was, therefore, correlated with poorer viral control in this case. The fact that HSV-2 shedding can persist even 10 years or more after first occurrence of genital herpes³⁴ suggests that viral replication and reactivation can continue long after primary infection and, therefore, can cumulatively render pathologic and inflammatory insults to the host over the long run.

The frequency and severity of HSV reactivation are mediated by CD8+ T cell responses at the ganglion³⁵ and the peripheral mucosa.³⁶ While CD8+ T cells play a key role in controlling severity, duration, and rates of reactivation,³⁷ thus preventing disseminated, potentially life-threatening disease as seen in immunocompromised patients,⁴ HSV-specific antibodies also contribute to viral control by restricting the movement of virus from local infection to the nervous system.³⁸ Even in immunocompetent adults, HSV reactivations occur quite frequently, with rapid clearance of the virus within 12 hours,³⁹ and are mostly asymptomatic.^{39,40} A current view of HSV latency proposes a persistent or intermittent low-level viral gene expression in latently infected neurons that are surveyed by antigen-specific CD8+ T cells that prevent full viral reactivation.⁴¹

The extent to which HSV reactivations can cause systemic pathologic insults in immunocompetent adults, either overtly or subclinically, and thus influence long-term

morbidity and mortality is not clear. HSV viremia, reflecting systemic dissemination of HSV through the circulatory system, has been detected in immunocompetent adults with both primary⁴² and recurrent infections,⁴³ and in immunocompromised and immunocompetent hospitalized patients.⁴⁴ In hospitalized patients, HSV viremia was associated with systemic clinical manifestations and higher mortality rates in both immunocompromised and immunocompetent individuals.⁴⁴ Persistent herpesvirus infection may not always have a detrimental consequence; in a murine model, latent infection with herpesviruses similar to human EBV and CMV provided protection against bacterial infections by *Listeria monocytogenes* and *Yersinia pestis*, through prolonged basal activation of the innate immune system against subsequent infections.⁴⁵

The fact that the association between the continuous HSV-1 antibody level and mortality risk became statistically insignificant after adjustment for covariates, while that between HSV-2 and mortality risk remained significant, could be a result of an inadequate power of this study to detect this association between HSV-1 and mortality or a result of a true underlying pathophysiologic difference between HSV-1 and HSV-2 infections. HSV-2 genital infections recur more frequently than HSV-1 orolabial infections.⁴⁶ Differences in HSV-1 and HSV-2 epidemiology could also account for distinct sets of hazardous socio-behavioral risk factors that are associated with each virus: While HSV-1 infection is primarily contracted during childhood,¹¹ the acquisition of HSV-2 parallels the onset of sexual activity.⁴⁷ The association of HSV-2 seropositivity with a greater lifetime number of sexual partners and a greater number of past sexually transmitted diseases⁴⁷ suggest that a higher HSV-2 antibody level could denote a profile of risky behaviors that eventually lead to higher mortality risk. Nevertheless, the similarities between HSV-1 and HSV-2 seroprevalence and hazard ratios in this study, in contrast with the results for VZV and EBV, suggest that HSV-1 and HSV-2 infections are more likely to contribute to mortality risk in a similar manner.

An alternative interpretation of our data is that higher HSV antibody levels and thus more frequent or more severe HSV reactivations could be a bystander, rather than an initiator, of pathologic changes in human hosts. HSV reactivation is closely restrained by virus-specific cytotoxic CD8+ T cells.³⁷ Thus, poorer viral control could simply be consequent to an overall state of relatively impaired cellular immunity in an older adult with deteriorating health who, regardless of HSV reactivations, is prone to higher mortality risk.

It is also important to consider the possibility that HSV serostatus could simply be a surrogate marker of each person's underlying socio-behavioral risk. In our study, older women seropositive for HSV were more likely to be associated with indicators of lower socioeconomic status. This observation is consistent with data from NHANES, in which individuals who were seropositive for HSV-1 and HSV-2 were more likely to have attained a lower level of education and to have lived below the poverty level.^{41,43} It has long been noted that socioeconomic status is inversely related to mortality.^{48,49} A higher prevalence of health risk behaviors among individuals with lower levels of education and income is thought to contribute to, but not fully explain, the higher mortality in this group;⁴⁹ low income still significantly increased mortality risk when health risk behaviors were taken into consideration. Although several indicators of socioeconomic status were adjusted for as potential confounders in our Cox models, other unmeasured socioeconomic status variables

or measurement errors in these covariates could result in residual confounding of the association between herpes simplex viruses and mortality. Conversely, the possibility that greater herpesvirus reactivations among socioeconomically disadvantaged individuals could be a potential mechanistic explanation for the poorer health outcomes in this group has also been postulated.⁵⁰ Thus, the complex interrelationship between herpesvirus reactivation, socioeconomic status, and mortality should be recognized in evaluating these results.

The difference between the seroprevalence of VZV, HSV-1, and HSV-2 in our cohort and those reported in some previous studies constitute a potential limitation of our study. However, herpesvirus seroprevalence can vary significantly among different populations and sociodemographic groups.^{11,51} Although VZV seroprevalence is generally high in the U.S., VZV seroprevalence can vary from 73% to 100% even among cohorts of the same age group.⁵² Nevertheless, the seroprevalence data reported herein confirm the associations between herpesvirus seropositivity and sociodemographic factors reported previously in the literature. It should also be noted that antibody measurement at a single time point does not capture potential changes in serologic status (e.g, seronegative individuals' becoming seropositive) or changes in antibody level over the duration of our follow-up.

In conclusion, regardless of the underlying etiology, HSV-1 and HSV-2 antibody levels are associated with unadjusted risks of incident frailty and mortality in community-dwelling older women. HSV-2 antibody level alone is independently predictive of five-year mortality risk, with each incremental increase in the antibody level adding further risk. Given the wide prevalence of herpesvirus infections and the complex pathophysiologic mechanisms that may underlie the relationship between herpesvirus latency/persistence and health outcomes in older adults (particularly in view of the possibility of competing detrimental and beneficial effects), further research into the mechanisms of herpesvirus pathogenesis in immunocompetent adults is warranted, before rational and safe interventions such as vaccines can be designed and justified.

Acknowledgments

This work was supported by National Institutes of Health (NIH), National Institute on Aging (NIA) Grants K23 AG033113 and P30 AG021334 (through the Johns Hopkins Older Americans Independence Center); T. Franklin Williams Research Scholars Award, sponsored by Atlantic Philanthropies, American Geriatrics Society, the John A. Hartford Foundation, and the Association of Subspecialty Professors; John A. Hartford Foundation's Center of Excellence in Geriatric Medicine Scholars Award; and the Johns Hopkins Biology of Healthy Aging Program.

Sponsor's Role: The investigators retained full independence in the conduct of this research.

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Table 1

Baseline characteristics of study participants in the analytic sample selected from the Women's Health and Aging Studies I and II.

Characteristic ^b	Herpesvirus Seropositivity ^d							
	All participants (n = 633)	VZV		EBV		HSV-1		HSV-2
		+	-	+	-	+	-	+
		(79.9%)	(20.1%)	(72.7%)	(27.3%)	(78.7%)	(21.4%)	(81.7%)
								(18.3%)
Age, years (mean)	73.9	74.0	73.9	74.0	73.9	74.0	73.6	74.0
White race, %	76.5	76.2	77.9	77.3	74.5	73.7*	87.2*	72.3**
Completion of high schooleducation, %	58.0	58.6	55.9	58.2	57.7	52.1**	80.0**	53.1**
Coverage by private medical insurance, %	80.9	91.3	84.4	80.8	81.3	77.3**	94.3**	77.4**
Body mass index (mean)	27.6	27.5	28.4	27.9	27.0	28.1*	26.1*	28.1**
Current or former smoking, %	49.6	50.1	47.7	50.2	48.3	52.1*	40.5*	51.5*
Diabetes mellitus, %	14.3	12.6*	20.9*	14.3	14.3	16.5*	6.1*	15.6*
Cardiovascular disease, %	46.2	44.8	51.5	45.8	47.0	46.3	45.8	47.2
Osteoarthritis, %	72.2	72.4	71.5	71.6	73.8	72.5	70.9	71.6
Osteoporosis, %	42.3	44.8	32.2	41.7	43.8	41.8	44.1	41.8
Pulmonary disease, %	38.4	38.1	39.9	37.6	40.7	38.4	38.6	37.1
Mini-mental status exam score (median)	29	29	29	28	29	28**	30**	28*
Plasma IL-6 concentration, pg/mL (median)	2.74	2.74	2.66	2.74	2.69	2.77	2.61	2.78

Abbreviations: VZV, varicella zoster virus; EBV, Epstein-Barr virus; HSV-1, herpes simplex virus-1; HSV-2, herpes simplex virus-2; IgG, immunoglobulin G; IL-6, interleukin-6.

* $P < 0.05$;

** $P < 0.001$ (P for difference between the seropositive and seronegative groups).

^a Seropositivity was defined as an IgG index > 1.1 .

^b Percentages were those among all participants or within each category of herpesvirus antibody concentration and were calculated with the use of study-specific probability weights.

Table 2

Prevalence of frailty states by herpesvirus IgG antibody concentration.

Frailty status ^a	Herpesvirus Seropositivity							
	VZV		EBV		HSV-1		HSV-2	
	+	-	+	-	+	-	+	-
All participants (n = 633)								
Nonfrail, %	44.0	44.8	44.2	44.1	42.7	49.7	42.8	49.8
Prefrail, %	44.1	45.8	43.8	46.0	45.6	40.1	45.2	41.3
Frail, %	11.9	9.4	12.0	9.9	11.8	10.2	12.0	9.0

^aPrevalent frailty states were measured at baseline. Percentages were those among all participants or within each serostatus category and were calculated with the use of study-specific probability weights.

Table 3

Incident frailty rates by herpesvirus serostatus.

	Herpesvirus Seropositivity					
	VZV	EBV	HSV-1	HSV-2		
Incident Frailty (3 Years)	All participants					
No. at risk	481	387	94	347	134	106
No. of events	81	69	12	59	22	10
Incidence (95% CI) per 1000 person-years	60.8 (49.0–76.3)	63.3 (50.2–80.9)	50.1 (27.8–99.6)	59.2 (45.9–77.7)	51.9 (43.7–101.7)	35.9 (19.6–73.1)
Unadjusted hazard ratio (95% CI)		1.24 (0.64–2.43)	Ref.	0.89 (0.54–1.48)	Ref.	Ref.
Adjusted hazard ratio ^a (95% CI)		1.67 (0.75–3.71)	Ref.	0.89 (0.51–1.55)	Ref.	Ref.

Abbreviations: VZV, varicella zoster virus; EBV, Epstein-Barr virus; HSV-1, herpes simplex virus-1; HSV-2, herpes simplex virus-2; CI, confidence interval.

^aHazard ratios were derived from Cox proportional hazards models, adjusting for age, race, high school education, coverage by private medical insurance, body mass index, pack-years of smoking, cardiovascular disease (angina, myocardial infarction, congestive heart failure, peripheral artery disease, or stroke), diabetes mellitus, and plasma interleukin-6 concentration.

Table 4

Mortality rates by herpesvirus serostatus.

	Herpesvirus Seropositivity					
	VZV	EBV	HSV-1	HSV-2		
All-Cause Mortality (5 Years)	All participants					
No. at risk	633	509	124	454	179	134
No. of events	90	67	23	63	27	13
Incidence (95% CI) per 1000 person-years	27.9 (22.5, 34.8)	26.0 (20.4, 33.7)	35.5 (23.2, 56.8)	27.1 (21.0, 35.6)	29.8 (20.4, 45.0)	17.8 (10.3, 33.2)
Unadjusted hazard ratio (95% CI)	0.73 (0.44, 1.21)	Ref.	Ref.	0.91 (0.56, 1.45)	Ref.	Ref.
Adjusted hazard ratio ^a (95% CI)	0.80 (0.45, 1.41)	Ref.	Ref.	0.78 (0.48, 1.27)	Ref.	Ref.

Abbreviations: VZV, varicella zoster virus; EBV, Epstein-Barr virus; HSV-1, herpes simplex virus-1; HSV-2, herpes simplex virus-2; CI, confidence interval.

^aHazard ratios were derived from Cox proportional hazards models, adjusting for age, race, high school education, coverage by private medical insurance, body mass index, pack-years of smoking, cardiovascular disease (angina, myocardial infarction, congestive heart failure, peripheral artery disease, or stroke), diabetes mellitus, and plasma interleukin-6 concentration.

Table 5
Hazard ratios for incident frailty and mortality with unit increases in herpesvirus IgG index.

	Incident Frailty			Mortality		
	Unadjusted HR ^a (95% CI)	Adjusted HR ^b (95% CI)	Adjusted HR with MMSE in model ^c (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Adjusted HR with MMSE in model (95% CI)
VZV	1.17 (0.70–1.97)	1.44 (0.78–2.68)	1.34 (0.73–2.50)	0.80 (0.49–1.32)	0.81 (0.46–1.41)	0.79 (0.46–1.37)
EBV	1.02 (0.77–1.37)	0.96 (0.72–1.28)	0.91 (0.69–1.21)	1.16 (0.89–1.51)	0.96 (0.75–1.22)	0.93 (0.73–1.20)
HSV-1	1.81 (1.08–3.04)	1.32 (0.71–2.46)	1.21 (0.66–2.24)	1.96 (1.10–3.48)	1.43 (0.72–2.84)	1.38 (0.70–2.74)
HSV-2	1.57 (1.21–2.04)	1.28 (0.85–1.94)	1.21 (0.79–1.85)	1.51 (1.19–1.91)	1.50 [*] (1.04–2.18)	1.47 ^{**} (1.02–2.13)

^aHR with each one unit increase in IgG index

^bHazard ratios were derived from Cox proportional hazards models, adjusting for age, race, high school education, coverage by private medical insurance, body mass index, pack-years of smoking, cardiovascular disease (angina, myocardial infarction, congestive heart failure, peripheral artery disease, or stroke), diabetes mellitus, and plasma interleukin-6 concentration.

^cCovariates were the same as the “adjusted” model, with the addition of MMSE.

^{*} $P=0.03$

^{**} $P=0.04$