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Impact of Viral and Bacterial Burden on Cognitive Impairment in Elderly Persons With Cardiovascular Diseases

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Background and Purpose—Inflammation and infectious etiology have been implicated in the pathogenesis of dementia.

We sought to investigate whether the seropositivity of common infections was associated with cognitive function.

Methods—Viral burden (seropositivity for herpes simplex virus type 1 [HSV-1], herpes simplex virus type 2 [HSV-2], or cytomegalovirus [CMV]) and bacterial burden (*Chlamydia pneumoniae* and *Mycoplasma pneumoniae*) were related to cognitive status and its impairment among 383 home-dwelling elderly with cardiovascular diseases (mean age, 80 years). The Mini-Mental State Examination (MMSE) and its changes and the Clinical Dementia Rating (CDR) were used to define cognitive impairment.

Results—At baseline, 0 to 1, 2, and 3 positive titers toward viruses were found in 48 (12.5%), 229 (59.8%), and 106 individuals (27.7%), respectively. MMSE points decreased with increasing viral burden ($P=0.03$). At baseline, 58 individuals (15.1%) had cognitive impairment, which after adjustments was significantly associated with seropositivity for 3 viruses (hazard ratio, 2.5; 95% CI, 1.3 to 4.7). MMSE score decreased in 150 (43% of 348) during 12-month follow-up. After adjustment for MMSE score at baseline and with 0 to 1 seropositivities as reference (1.0), the hazard ratios were 1.8 (95% CI, 0.9 to 3.6) and 2.3 (95% CI, 1.1 to 5.0) for 2 and 3 seropositivities, respectively. The prevalence of possible or definite dementia according to CDR also increased with viral burden. No significant associations were observed between bacterial burden and cognition.

Conclusions—Viral pathogen burden of HSV and CMV was associated with cognitive impairment in home-dwelling elderly persons with cardiovascular diseases. The results need to be tested in larger databases, but they may offer a preventable cause of cognitive decline. (*Stroke*. 2003;34:2126-2131.)

Key Words: bacteremia ■ dementia ■ herpes simplex ■ neuropsychological tests ■ viral proteins

Dementias are growing healthcare problems in aging societies.¹ These disorders have a number of possible causes, and inflammatory or infectious factors have been implicated in their pathogenesis as well. Expression of the neurotropic cytokine interleukin-6 has been connected with Alzheimer disease,² and higher levels of serum C-reactive protein (CRP) levels were observed in subjects with dementia (half of them with Alzheimer disease) in a population-based study of cohorts aged ≥ 75 years.³ The inflammation may be triggered by the formation of β -amyloid plaques seen in the brains of Alzheimer disease patients,^{2,4} and this may explain the neuroprotective effects of nonsteroidal anti-inflammatory drugs.⁵ On the other hand, one may also speculate that a chronic, perhaps lifelong, infection would maintain inflammation and induce amyloid fibrils in susceptible persons. Of specific microbes, herpes simplex virus type 1 (HSV-1) has been a

prime suspect,⁶⁻¹¹ although other viruses have been implicated as well.¹¹ In this respect, the positive studies in Alzheimer disease have been mostly neuropathological case-control studies¹⁰ that were not adjusted for such factors as educational level. Cytomegalovirus (CMV) has been found in the brains of patients suffering from vascular dementia,¹² infection with human immunodeficiency virus (HIV) has been connected with dementia,¹³ and CMV may also contribute to dementia in these patients.¹⁴ Furthermore, in a large community survey, past exposure to vaccines toward both viruses and bacteria (diphtheria, tetanus, poliomyelitis, and influenza) was associated with lower risk for Alzheimer disease during follow-up.¹⁵ In contrast, 1 study including 33 Alzheimer patients and 28 nondemented controls found no differences in seropositivity toward common infectious agents (HSV, CMV, and influenza) between cases and controls.¹⁶

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Of various bacteria, *Chlamydia pneumoniae*¹⁷ in particular and also *Mycoplasma pneumoniae*¹⁸ have been associated with the pathogenesis of atherosclerotic vascular diseases. These associations are potentially interesting for cognitive disorders because late-onset dementia, including Alzheimer disease, is increasingly considered a vascular disorder.^{19,20} However, reports of *C pneumoniae* in the brains of Alzheimer disease patients have been conflicting.^{21,22}

In the present study we investigated the relationships between systemic microbial burden (serum antibodies) and cognitive function in a sample of elderly persons with vascular diseases retrieved from the community. At the time of the investigations they lived at home, and thus vulnerability for infections in an institutional setting does not confound the results. In the analyses we compared the effects of the pathogen burden of viruses implicated particularly in the development of Alzheimer disease (HSV) or vascular dementia (CMV) and of the pathogen burden of bacteria implicated in the pathogenesis of atherosclerotic diseases (*C pneumoniae*, *M pneumoniae*).

Subjects and Methods

Participants

The study participants were recruited from 2 random samples of residents (n=4821) in Helsinki, Finland, who were born in 1924 or 1925 (n=1450), 1919 or 1920 (n=1450), 1914 (n=1000), 1909 (n=774), and 1904 (n=147). The random sampling was performed and the addresses provided by the Central Population Register of Finland. From this population we randomized 400 home-dwelling individuals with cardiovascular diseases to a multifactorial prevention study, the Drugs and Evidence-Based medicine in The Elderly (DEBATE) study, which has been described in detail elsewhere.²³ All stages of the DEBATE study have been approved by the Ethics Committee of the Department of Medicine, University of Helsinki. Informed consent according to the Declaration of Helsinki was obtained from all patients before any study procedures were performed. The only inclusion criteria for the DEBATE study were the presence of atherosclerotic disease, home as place of residence, and informed consent to participate. Participants were not included or excluded on the basis of past or present infections.

Measurements

In the DEBATE study, cognition is evaluated yearly with the use of the CERAD (Consortium to Establish a Registry for Alzheimer's Disease),²⁴ including the Finnish translation of the Mini-Mental State Examination (MMSE)^{25,26} and the Clinical Dementia Rating (CDR), in which a score >0 implicates possible or definite dementia. MMSE is a validated instrument used widely to assess global cognitive status and to screen for cognitive dysfunction. The test consists of parts covering orientation, memory, and attention. MMSE also tests the ability to name, follow verbal and written commands, write a sentence spontaneously, and copy a geometric figure. In regard to its psychometric properties, both reliability and construct validity of the MMSE have been shown to be satisfactory.²⁶ Possible scores of MMSE range from 0 to 30, with lower scores indicating worse cognitive status. A score <24 points is usually considered to be indicative of clinically significant cognitive impairment. However, even smaller decreases in MMSE have prognostic significance,²⁷ and education- and age-related cut points have also been suggested.²⁸ In the present study we adjusted results for age and education, and therefore a fixed MMSE score and proportion <24 points were used to mark cognitive impairment in cross-sectional studies. Decrease of MMSE score (MMSE points at baseline minus

MMSE points at 1 year) was used as the end point in the prospective part of the study. All tests at baseline and after 1 year were performed by the same nurse trained to use the CERAD test battery.

Routine laboratory measurements including serum lipids, plasma glucose, and high-sensitivity CRP were performed in the central laboratory of Helsinki University Central Hospital.

Microbial Antibody Assays

To determine whether the participants had had earlier infections, immunity assays for HSV-1 and herpes simplex virus type 2 (HSV-2), CMV, *C pneumoniae*, and *M pneumoniae* were performed by testing the presence of immunoglobulin G (IgG) antibodies against these microbes. The serum samples collected at baseline were stored at -20°C until tested in random order by technicians blinded to the clinical data of the patients. The antibody assays were done at the Department of Virology of Helsinki University Central Hospital with the use of the enzyme immunoassay (EIA) method. We used commercial EIA test kits according to the manufacturers' instructions with some slight modifications. The following tests were used: HSV: HerpeSelect 1 enzyme-linked immunosorbent assay (ELISA) IgG and HerpeSelect 2 ELISA IgG (Focus Technologies); CMV: VIDAS CMV IgG (BioMerieux); *M pneumoniae*: *Mycoplasma pneumoniae* IgG EIA (ThermoLabsystems); and *C pneumoniae*: *Chlamydia pneumoniae* IgG EIA (ThermoLabsystems). The results are presented in a qualitative negative/positive scale. Cut points for positive titers were determined by an investigator (K.H.L.) without knowledge of the patients or their cognitive status.

Viral burden was defined as the number of seropositivities toward HSV-1, HSV-2, and CMV divided into 3 categories (0 to 1, 2, or 3). The lowest category was a combination because few people had zero viral seropositivities. Bacterial burden was defined as seropositivities toward *C pneumoniae* and *M pneumoniae* divided into 3 categories (0, 1, or 2).

Statistical Analysis

All analyses were performed with the NCSS statistical program (Internet Web site: www.ncss.com) with the use of descriptive statistics, χ^2 statistics, multiple and logistic regression, and ANCOVA. In analyses with cognitive impairment (MMSE score <24 points) at baseline or after 12 months as dependent variable, logistic regression was performed with the categories of viral or bacterial burden, various risk factors, and demographic data as independent variables. The lowest category of microbial burden was used as reference (hazard ratio=1.0), and 95% CIs were calculated. Backward selection was used to find significant predictors. In prospective analyses of MMSE decrease during 1 year and viral or bacterial burden at baseline, only baseline MMSE score (squared to obtain a normal distribution) was used as covariate because this score was already dictated by age and educational level.

Results

Clinical Characteristics at Baseline

Antibodies were measured in 383 individuals (96% of total population), and they form the sample of this study. The mean age was 80 years, 65% were women, and 42% had education more than primary school (Table 1). Eighty-two percent and 37% had a history of coronary heart disease or stroke, respectively, and 18% had type 2 diabetes mellitus. Median MMSE score at baseline was 27 points, and 58 individuals (15.1%) had MMSE score <24. The values for prevalence of positive titers for the measured pathogens are shown in Table 2. Seropositivity for both herpesviruses was significantly more common among individuals with cognitive decline. At baseline, 0 to 1, 2, and 3 positive titers toward viruses were found in 48 (12.5%), 229 (59.8%), and 106

TABLE 1. Baseline Characteristics According to the Cognitive Status*

Variable†	No Cognitive Impairment (n=325)	Cognitive Impairment (n=58)	P
Age, y (SD)	80 (5)	83 (5)	<0.0001
Women (%)	211 (65)	40 (69)	0.55
Education (%)			
Less than primary school	4 (1)	4 (7)	...
Primary school	165 (51)	48 (83)	...
More than primary school	156 (48)	6 (10)	<0.0001 (global)
Coronary heart disease (%)	266 (81.8)	50 (86.2)	0.42
History of stroke or TIA (%)	124 (38.2)	19 (38.2)	0.43
Diabetes (%)	57 (17.5)	16 (27.6)	0.07
Current smokers (%)	19 (5.8)	4 (6.9)	0.43
Never smokers (%)	177 (54.5)	37 (63.8)	0.19
Current alcohol abstainers (%)	92 (28.3)	26 (44.8)	0.01
MMSE, points, median (IQR)	27 (26–28)	22 (21–23)	<0.0001
Body mass index, kg/m ² (SD)	26.5 (4.2)	26.8 (4.3)	0.60
Blood pressure, mm Hg (SD)			
Systolic	153 (26)	148 (24)	0.11
Diastolic	76 (12)	71 (12)	0.008
High-sensitivity CRP, mg/L, median (IQR)†	1.36 (0.72–2.86)	1.64 (0.75–3.45)	0.36
Blood glucose, mmol/L (SD)	5.3 (1.1)	6.1 (2.5)	0.0007
Lipid values, mmol/L (SD)			
Total cholesterol	5.4 (1.0)	5.5 (1.0)	0.81
LDL cholesterol	3.3 (0.8)	3.3 (0.9)	0.95
HDL cholesterol	1.5 (0.4)	1.5 (0.4)	0.61
Triglycerides†	1.4 (0.6)	1.6 (0.9)	0.30

*Cognitive impairment was defined as Mini-Mental State Examination (MMSE) score <24 points. Scores may range from 0 to 30, with lower scores indicating worse cognitive status. P values are shown for the difference between cognitive impairment and no cognitive impairment.

†Log-transformed values used in statistical analyses.

TIA denotes transient ischemic attack; IQR, interquartile range.

individuals (27.7%), respectively. Positive titers toward 0, 1, and 2 bacteria were found in 79 (20.6%), 182 (47.5%), and 122 (31.9%), respectively.

Cross-Sectional Associations Between Cognitive Function and Pathogen Burden

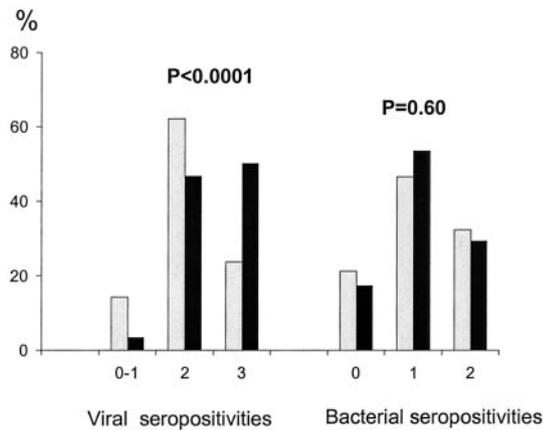
Cognitive status was significantly associated with viral burden at baseline. None of these associations were observed between cognitive status and bacterial burden. First, there was a statistically significant graded impairment of MMSE points (squared values used in calculations adjusted for age, sex, and education) with increasing viral burden: average values were 26.9, 26.5, and 25.8 ($P=0.03$). Second, an increasing viral burden was significantly associated with cognitive impairment, defined as MMSE score <24 points (Figure), and the association remained significant when only persons with less education were included. Abnormal CDR score (≥ 0.5) was seen increasingly with increasing viral burden (in 4.9%, 16.2%, and 26.9%; $P=0.008$). In logistic regression analyses after adjustment for cardiovascular risk

factors, age, and education, seropositivity for 3 viruses was significantly associated with cognitive impairment at baseline and at 1 year when 348 individuals were retested for MMSE (Table 3). The hazard ratios remained virtually unaltered after baseline serum CRP (log transformed) was added to the

TABLE 2. Seropositivity for Pathogens*

Pathogen (%)	No Cognitive Impairment (n=325)	Cognitive Impairment (n=58)	P
Herpes simplex virus 1	279 (85.8)	58 (100)	0.002
Herpes simplex virus 2	102 (31.4)	30 (51.7)	0.003
Cytomegalovirus	293 (90.2)	55 (94.8)	0.26
<i>Chlamydia pneumoniae</i>	206 (63.4)	39 (67.2)	0.57
<i>Mycoplasma pneumoniae</i>	155 (47.7)	26 (44.8)	0.69

*Cognitive impairment was defined as Mini-Mental State Examination (MMSE) score <24 points. Scores may range from 0 to 30, with lower scores indicating worse cognitive status. P values are shown for the difference between cognitive impairment and no cognitive impairment.



Percent distribution of viral and bacterial burden and cognitive impairment. Cognitive impairment was defined as MMSE score <24 points at baseline. Viral burden consists of 0 to 1, 2, or 3 seropositivities toward HSV-1, HSV-2, and CMV. Bacterial burden consists of 0, 1, and 2 seropositivities toward *C pneumoniae* and *M pneumoniae*. Probability values compare the distributions of seropositivities between individuals with normal (gray bars) or impaired (black bars) cognition.

model. All analyses of cognitive function including MMSE and CDR were repeated with bacterial burden substituted for viral burden, but no significant associations were found. *C pneumoniae* seropositivity alone was not associated with cognitive impairment (univariate hazard ratio at baseline, 1.2; 95% CI, 0.7 to 2.1; at 1 year, 1.2; 95% CI, 0.6 to 2.1).

Follow-Up

During the first year 22 individuals died. Among the decedents there were more persons with cognitive impairment at baseline (22.7%) than among survivors (14.7%), but the difference was not statistically significant ($P=0.3$). Mortality was not significantly ($P=0.7$) associated with pathogen burden at baseline. After 12 months, MMSE could be repeated in 348 individuals (91% of the baseline sample), of whom 58 individuals (16.7%) had MMSE score <24 points. Among those failing the test at 1 year, there were 11 individuals with MMSE score <24 points at baseline. During 1 year, the mean change of MMSE score (MMSE score at baseline minus MMSE score at 1 year) was 0.42 points (SD 2.07). Overall, MMSE score was decreased in 150 individuals (43.1% of 348).

After adjustment for MMSE score at baseline, the decrease of MMSE score during 1 year was associated with viral burden in a stepwise manner. With 0 to 1 seropositivities as reference (1.0), the hazard ratios were 1.8 (95% CI, 0.9 to 3.6) and 2.3 (95% CI, 1.1 to 5.0) for 2 and 3 seropositivities, respectively. The results were essentially similar when 1-year mortality or treatment group (indicating more efficient lipid-lowering and antihypertensive therapies) was added to the model. No similar associations were observed with bacterial burden.

Discussion

In this elderly cohort with cardiovascular diseases, viral burden consisting of certain herpesviruses (HSV-1 and HSV-2) and CMV was significantly associated with cognitive impairment. The association was strong and graded and was independent of age, sex, education, and cardiovascular risk factors, including CRP. The finding was consistent both cross-sectionally and during a 12-month follow-up. Viral burden was not associated with mortality. No association was observed between cognition and bacterial burden consisting of *C pneumoniae* and *M pneumoniae*, either alone or together.

In our study the strength of the association, the stepwise increase in the risk of cognitive impairment with increasing viral burden, the temporal association between baseline viral burden and cognitive decline during 12 months, and consistency of data in multiple analyses suggest a causal relationship between viral burden and cognitive impairment. That the relation was independent of cardiovascular risk factors, CRP, and bacterial burden suggests that the pathogenetic sequence may not be via atherosclerosis. On the other hand, all our study participants had some kind of atherosclerotic disease at baseline, which may attenuate the observed association between infection and cognitive impairment. This may also explain the absence of an association between *C pneumoniae* and cognition. However, only 63% were seropositive for *C pneumoniae* at baseline.

There are plausible mechanisms for associations between HSV, CMV, and cognitive function. HSV and CMV are neurotropic viruses, and their activation in the brain may lead to increased neuronal loss and hence may predispose to dementia. A significant homology has been observed between the β -amyloid protein characteristic of Alzheimer disease and an HSV-1 glycoprotein B (gB⁸). It has been hypothesized that

TABLE 3. Hazard Ratios for the Association Between Maximal Viral Burden and Cognitive Impairment*

Baseline Viral Burden†	Hazard Ratio (95% CI) of Cognitive Impairment					
	Baseline, 58 out of 383			At 12 Months, 58 out of 348		
	Univariate	Adjusted I‡	Adjusted II§	Univariate	Adjusted I‡	Adjusted II§
<3, n=277	1.0	1.0	1.0	1.0	1.0	1.0
3 seropositivities, n=106	3.2 (1.8–5.7)	2.4 (1.3–4.6)	2.5 (1.3–4.7)	2.6 (1.4–4.6)	2.3 (1.2–4.5)	2.3 (1.2–4.6)

*Cognitive impairment was defined as Mini-Mental State Examination (MMSE) score <24 points. Scores may range from 0 to 30, with lower scores indicating worse cognitive status.

†Seropositives for herpes simplex virus 1 and 2 and for cytomegalovirus.

‡Adjusted for age, education, body mass index, log-transformed blood glucose, LDL cholesterol, systolic blood pressure, smoking, and alcohol use. Backward selection of variables was used in the logistic regression analysis. Final model included age, education, body mass index, glucose, and viral burden.

§As in model I, but additionally adjusted for baseline serum C-reactive protein (log transformed).

gB may initiate the accumulation of β -amyloid fibril formation in the brain leading to Alzheimer disease.¹⁰ On the other hand, cognitive improvement has been reported when HSV-1 brain infections have been treated with acyclovir,²⁹ but medication may not be effective against latent viruses. In experimental studies, vaccination of mice with mixed HSV-1 glycoproteins significantly protected the animals from latent HSV-1 infection in the central nervous system.³⁰ A vaccine against HSV-1 to prevent development of Alzheimer disease would be a highly interesting option, but if multiple viruses are associated with cognitive impairment, the development of vaccines may be complicated.

Other mechanisms between viruses and brain cells must also be considered. The deposition of β -amyloid peptide in Alzheimer disease may make neurons more susceptible to infection,³¹ whereupon the virus infection would be a consequence rather than a cause of dementia. An interesting mechanism is a combination of pathogen exposure and genetic predisposition. The combination of apolipoprotein E (apoE) e4 allele and HSV-1 has been reported to confer a major risk for Alzheimer disease,^{7,8} and a similar connection may exist between apoE4 and HIV.¹³ In the DEBATE study we have not yet analyzed genetic data of the participants and do not have apoE genotypes available.

The strengths of our study include the relatively large sample retrieved from the community, standardized methods to measure cognitive status in all participants by the same person, and both cross-sectional and prospective analyses. The investigation has limitations, however. All our participants had a history of vascular disease, primarily coronary heart disease or stroke. While this diminishes the generalizability of the results, it may also make the effects of infections more visible because the possible atherosclerotic etiology of cognitive decline is more standardized. The main end point in our study was MMSE score, and at the moment we do not have extensive neurological workup data or brain imaging to confirm Alzheimer disease or vascular dementia. However, our aim in this epidemiological investigation was to focus on cognitive impairment, and the results support earlier, more detailed neuropathological studies. While MMSE is a widely used and validated method to screen cognitive impairment and its changes, it is not necessarily sensitive to pure memory disturbances and does not substitute for wider neuropsychological measurements. Measurement variations during follow-up have been minimized by use of the same nurse at baseline and follow-up examinations. During follow-up, regression toward the mean may occur in the MMSE score, but this will only move the hypothesis toward null. In the DEBATE study it is possible to follow the course of cognitive decline. Finally, half of the sample participates in a multifactorial prevention study after baseline examinations,²³ but the main interventions used are not cognition specific, such as acetylcholinesterase inhibitors. Moreover, no significant differences have been observed in the cognitive function between the control and intervention groups at 1 year (T.E. Strandberg, MD, PhD, et al, unpublished data, 2003). In the prospective part of the present study, the inclusion of the treatment group as a covariate did not essentially change the relationship between viral burden and cognition.

In conclusion, our results support earlier hypotheses that certain infections are associated with cognitive impairment and consequent dementia in old age. However, this may only apply to neurotropic viruses, such as HSV and CMV. In our elderly cohort, these viruses were probably contracted in childhood or young adulthood, but in modern society these are increasingly contracted later in life. If these results are verified in larger population-based studies, they may open new avenues to prevent dementia with antiviral drugs or possibly vaccinations.

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