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Systemic Markers of Inflammation and Cognitive Decline in Old Age

Miranda T. Schram, PhD,† Sjoerd M. Euser, MSc,*† Anton J. M. de Craen, PhD,* Jacqueline C. Witteman, PhD,† Marijke Frölich, PhD,* Albert Hofman, MD, PhD,† Jelle Jolles, MD, PhD,‡ Monique M. B. Breteler, MD, PhD,† and Rudi G. J. Westendorp, MD, PhD**

OBJECTIVES: To investigate whether higher circulating levels of C-reactive protein (CRP), interleukin-6 (IL-6), and α1-antichymotrypsin (ACT) are associated with worse cognitive function and decline in old age.

DESIGN: Two independent population-based cohort studies.

SETTING: The Rotterdam Study (mean follow-up 4.6 years) and the Leiden 85-plus Study (maximal follow-up 5 years).

PARTICIPANTS: Three thousand eight hundred seventy-four individuals, mean age 72, from the Rotterdam Study, and 491 individuals, all aged 85, from the Leiden 85-plus Study.

MEASUREMENTS: Both studies assessed global cognition, executive function, and memory. Linear regression analyses were used in the current study to investigate the associations between inflammatory markers and cognitive function and decline.

RESULTS: In the Rotterdam Study, higher levels of CRP and IL-6 were cross-sectionally associated with worse global cognition and executive function ($P < .05$). ACT was not associated with cognitive function. In the Leiden 85-plus Study, estimates were similar for CRP, although not statistically significant. Higher IL-6 levels were related to a steeper annual decline in memory function in the longitudinal analysis in the Leiden 85-plus Study ($P < .05$). The effect of higher IL-6 levels on global and memory function decline was stronger in apolipoprotein E (APOE) ε4 carriers (P-interaction = .01) than in those who were not (P-interaction = .05). In the Rotterdam Study, higher IL-6 levels were related to a steeper annual decline in global cognition in APOE ε4 carriers only.

CONCLUSION: Systemic markers of inflammation are only moderately associated with cognitive function and decline and tend to be stronger in carriers of the APOE ε4 allele. Systemic markers of inflammation are not suitable for risk stratification. **J Am Geriatr Soc 55:708–716, 2007.**

Key words: C-reactive protein; interleukin-6; α1-antichymotrypsin; cognitive decline; atherosclerosis; APOE ε4

Several lines of investigation have suggested that inflammation is involved in the pathogenesis of dementia. Animal models expressing high levels of proinflammatory cytokines in the brain suffer from neurodegeneration,[1] whereas upregulation of proinflammatory cytokines in tissue cultures leads to microglial activation and neuronal damage.[2] Furthermore, markers of inflammation are found in and around plaques in the brain,[3] and several population-based studies have shown an association between plasma levels of inflammatory markers and the risk of dementia.[4,5] However, dementia develops over a long preclinical period, and its association with inflammatory markers may reflect a consequence of the disease process rather than a causal association. In addition, elderly individuals with cognitive dysfunction often have comorbidities, which might underlie their elevated inflammatory markers. Therefore, it is important to investigate the role of inflammatory markers in the early stages of the disease process, where there is cognitive decline but no full-blown clinical dementia syndrome.

Several studies have reported an association between inflammatory markers and cognitive decline, but these studies were small,[6–8] had a short follow-up,[6,9] included only one marker of inflammation,[7] and typically did not include substantial numbers of individuals aged 80 and older. Moreover, these studies, except for one,[6] did not account for the possible interrelationships between inflammatory markers, atherosclerosis, and cognitive decline. Many elderly individuals have atherosclerosis, which is a

From the *Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands; †Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, the Netherlands; and ‡Department of Psychiatry and Neuropsychology, Maastricht University, Maastricht, the Netherlands.

Address correspondence to M.T. Schram, Department of Gerontology and Geriatrics C-02-R, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, the Netherlands. E-mail: m.vanmeeteren@lumc.nl

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strong risk factor for the development of cognitive decline[10] and is strongly related to inflammatory markers such as C-reactive protein (CRP).[11] Therefore, the observed associations between inflammatory markers and cognitive decline may be nonspecific (i.e., due to the presence of atherosclerosis).

In view of these considerations, the hypothesis that higher levels of the inflammatory markers CRP, interleukin-6 (IL-6), and α1-antichymotrypsin (ACT) are associated with cognitive decline, and that atherosclerosis mediates this association was investigated in two large prospective population-based studies, the Rotterdam Study and the Leiden 85-plus Study. Both studies used a dedicated neuropsychological test battery to assess cognitive decline. Because the apolipoprotein E (APOE) ε4 allele is an important risk factor for cognitive decline,[12,13] which may assert its effects via inflammatory mechanisms, the effect of whether an individual carries the APOE ε4 allele on the association between inflammatory markers and cognitive decline was also investigated.

METHODS

Population

Rotterdam Study

The Rotterdam Study is a large, prospective, population-based cohort study in which all inhabitants aged 55 and older in Ommoord, a district of Rotterdam, the Netherlands, were invited to participate.[14] The medical ethics committee of Erasmus University of Rotterdam approved the study, and informed consent was obtained from all participants. A total of 7,983 individuals (response rate 78%) participated in the baseline examinations between 1989 and 1993 (mean age 71 ± 25, range 55–106). All individuals were interviewed at home and invited to visit the research center for further examinations. In the third (1997–99) and fourth surveys (2001–04), cognitive function was assessed at the research center using a dedicated neuropsychological test battery.

Source Population

The current analyses on inflammation and cognition were conducted on participants who underwent the neuropsychological test battery and had blood drawn at the third survey, in which 4,797 individuals participated, of whom 4,206 underwent neuropsychological testing. Blood samples were obtained from 3,993 of these individuals. Individuals with dementia (n = 119) were excluded, resulting in 3,874 individuals available for analyses.

Of these 3,874 individuals, 2,433 completed the neuropsychological test battery at the fourth survey and thus were available for longitudinal analyses. Of the 1,441 individuals who did not participate, 444 had died, 396 had incomplete data on cognitive function tests, 297 refused to participate, 193 were too ill to visit the research center, and 111 could not be contacted.

Additional Measurements

Education was measured at the baseline examination (1989–93) and dichotomized into at more or less than 6 years of schooling. The following measures were all assessed at the third survey (1997–99). Depressive symptoms were assessed during the home interview, using a depression questionnaire (the 20-item version of the Center for Epidemiologic Studies Depression Scale). The use of cardiovascular or antiinflammatory drugs was assessed by questionnaire during the home interview. The presence of cardiovascular disease (CVD) was defined as a positive history of myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass graft, stroke, or the presence of angina pectoris or intermittent claudication as assessed using the Rose questionnaire. Furthermore, carotid intima medial thickness greater than 1.0 mm and the presence of plaques in the carotid arteries, as assessed using ultrasonography,[15] were used as measures of atherosclerosis. APOE genotypes were assessed as previously described[16] and were available in 3,664 of the 3,874 individuals.

The diagnosis of dementia was made following a three-step protocol.[17] In short, two brief cognition tests (Mini-Mental State Examination (MMSE)[18] and Geriatric Mental State schedule (GMS))[19] were used to screen all subjects. If persons screened positive (MMSE score <26 or GMS organic level >0), a physician examined them using the Cambridge Examination for Mental Disorders of the Elderly and obtained an interview with an informant.[20] A neuropsychologist examined subjects who were suspected of having dementia if additional neuropsychological testing was required for diagnosis. An adjudication panel made final diagnoses.

Leiden 85-plus Study

The Leiden 85-plus Study is a prospective population-based cohort study of inhabitants of Leiden, the Netherlands. The medical ethical committee of the Leiden University Medical Center approved the study, and informed consent was obtained from all participants. Between September 1997 and September 1999, all inhabitants of Leiden born between 1912 and 1914 (n = 705) were contacted within a month after their 85th birthday. A total of 599 individuals (response rate 87%) agreed to participate. From age 85 to 90, annual neuropsychological tests were performed during home visits.

Source Population

The current analyses on inflammation and cognition were conducted on participants who underwent the neuropsychological test battery and had blood samples drawn at age 85. Five hundred ninety-nine individuals underwent neuropsychological testing, of whom 563 had blood samples taken, 29 refused, and seven died before blood samples could be taken. Individuals with dementia at age 85, as defined according to a clinical diagnosis of the treating physician (n = 72), were excluded, resulting in 491 individuals available for analyses.

Data on cognitive function with at least one follow-up collection were available in 440 individuals. At age 86, cognitive function was assessed in 437 individuals; at age 87, in 392 individuals; at age 88, in 374 individuals; at age 89, in 299 individuals; and at age 90, in 255 individuals. Of the remaining 51 individuals without follow-up, 35 died before the age of 86, 13 refused to participate, and three had no data on cognitive function tests at follow-up visits.

Additional Measurements

The following measures were assessed at age 85. Education was dichotomized into more or less than 6 years of schooling. Depressive symptoms were assessed using the 15-item Geriatric Depression Scale[21] during the home interview. The use of cardiovascular or antiinflammatory drugs was determined according to pharmacy records. The presence of CVD was defined as a positive medical history of myocardial infarction, arterial surgery, stroke, angina pectoris, or intermittent claudication. APOE genotypes were assessed as previously described[22] and were available in 479 of the 491 individuals.

Cognitive Function

Global cognitive function was measured using the MMSE in both cohorts. In addition, a neuropsychological test battery was used to assess global cognitive function, executive function, and memory function. The test battery included the abbreviated Stroop test part 3 and the Letter Digit Substitution Task in both cohorts.[23]

In the Rotterdam Study, word fluency[24] was additionally assessed. Because no separate test was administered at the third survey to measure memory function, the items from the MMSE and GMS (an organic level test)[25] that assess memory were used. The resulting memory score had a range of 0 (worst score) to 4 points (best score).

In the Leiden 85-plus Study, memory function (immediate and delayed recall) was assessed using the 12-Picture Learning Test.[26]

Compound cognitive test scores were constructed by transforming individual test scores into standardized Z-scores (Z-score = (individual score–mean population score)/standard deviation of the population score). Compound scores were estimated for global cognitive function and executive function. In the Rotterdam Study, global cognitive function was calculated by averaging the Z-scores of the Stroop test, the Letter Digit Substitution Task, the word fluency test, and the memory score; in the Leiden 85-plus Study, global cognitive function was calculated by averaging the Z-scores of the Stroop test, the Letter Digit Substitution Task, and the 12-Picture Learning Test immediate and delayed recall. Executive function included the Z-scores of the Stroop test and the Letter Digit Substitution Task in both cohorts.[27]

Inflammatory Markers

High-sensitivity CRP was measured using a Rate Near Infrared Particle Immunoassay (IMMAGE, Immunochemistry System, Beckman Coulter, San Diego, CA, detection limit 0.2 mg/L, coefficient of variation (CV) 3.2%) in the Rotterdam Study. CRP was measured using a fully automated Hitachi 747 system (Hitachi, Tokyo, Japan, detection limit 1 mg/L, CV < 5%) in the Leiden 85-plus Study.

IL-6 plasma levels were determined using a quantitative enzyme-linked immunosorbent assay (ELISA) technique (Quantikine HS IL-6 kit, R&D Systems, Oxon, UK, detection limit 0.094 pg/mL, CV 8.7%) and ACT plasma levels using kinetic nephelometry (Behring Nephelometer BN200, Marburg, Germany, detection limit 1.5 mg/dL, CV 2.8%) in a random sample of the Rotterdam Study (n = 491). IL-6 levels in the Leiden 85-plus Study were obtained from an ex vivo whole blood stimulation assay at age 85.[28] In short, after incubation of venous blood samples at 37°C and 5% carbon dioxide for 24 hours, supernatants were collected and stored at −80°C until IL-6 was measured using a standard ELISA (Sanquin, Amsterdam, the Netherlands, detection limit 4 pg/mL, CV 5–10%). Unstimulated IL-6 levels were used as an estimate of circulating IL-6 levels. In addition, circulating IL-6 levels were measured at age 86 using the same ELISA.

Statistical Analyses

First, the cross-sectional association between systemic markers of inflammation and cognitive function was investigated using linear regression analyses and markers of inflammation both in categories and as continuous variables in the models. CRP and IL-6 were log-transformed because of their skewed distribution.

Second, linear regression was used to investigate the longitudinal association between systemic markers of inflammation and cognitive decline, with the annual change in cognitive function as the dependent variable and inflammatory markers as the independent variable. In the Rotterdam Study, annual cognitive decline was calculated as the difference between the test scores at the fourth and the third survey divided by follow-up time (mean follow-up 4.6 ± 0.5 years). In the Leiden 85-plus Study, annual cognitive decline was calculated by subtracting test scores at the latest follow-up examination from the test scores at age 85 and dividing them by the follow-up time (mean follow-up 3.4 ± 1.8 years).

All analyses were adjusted for age (Rotterdam Study only), sex, and education level, because these factors strongly relate to cognitive function. On further analysis, body mass index, diabetes mellitus, and prevalent CVD were adjusted for. Finally, whether the association between inflammatory markers and cognitive decline is different in the presence of APOE ε4, CVD, and atherosclerosis was tested for.

RESULTS

Table 1 gives principal features of both study populations. Table 2 shows the clinical characteristics of both study samples used for cross-sectional and longitudinal analyses. In the sample from the Rotterdam Study, mean age was 72.1, 58% were women, and 29% had primary education only. In the Leiden 85-plus Study, mean age was 85, 65% were women, and 62% had primary education only. Cognitive function in the Rotterdam Study was better than in the Leiden 85-plus Study, reflecting the younger age range and higher education level in the Rotterdam Study.

As expected, participants who were included in the follow-up examinations of the Rotterdam Study were younger and had better cognitive function than the complete sample. Median follow-up was 4.6 years (range 1.5–7.1). In the Leiden 85-plus Study, individuals with at least 1 year of follow-up had only slightly better cognitive function than the complete sample. Median follow-up was 5.0 years (range 1.0–5.0).

Table 1. Overview of Both Study Populations

Characteristics	Rotterdam Study	Leiden 85-plus Study
Baseline investigation	1989–1993	1997–1999
Inclusion	All individuals aged ≥55 living in Ommoord, a district of Rotterdam	All individuals born between 1912 and 1914 living in Leiden
Age at baseline	≥55 (range 55–99)	85
Cognitive function		
Measurements	Mini-Mental State Examination Stroop test Letter Digit Substitution Task Memory score Word Fluency	Mini-Mental State Examination Stroop test Letter Digit Substitution Task Immediate and delayed recall of the 12-Picture Learning Test
Number of measurements	Two	Annual (maximum 5)
Measurements used	Third survey (1997–1999) Fourth survey (2001–2004)	Baseline (1997–1999) Latest follow-up examination
Follow-up	4.6 years	Maximum 5 years
Markers of inflammation		
Measurements	High-sensitivity CRP IL-6 ACT	CRP Unstimulated IL-6
Measured at:	Third survey (1997–1999)	Baseline
Number	3874 for high-sensitivity CRP 491 for IL-6/ACT	491 for CRP 491 for unstimulated IL-6

CRP = C-reactive protein; IL-6 = interleukin-6; ACT = α1-antichymotrypsin.

Inflammatory Markers and Cognitive Function

In the Rotterdam Study, higher CRP and IL-6 levels were associated with worse cognitive function (Table 3). Estimates were similar in the Leiden 85-plus Study for CRP, although not statistically significant (Table 3). ACT was not related to cognitive function (all $P > .37$).

Additionally, the study populations were stratified for the presence or absence of an APOE ε4 allele and according to the presence or absence of CVD or atherosclerosis at baseline. The association between inflammatory markers and cognitive function did not differ between those with and without an APOE ε4 allele or between those with or without CVD or atherosclerosis. This held true for both cohorts (data not shown).

Inflammatory Makers and Cognitive Decline

Table 4 shows the association between CRP and IL-6 and cognitive decline, as measured during follow-up. In both cohorts, nearly all point estimates of cognitive function were negative, indicating worse outcome, when levels of inflammatory markers at baseline were higher. There may be a significant association between IL-6 and memory decline in the Leiden 85-plus Study. Higher levels of ACT were not related to a steeper annual cognitive decline (all $P > .19$).

Table 5 shows the associations between inflammatory markers and cognitive decline stratified for carriers and noncarriers of the APOE ε4 allele. In the Rotterdam Study, IL-6 was associated with a decline in global cognitive function in those who carry the APOE ε4 allele but not in those who do not. In the Leiden 85-plus Study, the associations of CRP and IL-6 with global cognitive decline and memory decline were stronger in carriers of the APOE ε4 allele than in noncarriers (P-interaction $< .05$). ACT was associated with a decline in executive function in APOE ε4 carriers (annual decline –0.020, 95% confidence interval (CI) = –0.039 to –0.002), but not in noncarriers (annual decline –0.001 95% CI = –0.017–0.019), P-interaction = .15).

The presence of CVD or atherosclerosis at baseline did not consistently influence the relationship between inflammatory markers and cognitive decline in either of the two cohorts (data not shown).

Additional Analyses

Additional adjustment for symptoms of depression, use of antiinflammatory drugs, body mass index, CVD, and diabetes mellitus did not materially change the results (data not shown). Furthermore, in the Rotterdam sample, whether the association between inflammatory markers and cognitive function and decline was stronger at old age was investigated; no consistent influence of age on this association was found.

In this follow-up study on inflammation contributing to cognitive decline, those who suffered from dementia at baseline were excluded (Rotterdam Study: n = 119, Leiden 85-plus Study: n = 72), but when individuals with dementia at baseline were reintroduced into the analyses, the results did not materially change (data not shown).

In addition to unstimulated production levels of IL-6, circulating IL-6 was also measured at age 86 in the Leiden 85-plus Study (n = 427). In this sample, all analyses of the relationship between cognitive function and decline and circulating IL-6 were repeated. The key question was whether the association between higher unstimulated IL-6 production levels and greater annual cognitive decline could

Table 2. Characteristics at the Third Survey of the Rotterdam Study and at Age 85 of the Leiden 85-plus Study

Characteristics	Rotterdam Study		Leiden 85-plus Study	
	Study Sample at Third Survey (n = 3,874)	Study Sample at Third Survey with 4.6-Year Follow-Up (n = 2,433)	Study Sample at Baseline (n = 491)	Study Sample at Baseline with at Least 1-Year Follow-Up (n = 440)
Clinical characteristics				
Age, mean ± SD	72.1 ± 6.9	70.2 ± 5.7	85	85
Female, %	58	58	65	67
Low level of education, %	29	26	62	60
Cardiovascular disease, %	37	31	62	61
Stroke, %	5	3	9	8
Present smoking, %	18	17	15	14
Diabetes mellitus, %	12	10	16	16
Medication use				
Use of cardiovascular drugs, %	48	45	53	52
Use of nonsteroidal antiinflammatory drugs, %	8	8	28	27
Cognitive function				
MMSE score, mean ± SD	27.7 ± 2.0	28.1 ± 1.6	25.4 ± 4.6	25.5 ± 4.4
Center for Epidemiological Studies Depression Scale score, median (interquartile range)	1 (0–6)	1 (0–5)	—	—
Geriatric Depression Scale score, median (interquartile range)	—	—	2 (1–3)	2 (1–3)
Stroop Test Part 3, seconds, mean ± SD	57.5 ± 21.6	53.6 ± 17.6	81.5 ± 32.9	81.1 ± 33.3
Letter Digit Substitution Task, correct answers, mean ± SD	26.6 ± 7.1	28.1 ± 6.5	17.3 ± 7.0	17.6 ± 7.0
Word Fluency, words, mean ± SD	20.9 ± 5.5	21.8 ± 5.3	—	—
Memory Score delayed recall, words, mean ± SD	3.2 ± 0.9	3.3 ± 0.8	—	—
12-Picture Learning Test immediate recall, total pictures of 3 trials, mean ± SD	—	—	24.4 ± 5.4	24.4 ± 5.5
12-Picture Learning Test delayed recall, pictures, mean ± SD	—	—	8.9 ± 2.5	8.9 ± 2.5
Inflammatory markers				
High-sensitivity CRP, mg/L, median (interquartile range)	2.4 (1.2–4.6)	2.2 (1.1–4.2)	—	—
CRP, mg/L, median (interquartile range)	—	—	4 (1–8)	3 (1–7)
IL-6, pg/mL, median (interquartile range)	2.11 (1.44–3.32)*	1.96 (1.37–2.88)†	—	—
Unstimulated IL-6, pg/mL, median (interquartile range)	—	—	11 (0–53)	10 (0–57)
ACT, mg/dL, mean ± SD	39.8 ± 11.3*	38.9 ± 10.8†	—	—

Note: Results in the Rotterdam Study for neuropsychological tests and C-reactive protein (CRP) were available in 3,874 individuals for the Mini-Mental State Examination (MMSE), 3,720 for the Stroop Test, 3,766 for the Letter Digit Substitution Task, 3,802 for Word Fluency, and 3,871 for the Memory Score delayed recall. Results for neuropsychological tests, interleukin-6 (IL-6) and α1-antichymotrypsin (ACT) were available in 491 individuals for the MMSE, 487 for the Stroop Test, 489 for the Letter Digit Substitution Task, 487 for Word Fluency, and 491 for the Memory Score delayed recall. Results in the Leiden 85-plus Study for neuropsychological tests and inflammatory markers were available in 491 individuals for MMSE, 413 for the Stroop test, 408 for the Letter Digit Substitution Task, and 426 and 425 for immediate and delayed recall, respectively, of the 12-Picture Learning Test.

* In a random sample of 491.

† In a sample of 300.

SD = standard deviation.

Table 3. Association Between Inflammatory Markers and Cognitive Test Performance

	Rotterdam Study				Leiden 85-plus Study			
Inflammatory Marker	Difference in Test Performance (95% CI)	*R*	R^2	*P*-value	Difference in Test Performance (95% CI)	*R*	R^2	*P*-value
CRP per SD*	(n = 3,874)				(n = 491)			
MMSE score	− 0.04 (− 0.10–0.02)	0.38	0.14	.15	− 0.14 (− 0.52–0.23)	0.32	0.10	.46
Global cognitive function (Z-score)	− 0.03 (− 0.05 to–0.02)	0.53	0.29	< .001	− 0.05 (− 0.11–0.02)	0.30	0.09	.20
Executive function (Z-score)	− 0.04 (− 0.07 to–0.02)	0.54	0.29	< .001	− 0.05 (− 0.13–0.03)	0.40	0.16	.23
Memory (delayed recall)	− 0.03 (− 0.05–0.001)	0.29	0.09	.06	− 0.15 (− 0.39–0.09)	0.13	0.02	.21
Interleukin-6 per SD	(n = 491)				(n = 491)			
MMSE score	− 0.22 (− 0.41 to − 0.03)	0.38	0.15	.03	0.16 (− 0.22–0.53)	0.32	0.10	.41
Global cognitive function (Z-score)	− 0.08 (− 0.14 to − 0.02)	0.56	0.31	.009	0.01 (− 0.06–0.07)	0.29	0.09	.89
Executive function (Z-score)	− 0.10 (− 0.17 to − 0.03)	0.54	0.30	.008	0.02 (− 0.06–0.09)	0.40	0.16	.66
Memory (delayed recall)	− 0.05 (− 0.14–0.04)	0.31	0.10	.27	0.01 (− 0.22–0.25)	0.11	0.01	.92

Note: All analyses were performed using linear regression adjusted for age (Rotterdam Study only), sex, and education.
* Measured as high-sensitivity C- reactive protein (CRP) in the Rotterdam Study and as CRP in the Leiden 85-plus Study.
CI = confidence interval; SD = standard deviation; MMSE = Mini-Mental State Examination.

be replicated, especially in the memory domain. High circulating IL-6 levels were associated with an annual decline in MMSE of –0.418 points (95% CI = –0.829 to –0.133) and with an annual decline in memory function of –0.254 words (95% CI = –0.488 to –0.019). Corresponding estimates for unstimulated IL-6 levels were –0.352 points (95% CI = –0.756–0.061) for the MMSE and –0.334 words (95% CI = –0.588 to –0.080) for memory decline. The similar effect sizes suggest that the unstimulated production level of IL-6 in whole blood samples is a valid estimate of circulating IL-6.

DISCUSSION

In this study, systemic levels of CRP and IL-6 were only moderately associated with estimates of global cognition and executive function in cross-sectional analyses. Systemic levels of IL-6 were related to a longitudinal decline in memory function in the Leiden 85-plus Study only. In APOE ε4 carriers, greater IL-6 levels tended to be more strongly associated with the annual decline in global cognition and memory function than in noncarriers, although because of the large number of comparisons made and the large sample size, these results may be due to chance.

Table 4. Association Between Inflammatory Markers and Annual Cognitive Decline

	Rotterdam Study				Leiden 85-plus Study			
Inflammatory Marker	Annual Decline* (95% CI)	*R*	R^2	*P*-value	Annual Decline* (95% CI)	*R*	R^2	*P*-value
CRP per SD†	(n = 2,433)				(n = 440)			
MMSE score	0.001 (− 0.016–0.019)	0.14	0.02	.88	− 0.100 (− 0.290–0.090)	0.07	0.01	.30
Global cognitive function (Z-score)	− 0.002 (− 0.006–0.002)	0.18	0.03	.43	− 0.005 (− 0.032–0.021)	0.03	0.00	.69
Executive function (Z-score)	− 0.003 (− 0.007–0.002)	0.22	0.05	.21	− 0.020 (− 0.055–0.014)	0.13	0.02	.24
Memory (delayed recall)	0.002 (− 0.006–0.011)	0.06	0.00	.59	− 0.020 (− 0.137–0.097)	0.10	0.01	.74
Interleukin-6 per SD	(n = 304)				(n = 440)			
MMSE score	− 0.035 (− 0.087–0.020)	0.19	0.04	.22	− 0.133 (− 0.317–0.050)	0.08	0.01	.15
Global cognitive function (Z-score)	− 0.008 (− 0.021–0.006)	0.14	0.03	.26	− 0.024 (− 0.049–0.001)	0.11	0.01	.06
Executive function (Z-score)	− 0.005 (− 0.019–0.009)	0.24	0.06	.49	− 0.011 (− 0.043–0.022)	0.12	0.02	.52
Memory (delayed recall)	− 0.008 (− 0.038–0.022)	0.08	0.01	.60	− 0.123 (− 0.233 to − 0.013)	0.15	0.02	.03

Note: All analyses were performed using linear regression, adjusted for age (Rotterdam Study only), sex, and education level. In the Leiden 85-plus Study follow-up, data on cognitive function were available in 440 individuals for Mini-Mental State Examination (MMSE), 350 for global cognitive function, 328 for executive function, and 368 for memory.
* Estimates from a mean follow-up of 5 years.
† Measured as high-sensitivity C- reactive protein (CRP) in the Rotterdam Study and as CRP in the Leiden 85-plus Study.
CI = confidence interval; SD = standard deviation.

Table 5. Association Between Inflammatory Markers and Annual Cognitive Decline in Carriers and Noncarriers of the Apolipoprotein E (APOE) ε4 Allele

Inflammatory Marker	Rotterdam Study						Leiden 85-plus Study					
	APOE ε4 Noncarrier			APOE ε4 Carrier			APOE ε4 Noncarrier			APOE ε4 Carrier		
	Annual Decline* (95% CI)	*R*	R^2	Annual Decline* (95% CI)	*R*	R^2	*P* for Interaction	Annual Decline* (95% CI)	*R*	R^2	Annual Decline* (95% CI)	*R*	R^2	*P* for Interaction
CRP per SD†	(n = 1,698)			(n = 621)				(n = 342)			(n = 85)			
MMSE score	−0.001 (−0.022–0.019)	0.14	0.02	−0.007 (−0.045–0.031)	0.13	0.02	.78	−0.130 (−0.366–0.106)	0.08	0.01	−0.021 (−0.336–0.294)	0.07	0.01	.59
Global cognitive function (Z-score)	−0.002 (−0.007–0.003)	0.18	0.03	−0.004 (−0.012–0.004)	0.18	0.03	.72	0.017 (−0.010–0.045)	0.09	0.01	−0.061 (−0.136–0.015)	0.21	0.05	.01
Executive function (Z-score)	−0.006 (−0.011–0.000)	0.20	0.04	−0.001 (−0.010–0.008)	0.26	0.07	.32	−0.014 (−0.054–0.026)	0.14	0.02	−0.024 (−0.101–0.053)	0.09	0.01	.75
Memory (delayed recall)	0.006 (−0.005–0.016)	0.08	0.01	−0.007 (−0.025–0.012)	0.05	0.00	.22	0.054 (−0.076–0.183)	0.10	0.01	−0.228 (−0.519–0.063)	0.26	0.07	.03
Interleukin -6 per SD	(n = 204)			(n = 85)				(n = 342)			(n = 85)			
MMSE score	−0.013 (−0.077–0.052)	0.22	0.05	−0.094 (−0.206–0.018)	0.21	0.05	.27	−0.094 (−0.306–0.118)	0.07	0.01	−0.231 (−0.648–0.187)	0.14	0.02	.61
Global cognitive function (Z-score)	−0.002 (−0.018–0.014)	0.20	0.04	−0.028 (−0.054 to−0.001)	0.34	0.11	.20	−0.016 (−0.040–0.008)	0.10	0.01	−0.127 (−0.234 to −0.021)	0.30	0.09	.01
Executive function (Z-score)	−0.006 (−0.023–0.010)	0.22	0.05	−0.014 (−0.040–0.012)	0.31	0.09	.55	−0.012 (−0.046–0.023)	0.14	0.02	−0.018 (−0.129–0.094)	0.05	0.00	.98
Memory (delayed recall)	0.006 (−0.029–0.041)	0.16	0.02	−0.042 (−0.106–0.021)	0.21	0.04	.26	−0.099 (−0.212–0.014)	0.13	0.02	−0.486 (−0.892 to −0.079)	0.33	0.11	.05

Note: All analyses were performed using linear regression adjusted for age, sex, and education level. In the Leiden 85-plus Study, follow-up data on cognitive function were available in 440 individuals for Mini-Mental State Examination (MMSE), 346 for global cognitive function, 348 for executive function, and 364 for memory.

*Estimates from a mean follow-up of 5 years.

†Measured as high-sensitivity C-reactive protein (CRP) in the Rotterdam Study and as CRP in the Leiden 85-plus Study.

CI = confidence interval; SD = standard deviation; *R* = correlation coefficient; R^2 = coefficient of determination.

Previous studies also demonstrated minor associations between inflammatory markers and cognitive decline,[6–9,29] although the effect size being modest does not imply that the involvement of systemic inflammation in the pathophysiology of cognitive decline is modest as well.[30] In fact, the consistent finding of this association in different populations may suggest that inflammation is involved in the pathophysiology of cognitive decline, although the possibility that both inflammatory activation and cognitive decline reflect the consequence of an underlying common disease process cannot be excluded. In contrast, these findings imply that measurement of inflammatory markers is not suitable for risk prediction of cognitive decline in clinical practice.

Dementia, the end stage of severe cognitive decline, develops over a long preclinical period. Therefore, its association with inflammatory markers may reflect a consequence of the disease process rather than a causal path. This may play a role in cross-sectional studies and in studies with a short follow-up. To limit the possibility that individuals with dementia would affect the association between inflammatory markers and cognition, subjects with dementia were vigorously excluded from the analyses. It cannot be excluded that subjects who are early in the dementia process and do not fulfill the criteria for dementia yet affect the associations as described here, although when the analyses were repeated, and those with dementia at baseline were included, the observed associations did not change. It was previously demonstrated in the Rotterdam Study that ACT was strongly related to incident dementia, whereas CRP and IL-6 were only moderately associated with dementia.[5] The discrepancy with the current findings suggests that timing is important in the association between inflammatory markers and cognitive decline and dementia. It is possible that inflammation can only predict the late stages of cognitive decline, that of dementia, whereas it is not suitable for risk stratification of early cognitive decline.

A trend toward a stronger association between inflammatory markers and cognitive decline in carriers of the APOE ε4 allele was found. This is in agreement with a previous finding that the APOE ε4 allele is associated with an impaired response to cerebral damage,[31] which may lead to a steeper decline in cognitive function, although significant interactions were observed only in the Leiden 85-plus sample, which may imply an important influence of age on the relationship between inflammatory markers and cognitive decline. However, formal testing of this suggestion did not demonstrate a stronger association between inflammatory markers and cognitive decline in old age. Previously, the Longitudinal Aging Study Amsterdam investigated whether APOE ε4 modulates the association between inflammatory markers and cognitive decline[6] and did not find an interaction between APOE ε4, inflammatory markers, and cognitive decline. Taken together, the effect of the APOE ε4 allele on the relationship between inflammation and cognition needs further investigation.

No interaction was observed between atherosclerosis or CVD and the association between inflammatory markers and cognitive function and decline. This is in contrast to previous results from the Leiden 85-plus Study, which showed that inflammatory markers interact with atherosclerosis in their association with cognitive decline.[32]

However, these results were derived from cross-sectional data and are therefore more difficult to interpret with respect to causality.

The current study had several strengths. First, data on inflammation and cognitive function were available in a large population-based sample of 3,874 individuals from the Rotterdam Study over 4.6 years of follow-up and of 491 individuals of the Leiden 85-plus Study with annual follow-up measures over a period of 5 years. Second, cognitive function was measured using a neuropsychological test battery that measures separate cognitive domains—executive and memory function, as well as global function—and used cognitive decline as an early indicator of the dementia process. Third, by combining data from two population-based studies with a different age range, it can be concluded that the association between inflammatory markers and cognition is present over a large age range.

This study had also several limitations. First, plasma levels of CRP, IL-6, and ACT were measured only once, which may have diluted the associations found and may thus have led to an underestimation of the risk estimates. Second, different assays were used to determine CRP levels. The discriminative power of the Rotterdam Study for the analyses including CRP was larger because of the sample size and the use of a high-sensitivity CRP assay. This may explain why significant findings could not be replicated in both study samples. Third, in the Leiden 85-plus Study, unstimulated IL-6 production in a whole blood assay was used as an estimate of circulating plasma IL-6 levels. Although these production levels are higher than circulating plasma levels, this has been demonstrated to be a good estimate of plasma IL-6 levels. Fourth, a number of statistical analyses were performed, which carries the possibility of significant findings by chance (i.e., caused by random type 2 errors produced by the large sample size and number of statistical test performed). Although, the main interest was to replicate the findings in two independent populations, which automatically doubled the number of analyses performed, and the analyses were based on an a priori hypotheses, a stricter *P*-value, for instance of $<.01$, might have been more appropriate to use for these analyses. With this stricter interpretation, statistical significance in the prospective analyses would have been lost.

Although the cohorts were population based and prospectively followed, selection bias may have occurred by selective nonresponse. The participation rate was probably lowest in subjects with cognitive impairment. Individuals with follow-up data had a better cognitive performance than those who did not return for follow-up examinations, especially in the Rotterdam Study. This suggests that the observed cognitive decline is probably lower than the actual decline and therefore that the associations with the inflammatory markers may have been underestimated.

In conclusion, systemic markers of inflammation are only moderately associated with cognitive function and decline. These data show some suggestion that these associations may be stronger in the presence of the APOE ε4 allele, although these results should be interpreted with care and may be due to chance. Systemic markers of inflammation are not suitable to predict individual risk of cognitive decline.

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REFERENCES

1. Campbell IL, Stalder AK, Chiang CS et al. Transgenic models to assess the pathogenic actions of cytokines in the central nervous system. Mol Psychiatry 1997;2:125–129.
2. Ho GJ, Drego R, Hakimian E et al. Mechanisms of cell signaling and inflammation in Alzheimer's disease. Curr Drug Targets Inflamm Allergy 2005;4:247–256.
3. Eikelenboom P, Veerhuis R. The importance of inflammatory mechanisms for the development of Alzheimer's disease. Exp Gerontol 1999;34:453–461.
4. Schmidt R, Schmidt H, Curb JD et al. Early inflammation and dementia: A 25-year follow-up of the Honolulu-Asia Aging Study. Ann Neurol 2002; 52:168–174.
5. Engelhart MJ, Geerlings MI, Meijer J et al. Inflammatory proteins in plasma and the risk of dementia. The Rotterdam Study. Arch Neurol 2004;61: 668–672.
6. Dik MG, Jonker C, Hack CE et al. Serum inflammatory proteins and cognitive decline in older persons. Neurology 2005;64:1371–1377.
7. Weaver JD, Huang MH, Albert M et al. Interleukin-6 and risk of cognitive decline. Macarthur Studies of Successful Aging. Neurology 2002; 59:371–378.
8. Teunissen CE, van Boxtel MP, Bosma H et al. Inflammation markers in relation to cognition in a healthy aging population. J Neuroimmunol 2003;134: 142–150.
9. Yaffe K, Lindquist K, Penninx BW et al. Inflammatory markers and cognition in well-functioning African-American and white elders. Neurology 2003;61:76–80.
10. O'Brien JT, Erkinjuntti T, Reisberg B et al. Vascular cognitive impairment. Lancet Neurol 2003;2:89–98.
11. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–126.
12. Nussbaum RL, Ellis CE. Alzheimer's disease and Parkinson's disease. N Engl J Med 2003;348:1356–1364.
13. Henderson AS, Easteal S, Jorm AF et al. Apolipoprotein E allele epsilon 4, dementia, and cognitive decline in a population sample. Lancet 1995; 346:1387–1390.
14. Hofman A, Grobbee DE, de Jong PT et al. Determinants of disease and disability in the elderly. The Rotterdam Elderly Study. Eur J Epidemiol 1991;7:403–422.
15. van der Meer I, Bots ML, Hofman A et al. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction. The Rotterdam Study. Circulation 2004;109:1089–1094.
16. Slooter AJ, Cruts M, Kalmijn S et al. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study. The Rotterdam Study. Arch Neurol 1998;55:964–968.
17. Ott A, Breteler MM, van Harskamp F et al. Incidence and risk of dementia. The Rotterdam Study. Am J Epidemiol 1998;147:574–580.
18. Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–198.
19. Copeland JR, Kelleher MJ, Kellett JM et al. A semi-structured clinical interview for the assessment of diagnosis and mental state in the elderly. The

Geriatric Mental State Schedule. I. Development and reliability. Psychol Med 1976;6:439–449.
20. Roth M, Tym E, Mountjoy CQ et al. CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. Br J Psychiatry 1986;149:698–709.
21. Yesavage JA, Brink TL, Rose TL et al. Development and validation of a geriatric depression screening scale: A preliminary report. J Psychiatr Res 1982;17:37–49.
22. Bolla MK, Haddad L, Humphries SE et al. High-throughput method for determination of apolipoprotein E genotypes with use of restriction digestion analysis by microplate array diagonal gel electrophoresis. Clin Chem 1995;41:1599–1604.
23. Houx PJ, Shepherd J, Blauw GJ et al. Testing cognitive function in elderly populations: the PROSPER study. PROspective Study of Pravastatin in the Elderly at Risk. J Neurol Neurosurg Psychiatry 2002;73:385–389.
24. Luteijn F, van der Ploeg F. Groninger Intelligentie Test. Lisse, the Netherlands: Swets & Zeitlinger, 1983.
25. Copeland JR, Dewey ME, Griffiths-Jones HM. A computerized psychiatric diagnostic system and case nomenclature for elderly subjects: GMS and AGE-CAT. Psychol Med 1986;16:89–99.
26. Brand N, Jolles J. Learning and retrieval rate of words presented auditorily and visually. J Gen Psychol 1985;112:201–210.
27. van Boxtel MP, Buntinx F, Houx PJ et al. The relation between morbidity and cognitive performance in a normal aging population. J Gerontol A Biol Sci Med Sci 1998;53A:M147–M154.
28. van den Biggelaar AH, Huizinga TW, de Craen AJ et al. Impaired innate immunity predicts frailty in old age. The Leiden 85-plus study. Exp Gerontol 2004;39:1407–1414.
29. Ravaglia G, Forti P, Maioli F et al. Serum C-reactive protein and cognitive function in healthy elderly Italian community dwellers. J Gerontol A Biol Sci Med Sci 2005;60A:M1017–M1021.
30. Rothman KJ, Greenland S. Chapter 2. Causation and Causal Interference. In: Rothman KJ, Greenland S, eds. Modern Epidemiology, 2nd Ed. Philadelphia: Lippincott Williams & Wilkins, 1998, pp 9–11.
31. Poirier J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. Trends Neurosci 1994;17:525–530.
32. van Exel E, de Craen AJ, Remarque EJ et al. Interaction of atherosclerosis and inflammation in elderly subjects with poor cognitive function. Neurology 2003;61:1695–1701.