Neurology Publish Ahead of Print
DOI: 10.1212/WNL.0000000000201116

Association of Midlife Inflammatory Markers With Cognitive Performance at 10-Year Follow-up

Author(s):
Teemu Kipinoinen, MD; Sini Toppala, MD; Juha O. Rinne, MD, PhD; Matti H. Viitanen, MD, PhD; Antti M Jula, MD, PhD; Laura L Ekblad, MD, PhD

Corresponding Author:
Teemu Kipinoinen, tpkipi@utu.fi

This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Neurology® Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.

Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.
Affiliation Information for All Authors: 1. Turku PET Centre, University of Turku and Turku University hospital, Turku, Finland; 2. Kuopio City Home Care, Rehabilitation and Medical Services for Elderly, Kuopio, Finland; 3. City of Turku, Welfare Division, Department of Geriatrics, Turku City Hospital and University of Turku, Turku, Finland; 4. Division of Clinical Geriatrics, NVS, Karolinska Institutet, Stockholm, Sweden; 5. National Institute for Health and Welfare, Turku, Finland

Equal Author Contribution:
Teemu Kipinoinen, MD and Sini Toppala, MD contributed equally to this work; co-first authors

Contributions:
Teemu Kipinoinen: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data; Additional contributions: Writing the manuscript - Writing the manuscript - Sini Toppala
Sini Toppala: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data; Additional contributions: Writing the manuscript - Teemu Kipinoinen Writing the manuscript
Juha O. Rinne: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data
Matti H. Viitanen: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data
Antti M Jula: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Laura L Ekblad: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data

Figure Count:
1

Table Count:
4
Search Terms:

Acknowledgment:

Study Funding:
T. Kipinoinen received funding from Uulo Arhio foundation and University of Turku post graduate education (PGE) scholarship. S. Toppala was supported by personal grants from the Finnish Medical Foundation, the Juho Vainio Foundation the Finnish Brain Foundation (Suomen Aivosäätiö sr), the Maud Kuistila Foundation, and personal fees from Finnish State Research Funding (ERVA). J.O. Rinne was supported by grants from the Academy of Finland research grants (#310962 to J.O. Rinne), the Sigrid Juselius Foundation and Finnish State Research Funding (ERVA). M.H. Viitanen was supported by King Gustaf V’s and Queen Victoria’s Freemasons’ Foundation. L.L. Ekblad was supported by the Emil Aaltonen Foundation and Juho Vainio Foundation.

Disclosures:
J.O. Rinne serves as a neurology consultant for Clinical Research Services Turku (CSRT Oy). Other authors report no relevant disclosures.

Preprint DOI:

Received Date:
2022-03-07

Accepted Date:
2022-07-01

Handling Editor Statement:
Submitted and externally peer reviewed. The handling editor was Linda Hershey, MD, PhD, FAAN.
Abstract

Background and objectives

Chronic low-grade inflammation, commonly associated with cardiovascular diseases and risk factors, has been associated inconclusively with cognitive decline and dementia. The aim of our study was to evaluate whether low-grade inflammation, measured in midlife, is associated with a decline in cognitive performance after a 10-year follow-up. We hypothesized that low-grade inflammation, estimated by interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and high-sensitivity CRP (hs-CRP), is a predictor of cognitive decline in the general population.

Methods

This prospective cohort study is based on a Finnish nationwide, population-based Health 2000 Examination Survey, its supplemental examinations in 2000-2001 and the follow-up Health 2011 Survey. Cognitive performance at baseline and at follow-up was assessed with categorical verbal fluency (VF), Word-list learning (WLL) and word-list delayed recall (WLDR). Baseline low-grade inflammation was measured with IL-6, TNF-α and hs-CRP in 2001. Associations between low-grade inflammation and cognitive performance were analyzed with multivariable linear models adjusted for age, sex, education, APOEε4 genotype, type 2 diabetes, hypertension, hypercholesterolemia, body mass index, depressive symptoms, smoking, and baseline cognition.

Results

915 participants aged 45 to 74 years (median age 54, 55% women) were included in the analysis. Both higher IL-6 and TNF-α at baseline predicted poorer performance in VF and WLL at 10-year follow-up (VF: IL-6 β: -1.14, p=0.003, TNF-α β: -1.78, p=0.008; WLL: IL-6 β: -0.61, p=0.007, TNF-α β: -0.86, p=0.03). Elevated IL-6 also predicted a greater decline in VF and WLL after a 10-year follow-up (VF: β: -0.81, p=0.01; WLL: β: -0.53, p=0.008). Baseline TNF-α did not predict cognitive decline and hs-CRP did not predict cognitive performance or decline after 10-years.

Discussion

Our results suggest that low-grade inflammation in midlife is an independent risk factor of poorer cognitive performance later in life. Of the studied markers IL-6 and TNF-α seem to be stronger predictors for cognitive performance and decline than hs-CRP.

Introduction

Low-grade inflammation is associated with a number of disorders including cardiovascular disease, obesity, metabolic syndrome, depression and dementia. However, it is unclear whether low-grade inflammation is the cause or consequence of these conditions. There is also evidence that levels of circulating inflammatory markers are elevated before the onset of cognitive impairment or Alzheimer’s Disease (AD), although some studies have not been able to find association between inflammatory markers and cognitive decline. However, few prospective studies have assessed the
roles of different inflammatory markers during midlife as risk factors for cognitive impairment later in life. Further, many of the previous studies have been cross-sectional or have investigated older populations instead of middle-aged populations resulting in heterogeneity in outcomes. To date, the importance of low-grade inflammation in cognitive decline and dementia remains inconclusive.

It has been suggested that chronic inflammation is promoted by cytokines such as TNF-α and IL-6 excreted from adipose tissue. The presence of these circulating cytokines is hypothesized to activate microglia-cell-mediated neuroinflammation in the brain that is believed to be part of the pathophysiology of AD. We have previously shown that higher levels of metabolic risk factors and high sensitivity C-reactive protein (hs-CRP) are associated with microglial activation, measured with [{\textsuperscript{11}}C]PBR28 -PET, in brain regions typical for early beta-amyloid accumulation in AD. The hypothesis of the present study was that Low-grade inflammation in midlife would be associated with cognitive performance and decline later in life. To test this hypothesis, we examined 915 individuals aged 45 to 74 years at baseline and evaluated whether higher levels of three circulating proinflammatory mediators, TNF-α, IL-6 and hs-CRP, could be associated with cognitive decline after 10 years. The study was conducted on a prospective cohort design in a sample of the Finnish adult population.

Methods

Study population

This study is based on the Finnish Health 2000 examination survey, its supplemental examinations on a subpopulation in 2001-2002, and its follow-up survey, Health 2011 study, all of which were conducted by the Finnish Institute for Health and Welfare. A multidisciplinary epidemiologic Health 2000 survey was carried out in Finland in the years 2000 and 2001. It was a nationwide population-based survey that was representative of the Finnish adult population living in mainland Finland. 8028 adults, aged 30 and over, were randomly selected from the Finnish population registry using a two-staged stratified cluster sampling procedure. 84% (n=6770) attended a health examination proper or had a health examination at home. After the Health 2000 examinations, a subsample of the study population underwent further examinations between 2001-2002 for a more thorough study of cardiovascular diseases and diabetes. Of the participants aged 45 to 74 years who had participated in the Health 2000 examination and lived near one of the five central university hospital cities in Finland, 1864 were invited to a supplemental investigation of the cardiovascular system. 82% (n=1526) attended the supplemental survey. The participants in the original Health 2000 Survey, who were still living in Finland, and who had not declined to participate (n=1396), were invited to attend the Health 2011 follow-up survey.
Individuals who had attended all three investigations (the Health 2000 examination proper in 2000-2001, the supplemental investigation in 2001–2002, and the Health 2011 follow-up health examination or the home examination in 2011) were included in the present study. Participants with missing cognitive test results at baseline (n=15) or at follow-up (n=24) and those whose IL-6 and TNF-α -levels were not measured (n=25) at baseline were excluded from this study. Additionally, individuals with hs-CRP values >10 mg/L (in the supplemental investigation) (n = 66) were excluded in order to eliminate possible confounding effects of an infectious disease. Similarly, individuals who were currently using of systemic corticosteroids (N=22) were excluded in order to eliminate the potential distorting effect on inflammation markers. 105 individuals declined to participate in the follow-up examination or had died or were generally lost for the follow-up. In total 915 individuals aged from 45 to 74 were included in the analyses (Figure 1).

**Baseline measurements**

In the Health 2000 study, APOE genotyping was performed for those who gave their written consent for DNA sampling with the MassARRAY system (Sequenom, San Diego, California) with a modified protocol that has previously been described.\(^3\)\(^5\)

Between 2001-2002 participants’ body mass index (BMI) was determined and blood their pressure was measured in a sedentary position from the right upper arm three times with oscillometric OMRON M4 blood pressure monitor (Omron Matsusaka, Japan, OMRON Healthcare Europe B.V., Hoofddorp, The Netherlands).\(^3\)\(^6\) A mean of the three measurements was used in the analyses.

Information about the participants’ medical history, medication, education and smoking was obtained by interviews and questionnaires. Depressive symptoms were evaluated with the Beck depression inventory (BDI).

Fasting blood samples were taken after fasting for 10-12 hours. Serum cholesterol values were analysed as previously reported.\(^3\)\(^3\)\(^4\) Inflammatory markers, hs-CRP, IL-6 and TNF-α concentrations were determined using chemiluminescent immunometric assays (Immulite, Diagnostic Products Corporation, Siemens Healthcare Diagnostics, Deerfield, IL, USA). The detection limits of the assays were 0.20 mg/l for hs-CRP, 1.5 ng/l for TNF-α and 0.5 ng/l for IL-6. Values under the detection limit were given the value detection limit divided by 2, i.e. 0.1mg/l for hs-CRP, 0.75ng/l for TNF-α and 0.25ng/l for IL-6.

**Cognitive tests at baseline and at follow-up**

The methods used for cognitive evaluation were chosen to measure different aspects of cognition. The cognitive test battery consisted of tests for verbal fluency (VF) and learning and retaining verbal material from the Finnish version of the CERAD (Consortium to Establish a Registry for Alzheimer’s Disease) test battery.\(^3\)\(^7\) Cognitive performance was measured at baseline and at follow-up.\(^3\)\(^3\)\(^4\) In the
categorical VF test the participants were asked to produce a list of as many animals as possible within one minute. One animal indicated one point. In the word-list learning (WLL) task the participants were shown 10 words and asked to read them aloud and memorize them. Then within 90 seconds the participants were to recall as many words as possible (immediate recall). At baseline, this procedure was repeated twice if the participant could not recall all the words after the first round. If a participant had memorised all 10 words in the first round, the result was 30 points. Thus, the WLL score was 30 for those who recalled all the 10 words after the first round and the total of the recalled words in the three rounds for other participants. At follow-up, even though all ten words had been remembered after the first round, a total of three rounds were performed. One correct word indicated one point. In the word-list delayed recall (WLDR) test the participants were asked to recall all the 10 words after a five-minute delay.\textsuperscript{33,34} In order to evaluate the change in the cognitive test scores, the cognitive test score in 2000 was subtracted from that of 2011 so that negative change would indicate decline in the cognitive test scores during the follow-up.

\textbf{Covariates}

Previously reported risk factors for cognitive decline: age, education, APOE\textsubscript{4} -genotype, diabetes hypertension, hypercholesterolemia, depressive symptoms, BMI and smoking habits were used as covariates in the analyses. Education was determined as self-reported years of formal education. The APOE\textsubscript{4} genotype was defined as positive if the subject had one or two APOE\textsubscript{4} alleles. Hypertension was defined as systolic blood pressure \(\geq 140\) mmHg, diastolic blood pressure \(\geq 90\) mmHg or current use of antihypertensive medication. Hypercholesterolemia was defined as a serum total cholesterol level \(\geq 6.5\) or current use of antilipidemic medication during the Health 2000 examinations. Diabetes was defined as the current use of insulin or oral diabetes medication, fasting plasma glucose \(\geq 7.0\) mmol/l or 2-hour oral glucose tolerance test value \(\geq 11.1\). APOE\textsubscript{4}, hypertension, hypercholesterolemia, diabetes and smoking were analysed as dichotomic variables. Age, formal education in years, BMI and BDI score were analysed as continuous variables. The total number of participants with data available of each covariate is shown in Table 1. The adjusted models included all individuals with complete data for all covariates.

\textbf{Statistical analyses}

First, the normality of all variables was inspected from the histograms. In order to achieve normal distribution, logarithmic transformation was performed on the non-normally distributed variables (hs-CRP, IL-6, TNF-\(\alpha\) and BDI). Differences between the 45-74-year-old participants of the main Health 2000 study (n=4195) and those included in this study (n=915) as well as the differences between the individuals of the subpopulation who dropped out from the follow-up study (n=353) and those who
completed the follow-up (n=915) were analysed by the Wilcoxon rank sums test (age), Student’s t-test (other continuous variables), or Chi-Square -test (sex and APOE4 genotype).

Since previous studies have identified differences between sexes in the associations between metabolic risk factors and cognition\textsuperscript{14,38}, the study population characteristics are presented for the total population and for men and women separately. Differences between men and women were analysed with Student’s t-test for continuous variables and with the Chi-Square test for categorical variables. Pairwise correlations between inflammatory markers and covariates were calculated using Pearson’s correlation. Multivariable linear models were used to test the independent association between baseline inflammatory parameters and cognitive performance at follow-up. Explanatory variables were adjusted on three different levels. Model 1 was adjusted for age, sex and prior education in years. Model 2 was additionally adjusted for APOE\textsuperscript{ε4} -genotype, diabetes, hypertension, and hypercholesterolemia. The fully adjusted model 3 was adjusted for all the explanatory variables above and BMI, depressive symptoms, and current smoking.

The associations between inflammatory parameters and change in cognitive performance after follow-up were evaluated by adding also baseline cognitive function to the models above. Normality assumption for the analyses were verified from the studentized residuals. Age and sex interactions for each inflammatory marker on the association with cognitive performance at follow-up and with cognitive decline during the follow-up time were tested in Model 1. Age group (45 to 65 years, n=797 and 65 to 75 years, n=118) and sex stratified analyses were performed for the associations with significant interaction.

Statistical significance was set a $p<0.05$ for all analyses. Statistical analyses were performed with SAS JMP pro 14 (SAS Institute, Cary, NC, USA).

**Data availability**

Anonymized data can be requested on reasonable request for a study that has been approved by a local ethics committee and that corresponds with the research areas of the Finnish Institute for Health and Welfare Biobank. The applications are to be directed to the Finnish Institute for Health and Welfare (https://thl.fi/en/web/thl-biobank/for-researchers).

**Standard Protocol Approvals, Registrations, and Patient Consents**

The studies were approved by the Ethics Committee for Epidemiology and Public Health in the hospital district of Helsinki and Uusimaa, Finland. Each participant gave their written informed consent for participating in the studies.

**Results**

**Demographics**
The characteristics of the study population at baseline are presented in Table 1. The mean age of the study participants (n=915), consisting of 413 males and 502 females, was 55.6 years; the mean BMI was 26.9 kg/m² (SD 4.6). The men were more obese than the women 27.3 kg/m² vs 26.5 kg/m² (p=0.005), had hypertension more often 61.9% vs 47.8% (p<0.0001) and diabetes 51% vs 39% (p=0.02). 296 (33.4%) had one or two APOEε4 alleles. There was no difference in the prevalence of APOEε4 genotype between the men and women.

Compared to the 45-74-year-old participants of the main Health 2000 study, the participants of the present study were younger (mean age at baseline 55.6 years vs. 57.3 years, p<0.0001), more educated (education in years, mean 11.5 years vs. 10.4 years, p<0.0001), and performed better in the cognitive tests at baseline (mean VF score 25.3 vs. 23.4, p<0.0001; mean WLL score 21.3 vs. 20.1, p<0.0001; mean WLDR score 7.2 vs. 6.8, p<0.0001). There were no differences in sex (p=0.18) or carrying APOEε4 genotype (p=0.39). The individuals who did not attend the follow-up examinations in 2011 were older (mean age at baseline 58.9 years vs. 55.6 years, p<0.0001), less educated (education in years, mean 10.4 years vs. 11.5 years, <0.0001), had higher levels of inflammation (median TNF-α 6.0 ng/l vs. 5.5 ng/l, p=0.0002; median IL-6 1.7 ng/l vs. 1.3 ng/l, p=0.0001; median hs-CRP 1.6 mg/L vs. 1.4 mg/L, p<0.0001), and performed worse in the cognitive tests at baseline (mean VF score 22.9 vs. 25.3, p<0.0001; mean WLL score 19.7 vs. 21.3; p<0.0001; mean WLDR score 6.3 vs. 7.2, p<0.0001) than those who finished also the follow-up examination but there was no difference between the groups in sex (p=0.43) or APOE4 genotype (p=0.99).

**Correlations between inflammatory markers and covariates**

A moderate positive correlation was found between each inflammatory variable (for IL-6 and TNF-α: r=0.31, p<0.0001, for hs-CRP and TNF-α: r=0.25, p<0.0001, and for hs-CRP and IL-6: r=0.35, p<0.0001). Among the established risk factors for cognitive decline higher BMI, age and fewer years of education, showed a positive correlation with all the inflammatory markers. Hs-CRP seemed to have a stronger positive correlation with BMI than TNF-α and IL-6. There was also a positive correlation between BDI -score and IL-6 and TNF-α. (Table 2.)

**Baseline inflammatory markers as predictors of follow-up cognition**

All the studied inflammatory markers predicted poorer cognitive performance at follow-up on VF, WLL, but not on WLDR, in the model adjusted only for age, sex and education. (Table 3.) After further adjustments for APOEε4 genotype, diabetes, hypertension, and hypercholesterolemia (model 2), these associations remained significant between TNF-α and VF (β=-2.41, p=0.0007) and WLL (β=-1.03, p=0.005), and between IL-6 and VF (β=-1.03, p=0.004) and WLL (β=-0.60, p=0.0024. In the fully adjusted model 3, a higher baseline TNF-α predicted lower VF (β=-1.78, p=0.08) and WLL
(β=-0.86, p=0.03), and a higher baseline IL-6 predicted lower VF (β=-1.14, p=0.003) and WLL (β=-0.61, p=0.007). The standardized estimates for each inflammatory marker in each model are also provided in Table 3.

**Inflammatory markers as predictors of cognitive decline from baseline to follow-up**

In the model further adjusted for age, sex, education, and baseline cognition TNF-α predicted a greater decline in VF (β=-1.41, p=0.005) and WLL (β=-0.84, p=0.007). Similarly, IL-6 predicted a decline in VF (β=-0.87, p=0.002) and WLL (β=-0.52, p=0.003). Hs-CRP was associated with a decline in WLL (β=-0.25, p=0.04). After adjustments for the variables in model 2, higher TNF-α predicted poorer VF (β=-1.26, p=0.02) and WLL (β=-0.79, p=0.02). In addition, IL-6 predicted a larger decline in VF (β=-0.75, p=0.01) and WLL (β=-0.52, p=0.005). These associations remained significant in the fully adjusted model 3 with IL-6 and decline in VF (β=-0.81, p=0.01) and WLL (β=-0.53, p=0.008). In model 2 hs-CRP and in model 3 hs-CRP and TNF-α were not associated with a decline in any of the cognitive tests. (Table 4.)

**Explanatory values of the regression models**

Age, sex, and years of education were the driving covariates explaining variance of the cognitive test scores at follow-up (adjusted r^2: 17% for VF, 29% for WLL and 22% for WLDR) in a model combining these three covariates. Adding the inflammatory markers or the other covariates to the model improved the model only slightly (Table 3). Similar phenomena were seen when evaluating explanatory values of the aforementioned covariates and cognitive decline (Table 4). The explanatory value of a model adjusted for age, sex education and baseline cognitive test scores was 24% for VF, 22% for WLL and 18% for WLDR.

**Interactions**

There was an interaction for age and IL-6 on the association with all three cognitive tests at follow-up in Model 1 (VF: p=0.0006; WLL: p=0.003; WLDR: p=0.01), and with the change in cognitive test scores (VF: p=0.001; WLL: p=0.004; WLDR: p=0.02). No interactions were found for age and TNF-α or hs-CRP with the cognitive test scores at follow-up. In the models stratified according to age (45 to 65 years and over 65 years), the associations between IL-6 and VF (45-65 years: β=-1.03, p=0.01; over 65 years: β=-1.26, p=0.31) and IL-6 and WLL (45-65 years: β=-1.47, p=0.046; over 65 years: β=-1.41, p=0.11) were driven by the middle-aged population in the fully adjusted Model 3. The association between IL-6 and change in VF (45-65 years: β=-0.46, p=0.09; over 65 years: β=-2.33, p=0.03) was stronger in the elderly population, whereas the association between IL-6 and WLL (45-65 years: β=-0.45, p=0.04; over 65 years: β=-1.05, p=0.17) reached statistical significance only in the younger age group. There was no association between IL-6 and WLDR in either age group.
The interaction for sex and hs-CRP was significant for WLL at follow-up (VF: p=0.08; WLL: p=0.004; WLDR: p=0.20) and the change in WLL (VF: p=0.17; WLL: p=0.04; WLDR: p=0.25). There were no sex interactions for IL-6 or TNF-α. However, sex stratified analyses showed no associations between hs-CRP and WLL in Model 3.

Discussion

In this prospective cohort study, IL-6 and TNF-α independently predicted poorer performance on VF and WLL after a 10-year follow-up. Higher IL-6 in midlife also predicted a greater decline on VF and WLL over a 10-year follow-up. Hs-CRP did not have any association with the 10-year follow-up performance nor did TNF-α or hs-CRP have any association with a decline on any of the cognitive tests in the fully adjusted model. These results suggest that TNF-α and especially IL-6 are predictors of cognitive decline. Our findings support the hypothesis that low-grade inflammation is associated with poorer cognitive performance later in life. Our findings further expand the results of previous studies indicating low-grade inflammation as a state preceding decline in cognitive functions.

Although many studies have evaluated the predictive value of circulating inflammatory markers and cognitive decline, most of the studies have focused on elderly populations, and none of the previous prospective studies on middle-aged populations have included all three inflammatory markers evaluated in the present study. The present study included mostly middle-aged individuals, and the associations between low-grade inflammation and cognitive performance were somewhat stronger in the middle aged populations in age-stratified analyses.

To date, only few studies have reviewed the relationship between low-grade inflammation and cognition in a longitudinal prospective population based cohort design on middle-aged individuals, and the inflammatory markers measured in these studies have varied. The previous studies have used only CRP, CRP combined with IL-6, elevated erythrocyte sedimentation rate (ESR), or a composite score consisting of different inflammatory markers. Walker et al. followed a total of 12,336 middle-aged individuals for a 20-year period using CRP, Von Willebrand factor, fibrinogen, factor VIII and a white blood cell count as markers for inflammation. In line with our results, they found that a higher midlife inflammation composite score was associated with a decline in the cognitive composite score measured with multiple cognitive tests. Their domain specific analyses revealed that a higher inflammation composite score was associated with a steeper decline in memory, but not executive function or language. Contrary to our findings, Walker et al. found that elevated CRP was associated with a steeper cognitive decline over a 20-year follow-up. In the Whitehall II study (n=5217), Singh-Manoux et al. reported an association between elevated midlife IL-6 levels and cognitive decline over a 10-year follow-up measured with multiple cognitive tests. In line with our study, Singh-Manoux et al. did not find an association between elevated CRP-levels and cognitive decline. Beydoun et al. followed 1719 participants for <1 to 8 years (mean 4.6 years, SD 0.93 years)
and found, that ESR predicted faster decline in verbal memory among older men. ESR was also associated with poorer performance on attention tests overall and verbal fluency among older women.\textsuperscript{14} The Honolulu-Asia Ageing Study (HAAS) reported a decline in cognition associated with inflammation measured with hs-CRP over 25 years of follow-up (n=691). Cognitive performance was evaluated with the Cognitive Abilities Screening Instrument (CASI). The results remained significant only when incident dementia cases were included.\textsuperscript{13}

One of the first studies indicating an association between low-grade inflammation and cognitive decline was the Health, Aging and Body Composition (Health ABC) study (n=2632) where elderly individuals with high level inflammation and metabolic syndrome were more likely to develop cognitive impairment than those with high level of inflammation but no metabolic syndrome.\textsuperscript{2} In contrast Metti et al. did not find any significant association between the slope or baseline level of CRP and cognitive decline; moreover Lima et al. even found that increased levels of CRP were associated with a decreased risk of a drop in cognitive performance.\textsuperscript{11,20} One reason for inconclusive results on the relationship between elevated levels of inflammatory markers and impaired cognition in the previous studies may be that the inflammation markers and cognitive tests were measured at an older age.\textsuperscript{2,19,20} Given the fact that the accumulation of beta-amyloid, a neuropathological hallmark of AD, is believed to begin during midlife, and that low-grade inflammation has been proposed to affect amyloid accumulation,\textsuperscript{39} it might be important that studies on the relationship between inflammation and cognitive impairment would include middle-aged individuals and a long follow-up period.

In the present study higher TNF-α and IL-6 levels predicted poorer cognitive performance whereas hs-CRP did not show a significant correlation with cognitive function. This may be due to the mechanism of synthetization and secretion of each mediator. TNF-α and IL-6 are both secreted by the adipose tissue whereas CRP is of hepatic origin. CRP concentration increases following IL-6 secretion, and it is argued that the production of CRP is stimulated by increased IL-6 secreted from the adipose tissue of the obese.\textsuperscript{3,40} Therefore, the elevation of CRP levels might occur later at the inflammation cascade, possibly indicating that TNF-α and IL-6 could be more sensitive mediators of inflammation associated with the adipose tissue.

Our study had several strengths. First, we were able to evaluate the prospective associations between all three inflammatory markers at baseline and cognition and its decline at the follow-up. Second, the inclusion of middle-aged individuals enabled the evaluation of midlife inflammation as a predictor for cognitive decline. Third, the Health 2000 examination and its supplementary examinations included detailed information on the study participants, which made it possible to adjust the analyses for previously reported risk factors of cognitive decline. Fourth, the long follow-up time with cognitive tests both at the baseline and the follow-up allowed us to evaluate the change in cognition from midlife to older age. There are also limitations that should be taken into consideration. Inflammatory mediators were measured only once at the supplemental examinations after the original Health 2000
survey. Because the blood samples were drawn at a different timepoint, it is possible that there could have been fluctuation in the levels of inflammatory markers due to the effects of comorbidities or acute infections at the time of cognitive tests. However, we assume that comorbidities would not have changed notably over a one-year time-period between the cognitive tests and the measurement of inflammatory markers. Additionally, the effort to exclude values that reflect acute inflammation may have led to the exclusion of hs-CRP levels reflective of high chronic inflammation. The subpopulation included in the present study was more educated, younger, and performed slightly better on the cognitive tests when compared to the original Health 2000 study population of the same age group. Similarly, those who had dropped out from the follow-up examinations had lower cognitive test scores, were less educated and older than those included in the present study. Considering these differences however, it is likely that our results would be diluted than that we would have found false-positive associations. Another limitation to this study is that in the original Health 2000 study, no data of biomarkers or other measures of AD pathology was collected, so the relationship between AD pathology in preclinical phase and low-grade inflammation remains unknown. The final aspect that should be considered is that more sensitive measurements of cognitive performance than CERAD might have been of use in this relatively young study sample. The CERAD WLL was performed slightly differently at baseline and at follow-up. However, none of the study participants had a score of total 10 on the first round of memorizing the word-list and thus, these differences did not affect our results.

In conclusion, our findings provide further evidence that a low-grade inflammation precedes and predicts the development of cognitive decline. Considering that the mean age of the study population was 55 years, these results further emphasize the role of midlife as a crucial period for predicting future cognitive decline and suggest that preventive efforts to reduce cognitive decline should be targeted at middle-aged populations.
References


41. mac Giollabhui N, Ellman LM, Coe CL, Byrne ML, Abramson LY, Alloy LB. To exclude or not to exclude: Considerations and recommendations for C-reactive protein values higher than 10 mg/L. *Brain, Behavior, and Immunity*. 2020;87:898-900. doi:10.1016/j.bbi.2020.01.023
Table 1.

The study population characteristics at baseline

<table>
<thead>
<tr>
<th></th>
<th>total</th>
<th>men</th>
<th>women</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n(%)</td>
<td>915 (100)</td>
<td>413 (45.1)</td>
<td>502 (54.9)</td>
<td></td>
</tr>
<tr>
<td>age in years, mean, n=915</td>
<td>55.6 (7.5, 45-74)</td>
<td>55.7 (7.4, 45-74)</td>
<td>55.5 (7.6, 45-74)</td>
<td>0.7</td>
</tr>
<tr>
<td>years of education, mean, n=910</td>
<td>11.5 (3.8, 2-26)</td>
<td>11.4 (3.8, 4-24)</td>
<td>11.6 (3.8, 2-26)</td>
<td>0.4</td>
</tr>
<tr>
<td>APOE ε4 genotype n(%), n=885</td>
<td>296 (33.4)</td>
<td>133 (32.8)</td>
<td>163 (34.0)</td>
<td>0.7</td>
</tr>
<tr>
<td>diabetes n (%), n=910</td>
<td>90 (9.9)</td>
<td>51 (12.4)</td>
<td>39 (7.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>hypertension n (%), n=914</td>
<td>495 (54.2)</td>
<td>255 (61.9)</td>
<td>240 (47.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>hypercholesterolemia n(%), n=872</td>
<td>225 (25.8)</td>
<td>109 (28.3)</td>
<td>116 (23.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>BMI, mean, kg/m², n=909</td>
<td>26.9 (4.3, 17.6-47.7)</td>
<td>27.3 (3.9, 19.1-41.1)</td>
<td>26.5 (4.6, 17.7-47.7)</td>
<td>0.005</td>
</tr>
<tr>
<td>BDI score, n=911</td>
<td>5 (2,10, 0-41)</td>
<td>5 (2, 9, 0-39)</td>
<td>6 (3, 11, 0-41)</td>
<td>0.07</td>
</tr>
<tr>
<td>current smoking, n (%), n=910</td>
<td>82 (9.0)</td>
<td>35 (8.5)</td>
<td>47 (9.4)</td>
<td>0.65</td>
</tr>
<tr>
<td>TNF-α, median, ng/l, n=915</td>
<td>5.5 (4.4, 6.6, 0.75-43.5)</td>
<td>5.6 (4.6, 6.6, 0.75-43.5)</td>
<td>5.3 (4.2, 6.5, 0.75-17)</td>
<td>0.01</td>
</tr>
<tr>
<td>IL-6, median, ng/l, n=915</td>
<td>1.3 (0.92, 1.9, 0.25-53.3)</td>
<td>1.4 (1, 2, 0.25-53.3)</td>
<td>1.25 (0.9, 1.9, 0.25-14.9)</td>
<td>0.008</td>
</tr>
<tr>
<td>hs-CRP, median, mg/l, n=912</td>
<td>1.37 (0.77, 2.66, 0.1-10)</td>
<td>1.38 (0.77, 2.48, 0.1-10)</td>
<td>1.37 (0.77, 2.71, 0.1-9.7)</td>
<td>0.50</td>
</tr>
<tr>
<td>At baseline 2000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>verbal fluency, mean, n=915</td>
<td>25.3 (7.0, 6-46)</td>
<td>24.7 (7.3, 6-45)</td>
<td>25.8 (6.6, 7-46)</td>
<td>0.02</td>
</tr>
<tr>
<td>Test</td>
<td>baseline</td>
<td>at follow-up 2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------</td>
<td>--------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>word-list learning sum, mean, n=915</td>
<td>21.3 (3.7, 11-29)</td>
<td>22.0 (3.5, 11-29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>delayed recall, mean, n=915</td>
<td>7.2 (1.7, 0-10)</td>
<td>7.5 (1.7, 2-10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>verbal fluency mean, n=915</td>
<td>23.8 (7.1, 0-51)</td>
<td>24.1 (6.8, 6-51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>word-list learning sum, n=914</td>
<td>20.4 (4.4, 0-30)</td>
<td>21.5 (4.0, 9-30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>delayed recall mean, n=915</td>
<td>6.9 (2.1, 0-10)</td>
<td>7.4 (2.0, 0-10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data are presented as mean (standard deviation and range) for variables with a normal distribution; as median (1st quartile, 3rd quartile, range) for variables with a skewed distribution; and as n (percentage) for categorical variables. The number of participants with data available for each variable are shown in the first column.

Abbreviations: BMI = Body Mass Index kg/m²; BDI = Beck Depression Inventory; TNF-α = Tumor necrosis factor alpha; IL-6 = Interleukin 6; hsCRP = High sensitivity C-reactive protein

Logarithmic transformation (log10) was performed on the non-normally distributed variables (hs-CRP, IL-6, TNF-α and BDI) before the analyses to achieve a normal distribution.

P-values indicate differences between men and women assessed with Student’s-test for continuous variables and the Chi-Square test for categorical variables.
Table 2.

Pearson correlation coefficients between inflammatory markers and risk factors for cognitive decline at baseline

<table>
<thead>
<tr>
<th></th>
<th>TNF-α</th>
<th>IL-6</th>
<th>hs-CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.20***</td>
<td>0.24***</td>
<td>0.20***</td>
</tr>
<tr>
<td>BMI</td>
<td>0.28***</td>
<td>0.25***</td>
<td>0.40***</td>
</tr>
<tr>
<td>BDI</td>
<td>0.08*</td>
<td>0.08*</td>
<td>0.07</td>
</tr>
<tr>
<td>Education in years</td>
<td>-0.19***</td>
<td>-0.11**</td>
<td>-0.13***</td>
</tr>
<tr>
<td>TNF-α</td>
<td>NA</td>
<td>0.31***</td>
<td>0.25***</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.31***</td>
<td>NA</td>
<td>0.35***</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>0.25***</td>
<td>0.35***</td>
<td>NA</td>
</tr>
</tbody>
</table>

The data are presented as Pearson’s correlation coefficients. Logarithmic transformation (logₑ) was performed on variables with a skewed distribution (hs-CRP, IL-6, TNF-α and BDI) to achieve a normal distribution before the analyses.

* = p<0.05, ** = p<0.01, *** = p<0.0001

Abbreviations: BMI = Body Mass Index kg/m²; BDI = Beck Depression Inventory; TNF-α = Tumor Necrosis Factor alpha; IL-6 = Interleukin 6; hs-CRP = High sensitivity C-reactive protein
Table 3. - Multivariate associations of inflammatory markers TNF-\(\alpha\), IL-6 and hs-CRP values at baseline with cognitive test scores at 10-year follow-up.

<table>
<thead>
<tr>
<th></th>
<th>verbal fluency 2011</th>
<th>word-list learning 2011</th>
<th>word-list delayed recall 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\beta) (95% CI)</td>
<td>Std (\beta)</td>
<td>(r^2_{adj})</td>
</tr>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>-1.19 (-1.86 to -0.52)**</td>
<td>-0.11</td>
<td>0.18</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>-2.29 (-3.48 to -1.11)**</td>
<td>-0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>-0.48 (-0.94 to -0.03)*</td>
<td>-0.06</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>-1.0 (-1.73 to -0.34)**</td>
<td>-0.10</td>
<td>0.20</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>-2.41 (-3.34 to -0.90)**</td>
<td>-0.11</td>
<td>0.20</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>-0.30 (-0.78 to 0.19)</td>
<td>-0.04</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>-1.14 (-1.90 to -0.37)**</td>
<td>-0.11</td>
<td>0.20</td>
</tr>
</tbody>
</table>
**Table 4.** – Multivariate associations of baseline inflammatory markers TNF-α, IL-6 and hs-CRP values with change in cognitive test scores from 2000 to 2011.

<table>
<thead>
<tr>
<th></th>
<th>change in verbal fluency</th>
<th>change in word-list learning 2011–2000</th>
<th>change in word-list delayed recall 2011–2000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>Std β</td>
<td>r² adj</td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.87 (-1.43 to -0.31)**</td>
<td>-0.09</td>
<td>0.25</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-1.41 (-2.40 to -0.42)**</td>
<td>-0.08</td>
<td>0.25</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>-0.21 (-0.59 to 0.17)</td>
<td>-0.03</td>
<td>0.24</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>TNF-α</td>
<td>hs-CRP</td>
</tr>
<tr>
<td>-----</td>
<td>--------------</td>
<td>--------------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>β (95% CI)</td>
<td>β (95% CI)</td>
</tr>
<tr>
<td></td>
<td>-0.75 (-1.33 to -0.18)*</td>
<td>-1.26 (-2.28 to -0.24)*</td>
<td>-0.07 (-0.47 to 0.34)</td>
</tr>
<tr>
<td></td>
<td>-0.08</td>
<td>-0.08</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>-0.52 (-0.89 to -0.15)**</td>
<td>-0.79 (-1.44 to -0.15)*</td>
<td>-0.17 (-0.43 to 0.08)</td>
</tr>
<tr>
<td></td>
<td>-0.09</td>
<td>-0.08</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>0.22</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>-0.12 (-0.30 to 0.06)</td>
<td>-0.20 (-0.52 to 0.11)</td>
<td>-0.06 (-0.19 to 0.06)</td>
</tr>
<tr>
<td></td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
</tr>
</tbody>
</table>

* = p<0.05, ** = p<0.01, *** = p<0.0001

N = 915. Estimates (β), confidence intervals (CI), standardized estimates (std β) and the adjusted explanatory value of each model (r² adj) are derived from linear regression analysis and are adjusted for multivariate linear models. Model 1 adjusted for age, sex, years of education, and cognitive test results at baseline. Model 2 adjusted as for Model 1 + APOEε4 genotype, diabetes, hypertension, and hypercholesterolemia. Model 3 adjusted as for Model 2 + body mass index, depressive symptoms, and current smoking. Logarithmic transformation (log e) is used for TNF-α, IL-6, hs-CRP and BDI-score to achieve a normal distribution.
Figure 1. – Study selection process and reasons for exclusion from the study.

Participants aged 45–74 who attended the health examination proper in Health 2000 study (N = 4,195)

Invited to the additional examination (n = 1,867)

Attended the additional examination (n = 1,526)

Attended the laboratory sampling (n = 1,396)

Excluded (n = 481):
- Cognitive tests not performed at baseline (15)
- Use of systemic corticosteroids (22)
- IL-6 and TNF-α not analyzed (25)
- CRP > 10 (66)
- Deceased or moved abroad (105)
- Did not attend the health examination proper or the home examination in 2011 (224)
- Cognitive tests interrupted or not performed at follow-up (24)

n = 915
Association of Midlife Inflammatory Markers With Cognitive Performance at 10-Year Follow-up
Teemu Kipinoenen, Sini Toppala, Juha O. Rinne, et al.
Neurology published online October 4, 2022
DOI 10.1212/WNL.0000000000201116

This information is current as of October 4, 2022

Updated Information & Services
including high resolution figures, can be found at:
http://n.neurology.org/content/early/2022/10/04/WNL.0000000000201116.full

Subspecialty Collections
This article, along with others on similar topics, appears in the following collection(s):
All Cognitive Disorders/Dementia
http://n.neurology.org/cgi/collection/all_cognitive_disorders_dementia
Cognitive aging
http://n.neurology.org/cgi/collection/cognitive_aging
Cohort studies
http://n.neurology.org/cgi/collection/cohort_studies
Memory
http://n.neurology.org/cgi/collection/memory
Risk factors in epidemiology
http://n.neurology.org/cgi/collection/risk_factors_in_epidemiology

Permissions & Licensing
Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
http://www.neurology.org/about/about_the_journal#permissions

Reprints
Information about ordering reprints can be found online:
http://n.neurology.org/subscribers/advertise