

Pro-Inflammatory Substances and Cognition in the Dallas Heart Study

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Abstract

Cognitive decline in late adulthood might be partially mediated by subclinical generalized vascular disease. If so, atherogenic factors such as pro-inflammatory cytokines might be mid-life targets for prevention or treatment. Dallas Heart Study subjects (n = 997; mean age = 42.94 ± 10.2 yrs) underwent blood assays of pro-inflammatory biomarkers associated with atherosclerosis and 8 years later completed a cognitive outcome measure, the Montreal Cognitive Assessment (MoCA). Markers included C-reactive protein (CRP), Interleukin-18 (IL-18), Lipoprotein-associated phospholipase (LP-PLA₂), and Monocyte Chemoattractant Protein (MCP-1), with Apolipoprotein E4 (ApoE4) as a potential modifier. We found weak evidence for LP-PLA₂ and CRP as predictors of cognitive scores. No relationship was found between elevated MCP-1, IL-18 and cognition. Presence of the ApoE4 allele did not impact the relationship between biomarkers and cognitive function. Levels of atherogenesis-related pro-inflammatory blood biomarkers did not predict cognitive function in middle-aged adults after an interval of 8 years.

Keywords

Montreal Cognitive Assessment, Cognition, Inflammation, Dallas Heart Study

1. Introduction

The present study examined the relationship of four pro-inflammatory factors, C-reactive protein (CRP), Interleukin-18 (IL-18), Lipoprotein-associated phospholipase (LP-PLA₂), and Monocyte chemoattractant protein

(MCP-1), with ApoE4 as a potential modifier, to cognitive function later in life.

The relationship between CRP and future cardiovascular events has been consistent throughout the literature, and appears to be independent of age, smoking, blood pressure, and diabetes [1]. Elevations in plasma CRP have also been associated with higher risk of Alzheimer disease (AD) and vascular dementia (VaD) [2]-[4] though the relationship between CRP and cognitive function remains unclear. For example, in a one-year study of 78 subjects with cardiovascular disease, high levels of CRP are associated with subtle declines in attention-executive-psychomotor performance [5]. Others, however, find that cognitive function scores do not vary in relation to CRP concentration measured 4 - 7 years earlier [6]. Elevated levels of IL-18 have been linked to risk for coronary heart disease [7] [8]. Researchers have also examined the relationship between IL-18, AD and VaD, with higher levels of IL-18 found in VaD patients compared with healthy controls [9]. IL-18 production by peripheral blood cells is found increased in AD patients and correlated with severity of cognitive impairment [10]. There has been a consistent linking of Lp-PLA₂ levels to risk of atherosclerotic disease [11]. The relationship between Lp-PLA₂ and cognitive function has been only recently examined; elevated levels of Lp-PLA₂ are associated with an increased risk of dementia [12] and increased risk of both Alzheimer's dementia and mixed dementia [13]. Plasma MCP-1 levels have been linked to acute coronary syndromes [14]. The relationship between MCP-1 and cognitive function has also not been well investigated. One study finds significantly increased MCP-1 levels in MCI and mild AD, but not in severe AD as compared with controls, suggesting that upregulation of this chemokine may be an early event in the pathogenesis of MCI and dementia [15].

Carriage of the apolipoprotein E 4 allele (ApoE4) is a risk factor for atherosclerosis [16]-[18]. The relationship between ApoE4 and risk of AD is also well-established [19], and there is evidence of a relationship between ApoE4 genotype and cognitive decline across the lifespan independent of AD [20].

2. Material and Methods

Participants in this study were enrollees in the Dallas Heart study; an investigation of the factors contributing to development of atherosclerotic cardiovascular disease [21]. The first wave of the Dallas Heart Study (DHS 1), initiated in 1999, recruited participants based on a stratified random sampling from Dallas County. DHS 1 participants were subsequently recruited to participate in DHS 2, which enrolled subjects between 2007 and 2010. The present investigation examined data from participants tested for the blood markers of interest obtained in DHS 1 who also had cognitive testing with the MoCA, which was first introduced as part of DHS 2. There was a mean time of approximately 8 years between blood marker collection and cognitive testing. Participants were fluent in English and signed informed consent as approved by the Institutional Review Board at the University of Texas Southwestern Medical Center. Persons with a history of stroke were excluded ($n = 38$). Complete data on all four pro-inflammatory markers drawn as part of DHS 1 and DHS 2 MoCA scores were available for 997 of these participants. Sample size varied for individual analyses depending on missing data.

Assays for selected substances included a high-sensitivity CRP assay [22], MCP-1 assay [23], Lp-PLA₂ assay [24], and IL-18 assay [25] for correlational analyses, biomarker concentration data were used as continuous variables. Following the DHS protocol, participants with CRP values >3 mg/L were considered to have CRP elevations. Significant elevations for MCP-1, Lp-PLA₂, and IL-18 were based on scores greater than 1 standard deviation above the mean for the entire sample, again following the standard DHS protocol.

The Montreal Cognitive Assessment (MoCA) was utilized to assess cognitive functioning. Developed as a screening tool for detection of Mild Cognitive Impairment (MCI) in individuals with cognitive complaints, the MoCA is a 30-point test [26]. It was selected for its brevity and ease of administration, requiring approximately 10 minutes to administer. It is comprised of items from the following cognitive domains: visuospatial ability, naming, memory, attention, language, abstraction, and orientation. MoCA scores were analyzed as a continuous variable, and were further divided into tertiles for comparison of group differences in demographic and biomarker data.

Hypertension was defined as average systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg or use of antihypertensive medication. Diabetes was defined as a fasting glucose level ≥ 126 mg/dl or use of any hypoglycemic medication. Hypercholesterolemia was defined as low-density lipoprotein cholesterol ≥ 160 mg/dl, total cholesterol ≥ 240 mg/dl, or the use of statin medication. Waist circumference was measured in centimeters on a horizontal plane 1 cm above the iliac crest. The National Cholesterol Education Program obesity threshold was used to determine abdominal obesity; 88 cm and 102 cm for women and men, respectively [27].

Apolipoprotein E genotyping was conducted by a method described elsewhere [28].

All data were analyzed using SPSS version 18 (SPSS, Inc., Chicago IL). Missing data were filtered out via list-wise deletion. Participants with missing data were compared to those with complete data using chi-square tests for categorical variables, and t-tests for continuous variables, with no significant differences noted. In order to account for potentially spurious findings in the large sample being investigated, a significance level of 0.01 was used in all analyses. CRP demonstrated a skewed distribution, and these data were logarithmically transformed (log10 transformation) for the use in parametric testing, though for clarity these results are reported without this transformation. Gender differences were examined using both chi square analysis and independent samples t-tests.

To investigate the relationship between CRP, IL-18, Lp-PLA₂, and MCP-1 and MoCA scores, and to account for the non-normality of data distribution, partial Spearman correlation analysis that controlled for education and age was utilized. The strength of significant correlation coefficients was designated as small (0.10 - 0.29), medium (0.30 - 0.49), and large (>0.50).

A new variable was created that identified participants with or without an elevation on each biomarker (as described above), and was used in ANCOVAs to determine if there were mean differences in MoCA scores between participants with and without elevated values. Logistic regressions were then conducted to identify which inflammatory markers (CRP, MCP-1, IL-18, and LpPLA₂), vascular risk factors (e.g., hypertension, hyperlipidemia, diabetes, waist circumference) and demographic variables best predicted MoCA scores. For these analyses, the lowest MoCA tertile group was used as the dependent variable. The above vascular risk factors and pro-inflammatory markers were entered in Step 1 of the analyses via entry method in order to obtain an unadjusted full model, followed by backward stepwise method in order to obtain a reduced model. In Step 2, demographic variables were added to obtain an adjusted model, and this final model was entered via entry method in order to maximize the sample size. The Hosmer-Lemeshow Goodness-of-Fit Test was used to identify the model with best overall fit.

ANCOVAs were used to compare mean MoCA scores between groups with or without an ApoE4 allele while controlling for age and education. To determine if the presence of the ApoE4 allele affected the relationship between pro-inflammatory blood markers and cognitive function, ApoE4 status was added to the regression models used in previous analyses.

3. Results

The majority of participants were African American (54%) and female (58%). Mean age of subjects was 42.94 years (SD = 10.52; range 18 - 65). Subjects had a mean education of 13.6 years (SD = 2.7; range = 1 to 20 years). Mean MoCA total score was 23.4 points (SD = 4.0; range 7 to 30). The most frequently occurring ApoE allele pattern in this population was E3/E3 (48%); 28% of the group had at least one E4 allele (see also [Table 1](#)).

There were significant differences between males and females for CRP, IL-18, and Lp-PLA₂. Females had significantly greater CRP concentrations [$F(1, 1654) = 35.92, p < 0.001$] and significantly lower IL-18 [$F(1, 1046) = 6.33, p = 0.012$] and LpPLA₂ [$F(1, 1608), p < 0.001$] concentrations than men. African-American subjects had significantly greater concentrations of CRP than Caucasians [$F(3, 1649) = 10.64, p < 0.001$]. Caucasians had significantly greater concentrations of Lp-PLA₂ than African Americans and Hispanics [$F(3, 1603) = 31.26, p < 0.001$]. There were no significant differences for gender in MCP-1. There were no ethnic differences in MCP-1 or IL-18 concentrations.

Education was significantly correlated with MoCA total score [$r = 0.43, p < 0.001$], as was age [$r = -0.199, p = 0.000$]. A partial Spearman's correlation controlling for age and education showed a statistically significant but weak correlation between MoCA total scores and levels of Lp-PLA₂ [$(n = 994; r = 0.09, p = 0.003)$] ([Table 2](#)). No significant correlations between concentration of CRP, MCP-1, IL-18 and MoCA total scores were found. Individuals with elevated CRP (>3 mg/L, $n = 765$) had significantly lower MoCA total scores than those without elevated CRP ($n = 889$), though the difference was small (about 1 point). MoCA scores otherwise did not differ based on the presence or absence of other elevated markers (see [Figure 1](#)).

Using ANCOVA to control for education and age. There was no significant difference between participants with or without an ApoE4 allele on MoCA total score [$F(2, 1643) = 0.244, p = 0.780$]. Logistic regression revealed no significant impact of the presence of the ApoE4 allele on cognitive function, and the presence of this allele did not strengthen the relationship between blood marker elevations and cognitive function.

Table 1. Descriptive characteristics of participants.

	N (%)	Minimum	Maximum	Mean (SD)
DHS 1 Age	1904	18	65	42.80 (10.53)
DHS 2 Age	1904	26	74	50.73 (10.40)
Education	1903	1	20	13.59 (2.69)
Gender				
Male	801 (42)			
Female	1103 (58)			
Ethnicity				
Black	1019 (53)			
White	637 (34)			
Hispanic	207 (11)			
Other	41 (2)			
Waist Circumference (cm)	1564	56	164	99.55 (17.04)
Diabetic	157 (8)			
Hypertensive	578 (30)			
ApoE Status				
E2/E2	13 (1)			
E2/E3	191 (12)			
E2/E4	51 (3)			
E3/E3	910 (55)			
E3/E4	417 (25)			
E4/E4	61 (4)			

Table 2. Partial spearman correlation between MoCA scores and inflammatory markers.

		MCP1 (n = 1646)	CRP (n = 1654)	IL-18 (n = 1046)	Lp-PLA ₂ (n = 1609)
MoCA Total Score	Correlation	−0.03	−0.06	0.02	0.09
	Sig.	0.345	0.079	0.567	0.003
	df	994	994	994	994

Variable controlled for in model: Age and education.

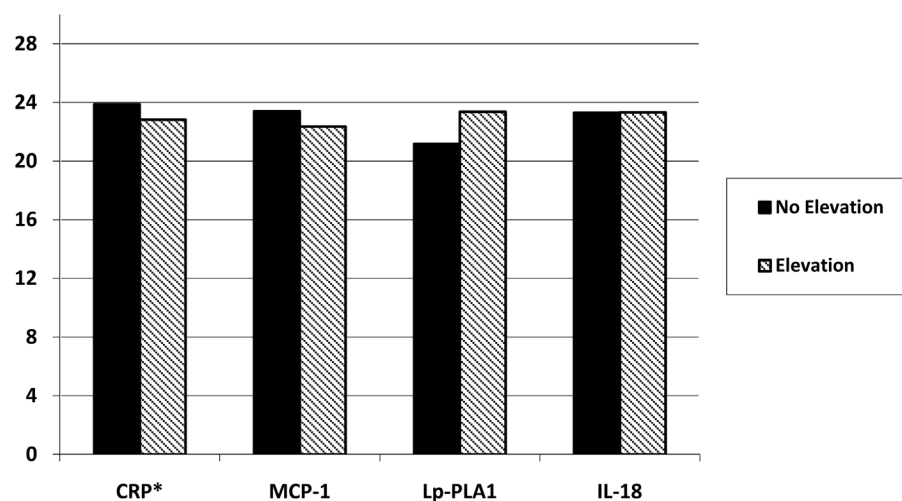


Figure 1. MoCA total score means by biomarker elevation versus no elevation. *Significantly lower MoCA score with elevated CRP [$F(1, 1650) = 6.24, p = 0.013$]. MCP-1 $F(1, 1641) = 0.377, p = 0.539$. Lp-PLA₂ $F(1, 1604) = 1.53, p = 0.216$. IL-18 $F(1, 1042) = 2.27, p = 0.133$.

4. Discussion

We did not observe a significant relationship between cognitive function and inflammatory biomarker levels ascertained 8 years earlier; the only correlation was between Lp-PLA2 and MoCA scores, but in the positive direction. There was a modest relationship between CRP and MoCA scores but no significant relationships between levels of MCP-1, IL-18 and cognition. Lack of significant correlations between MoCA scores and pro-inflammatory substance concentration in blood was surprising, in that we had previously reported a modest relationship between Framingham Coronary and Stroke scores in the same relatively young group of subjects [29]. However, other studies had also failed to find such associations, suggesting that inflammatory markers, at least in healthy samples, were not useful predictors of cognitive function [6]. Although information on biomarker concentrations was likely to inform pathologies that had inflammatory processes as a component, its utility as a predictive variable might only be detected when combined with other molecules or additional biochemical data. In the current study, only Lp-PLA2 was associated with cognitive function measured at a later time, but not in the expected direction. It was unclear if putative pro-inflammatory biomarkers contributed to the atherosclerotic process, were a reaction to it, or constituted a simultaneous parallel process [30].

A major limitation of this study was that it was neither cross-sectional nor longitudinal. During the 8 years between pro-inflammatory marker measurement and assessment of cognitive functioning, participants in the sample might have experienced significant changes in health, medications and lifestyles. Additionally, it was possible that individuals suffering health conditions that were caused or exacerbated by pro-inflammatory markers during DHS 1 did not return for follow-up in DHS 2. The current study, then, might have not assessed those individuals with biomarker elevations high enough to impact cognitive function. It was also possible that changes in cognition might have been too subtle to be detected by the MoCA, which was originally designed to detect MCI rather than to quantitate cognitive function in a non-clinical sample of community-dwelling adults [31]. Finally, it was possible that potential effects of pro-inflammatory substances on cognition might not manifest until later in life. Thus, a further study was planned that would assess the relationship of pro-inflammatory measures to cognition over time.

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