Inflammatory Biomarkers and Cognitive Decline: The Ginkgo Evaluation of Memory Study

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OBJECTIVES: To examine the association between inflammatory biomarkers and global cognitive function.

DESIGN: Case-cohort.

SETTING: Ginkgo Evaluation of Memory Study.

PARTICIPANTS: Individuals aged 75 and older free of neurological or neurodegenerative conditions recruited from 2000 to 2002 (N = 1,315).

MEASUREMENTS: Outcome was cognitive function assessed using the modified Mini-Mental State Examination (3MSE) every 6 months for up to 7 years. Exposures were 10 biomarkers measured at baseline: interleukin-2, -6, and -10 (general systemic inflammation); pentraxin 3 (PTX3) and serum amyloid P (SAP) (vascular inflammation); plasminogen activator inhibitor-1, adiponectin, and resistin (metabolic function); receptor for advanced glycation endproduct (oxidative stress); and endothelin-1 (endothelial function). Associations between biomarkers and 3MSE scores (stratified according to mild cognitive impairment (MCI) at baseline) were analyzed using Cox regression (outcome: 3MSE decline of ≥5 points) and mixed-model regression. Bonferroni correction was used to determine significance threshold ($P < .0025$).

RESULTS: In individuals with baseline MCI, PTX3 was associated with a 20% greater hazard of cognitive decline (95% confidence interval = 1.07–1.35), although this association was no longer statistically significant after adjustment for apolipoprotein (APO)E ε4 allele. Adiponectin was associated with faster rate of 3MSE decline in individuals without baseline MCI in mixed-model regression, but the association was similarly attenuated after adjustment for APOE-ε4.

CONCLUSION: This study did not find strong evidence of the utility of the biomarkers evaluated for identifying individuals at risk of cognitive decline. Future studies investigating the association between PTX3, SAP, and adiponectin and 3MSE scores may be useful. **J Am Geriatr Soc 64:1171–1177, 2016.**

Key words: cognition; dementia; inflammation; biomarkers; Alzheimer's; 3MSE

Changes in the brain of individuals who develop dementia may occur up to 20 years before symptom occurrence. Many experts believe that future treatments to slow or halt the progression of dementia will need to be administered in early phases of the disease—particularly the preclinical or mild cognitive impairment (MCI) stage—to be most effective. Biomarkers are essential to identify individuals in early stages of dementia so treatments can be effectively targeted.[1,2] Alzheimer's disease (AD) has traditionally been thought of as a purely neurodegenerative disease separate from vascular dementia, but growing evidence suggests that vascular risk factors contribute to AD development. The majority of individuals with dementia have vascular and neurodegenerative pathologies.[2] There is increasing evidence that inflammation plays a role in brain changes that precede dementia, partially through cerebrovascular atherosclerosis or neuronal cell damage stimulated by the inflammatory process.[3–5]

Recent studies have investigated a range of inflammatory biomarkers and their associations with dementia or cognitive decline with inconsistent findings,[6–14] but the majority of these studies have been of case–control or cross-sectional design. The few cohort or nested case–control studies generally have used two measures of cognitive decline—one at baseline and one several years later. In addition, biomarkers have been evaluated separately without assessment of the predictive ability of a combination of inflammatory markers.

This analysis sought to prospectively evaluate the ability of biomarkers of inflammation to predict cognitive

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levels and decline in elderly adults free of dementia at baseline. Using the Ginkgo Evaluation of Memory Study (GEMS), the following 10 biomarkers were assessed: interleukin (IL)-2, -6, and -10 (general systemic inflammation); pentraxin 3 (PTX3) and serum amyloid P (SAP) (vascular inflammation); plasminogen activator inhibitor (PAI)-1, adiponectin, and resistin (metabolic function); receptor for advanced glycation endproduct (RAGE) (oxidative stress); and endothelin-1 (ET-1) (endothelial function).

METHODS

Study Design and Population

GEMS has been described previously.[15,16] Briefly, it was a double-blind, placebo-controlled trial to evaluate the effect of Ginkgo biloba on dementia prevention in elderly adults. Individuals aged 75 and older free of neurological or neurodegenerative diseases were recruited from four academic medical centers (University of Pittsburgh, University of California at Davis, Johns Hopkins School of Medicine, Wake Forest University) from 2000 to 2002 (N = 3,069) and followed for an average of 6 years. Although participants were free of dementia at baseline, those with MCI were not excluded. Ginkgo biloba was not found to prevent dementia or reduce mortality. Loss to follow-up was low (6.3%), and perceived side effects were similar for the treatment and placebo groups.[16] Thus, this cohort remains a valuable resource for investigating other risk factors for dementia.

This ancillary study to GEMS used a case–cohort design that included a randomly selected subset of participants, 1,046 of whom were dementia free through the end of the study; all participants diagnosed with incident dementia during follow-up were also included (n = 523). Participants were excluded if they did not have at least two cognitive function scores during follow-up. A sample (n = 995) of participants who provided genetic consent and had sufficient deoxyribonucleic acid for analyses were tested for the presence of apolipoprotein E ε4 genotype (APOE-ε4), an allele known to be associated with cognitive decline.

Cognitive Assessment

The baseline neuropsychological battery measured language, mood, executive and visuospatial function, memory, psychomotor speed, and global cognitive function. Participants were reevaluated every 6 months for global cognitive functioning using an abbreviated cognitive test battery including the modified Mini-Mental State Examination (3MSE). If dementia was suspected, the baseline neuropsychological test battery was repeated, followed by an additional neurological and medical examination and brain magnetic resonance imaging. An expert consensus panel made the final diagnosis. Participants were no longer followed for cognitive decline once dementia was diagnosed.

Biomarker Assessment

Plasma biomarkers were tested using stored blood samples collected at baseline. Laboratory analyses were conducted at the University of Vermont using multiplex panel technology and an enzyme-linked immunosorbent assay.

Statistical Analysis

Relationships between each biomarker and cognitive scores and decline were analyzed using mixed-effects regression models to account for correlation between study visits. All analyses were stratified according to mild cognitive decline (MCI) because associations between biomarkers and 3MSE were found in previous analyses to differ according to MCI.[17] Study visits were centered at the median follow-up (visit 7, or 3.5 years), and a visit-squared term was used to allow for curvilinearity in 3MSE scores. To assess differences in the rate of cognitive decline by baseline biomarker level, interaction terms between biomarkers and centered study visit were generated; the resulting coefficient represented the difference in the instantaneous rate of 3MSE change at year 3.5 for each standard deviation (SD) higher in baseline biomarker level. Interactions between APOE and age and time were also assessed. Models were adjusted hierarchically as follows: first for demographic characteristics, second with the addition of cardiovascular disease (CVD) risk factors, and third with the addition of the APOE-ε4 genotype, which was available for 77% of all participants. Continuous variables were categorized or log transformed as needed to fulfill model assumptions. IL-2, -6, and -10 were analyzed as log transformed in the main analysis because they have been found to be right skewed. Because APOE allele has been observed to modify the effect of inflammation and dementia, interactions between the allele and biomarkers were evaluated.[18] The collective contributions of the domains of inflammation were analyzed to evaluate the predictive ability of a combination of markers; colinearity was determined using a correlation matrix. A sensitivity analysis was conducted with a third APOE-ε4 category to indicate missing values, as done in previous studies.[21] To further characterize the relationship between biomarkers and cognitive decline, survival analysis with Cox regression was used with the outcome of time to decline in 3MSE of 5 or more points during the observation period. A decline of 5 or more has been used as a clinically relevant decrease in cognitive function.[19] Robust standard errors were used to relax model assumptions. As a sensitivity analysis, interaction terms between biomarkers and the natural log of visit number were generated to allow for nonlinear variation over time. Because 10 biomarkers were evaluated in two types of models for the main analyses (mixed effects and Cox regression), a Bonferroni corrected p-value ($P = .0025$) was use as a threshold for statistical significance. All analyses were conducted in Stata version 12 (Stata Corp., College Station, TX).[20]

RESULTS

Study Population

Similar to the full GEMS, individuals in the subcohort were predominantly white (95%) and highly educated (64% completed some advanced studies after high school)

and had a mean age of 79.0 ± 3.4. The subcohort had high levels of comorbidities, with 55% diagnosed with hypertension and 35% having a history of heart disease. Individuals who experienced a decline in 3MSE score of 5 points or more at any time during follow-up were more likely to be older (79.4 vs 78.6), nonwhite (6.8% vs 3.4%), male (56.6% vs 53.2%), and nondrinkers (59.1% vs 56.4%); have lower education (14.8% vs 10.0% did not complete high school); and have MCI at baseline (27% vs 16%) (Table 1). Those with a decline of 5 or more points in 3MSE score also developed dementia at substantially higher rates (60% vs 22%). Overall, participants had an average of 10.4 study visits with 3MSE scores (range 2–15). Baseline 3MSE scores were similar in the two groups (93.0 in those with a decline of ≥5, 92.5 in those with a decline of <5).

Table 1. Characteristics of the Study Population According to Decline in Modified Mini-Mental State Examination (3MSE) Score

Characteristic	3MSE Decline ≥5, n = 620	3MSE Decline <5, n = 678
Sex, n (%)		
Male	351 (56.6)	361 (53.2)
Female	269 (43.4)	317 (46.8)
Age, mean ± SD	79.4 ± 3.6	78.6 ± 3.3
Race, n (%)		
White	578 (93.2)	655 (96.6)
Other	42 (6.8)	23 (3.4)
Hispanic, n (%)	11 (1.8)	7 (1.0)
Education, n (%)		
≤11th grade	92 (14.8)	68 (10.0)
High school graduate	152 (24.5)	160 (23.6)
Some college	158 (25.5)	155 (22.9)
College graduate	85 (13.7)	111 (16.4)
Graduate school	133 (21.5)	184 (27.1)
Body mass index, kg/m^2, n (%)		
<18.5 (underweight)	6 (1.0)	7 (1.0)
18.5–24.9 (normal)	192 (31.2)	206 (30.4)
25.0–29.9 (overweight)	277 (45.0)	327 (48.3)
≥30.0 (obese)	141 (22.9)	137 (20.2
Alcohol consumption, drinks per week, n (%)		
<1	358 (59.1)	378 (56.4)
1–7	135 (22.3)	155 (23.1)
8–14	55 (9.1)	67 (10.0)
>14	58 (9.6)	70 (10.5)
Smoking, n (%)		
Never	257 (42.5)	272 (40.5)
Former	330 (54.6)	369 (54.9)
Current	18 (3.0)	31 (4.6)
Apolipoprotein E-ε4, n (%)		
No	309 (49.8)	456 (67.3)
Yes	158 (25.5)	103 (15.2)
Missing	153 (24.7)	119 (17.6)
Hypertension, n (%)	344 (55.5)	367 (54.1)
Diabetes mellitus, n (%)	55 (8.9)	65 (9.6)
History of heart disease, n (%)	231 (37.3)	225 (33.2)
Baseline 3MSE score, mean ± SD	93.0 ± 4.7	92.5 ± 5.1
Mild cognitive impairment at baseline, n (%)	169 (27.3)	110 (16.2)
Incident dementia, n (%)	370 (59.7)	151 (22.3)

SD = standard deviation.

Difference in 3MSE Score

Table 2 presents mixed-effects analysis of the difference in 3MSE score at the median follow-up for 1 SD higher in baseline biomarker level for models hierarchically adjusted for demographic characteristics, CVD risk factors, and APOE genotype stratified according to MCI at baseline. None of the biomarkers were statistically significantly associated with 3MSE at a *P*-value threshold of .0025. PTX3, RAGE, and adiponectin tended to be negatively associated with 3MSE score in persons without baseline MCI. In persons with MCI at baseline, PTX3, PAI, and adiponectin tended to be negatively associated with 3MSE score, while SAP was associated with higher 3MSE score. In persons with MCI at baseline, every SD higher of baseline PTX3 was associated with a 1.37-point lower mean 3MSE score (95% confidence interval (CI) = −2.5 to −0.25, $P = .02$) at median follow-up after adjusting for demographic characteristics. The association remained stable after further adjustment for CVD risk factors and APOE allele. The associations were generally larger for persons with MCI than those free of MCI at baseline.

Decline in 3MSE Scores

For individuals without MCI at baseline, a strong relationship was not found between baseline biomarker and rate of cognitive decline for any of the biomarkers assessed, although the increase in the rate of decline associated with a 1-SD-higher value of adiponectin was statistically significant (Table 3). The difference in rate of 3MSE decline was 0.12 points for every SD-higher baseline adiponectin after adjustment for demographic and CVD risk factors, but this result was attenuated to a decline of 0.08 points after the addition of the APOE allele and was no longer statistically significant using the $P < .0025$ threshold. In a sensitivity analysis, when an indicator for missing APOE-ε4 was included in the model, the association remained significant ($P = .001$). PTX3 was also associated with an increase in the rate of 3MSE decline in those free of MCI at baseline (−0.1, 95% CI = −0.18 to −0.03, $P = .007$). In those with baseline MCI, none of the relationships were statistically significant at the threshold of $P = .0025$, although PTX3 and adiponectin tended to be associated with cognitive decline, whereas SAP tended to be inversely related. These associations were not attenuated after adjustment for CVD risk factors and APOE-ε4 allele and were generally stronger in absolute terms than those seen in individuals without MCI at baseline.

Survival Analysis of 3MSE Decline

The associations between baseline biomarkers and time to decrease in 3MSE score of 5 or more points during follow-up are shown in Table 4. Baseline hazards were stratified according to clinic, because clinic was found to violate the proportional hazards assumption. For persons free of MCI at baseline, PTX3 again tended to be associated with decline in 3MSE, with each SD higher of baseline PTX3 associated with a 9% greater hazard of decline in 3MSE score after controlling for demographic characteristics, CVD risk factors, and APOE-ε4 allele (95%

Table 2. Average Difference in Modified Mini-Mental State Examination (3MSE) Score for a 1-Standard Deviation –Higher Baseline Biomarker Level at Midpoint of Follow-Up Stratified According to Mild Cognitive Impairment (MCI) at Baseline

	Model 1[a]			Model 2[b]			Model 3[c]		
Biomarker	N	Difference in 3MSE (95% CI)	*P*-Value	N	Difference in 3MSE (95% CI)	*P*-Value	N	Difference in 3MSE (95% CI)	*P*-Value
No MCI at baseline									
PTX3	1,016	−0.36 (−0.68 to −0.04)	.03	982	−0.33 (−0.65 to −0.004)	.05	785	−0.25 (−0.58–0.08)	.14
RAGE	1,019	0.25 (−0.08–0.57)	.14	985	0.3 (−0.03–0.64)	.08	787	0.4 (0.05–0.75)	.03
ET-1	1,019	−0.09 (−0.38–0.20)	.56	985	−0.06 (−0.35–0.24)	.71	787	0.002 (−0.28–0.29)	.99
SAP	1,019	0.09 (−0.24–0.42)	.59	985	0.103 (−0.23–0.44)	.55	787	0.06 (−0.32–0.43)	.76
IL-10 (log)	1,018	−0.14 (−0.4–0.13)	.31	984	−0.11 (−0.38–0.16)	.44	786	−0.31 (−0.61 to −0.02)	.04
IL-6 (log)	1,018	0.08 (−0.2–0.37)	.57	984	0.11 (−0.19–0.4)	.47	786	0.15 (−0.16–0.46)	.35
IL-2 (log)	1,018	−0.03 (−0.34–0.28)	.85	984	−0.02 (−0.33–0.29)	.91	786	−0.07 (−0.41–0.27)	.69
PAI	1,018	0.10 (−0.23–0.42)	.56	984	0.05 (−0.29–0.39)	.77	786	0.03 (−0.33–0.38)	.89
Resistin	1,018	0.004 (−0.36–0.37)	.98	984	0.001 (−0.37–0.37)	.99	786	−0.11 (−0.5–0.27)	.57
Adiponectin	1,018	−0.33 (−0.66 to −0.08)	.04	984	−0.32 (−0.64 to −0.001)	.05	786	−0.19 (−0.54–0.17)	.30
MCI at baseline									
PTX3	279	−1.37 (−2.5 to −0.25)	.02	267	−1.26 (−2.43 to −0.09)	.03	202	−1.37 (−2.84–0.10)	.07
RAGE	279	0.11 (−0.84–1.06)	.82	267	0.15 (−0.85–1.15)	.77	202	0.71 (−0.53–1.95)	.26
ET-1	279	0.43 (−0.91–1.77)	.53	267	0.41 (−0.96–1.78)	.56	202	1.12 (−0.54–2.78)	.18
SAP	279	1.24 (0.26–2.21)	.01	267	1.20 (0.2–2.19)	.02	202	0.95 (−0.25–2.15)	.12
IL-10 (log)	279	−0.64 (−1.43–0.15)	.11	267	−0.72 (−1.55–0.1)	.08	202	−0.82 (−1.85–0.22)	.12
IL-6 (log)	279	−0.59 (−1.61–0.43)	.26	267	−0.72 (−1.76–0.33)	.18	202	−0.30 (−1.61–1.0)	.65
IL-2 (log)	279	−0.73 (−1.85–0.39)	.20	267	−0.8 (−1.94–0.35)	.17	202	−0.19 (−1.64–1.27)	.80
PAI	279	1.12 (0.09–2.15)	.03	267	1.21 (0.15–2.26)	.03	202	1.06 (−0.22–2.34)	.11
Resistin	279	0.38 (−0.39–1.15)	.33	267	0.43 (−0.34–1.2)	.28	202	0.73 (−0.18–1.64)	.12
Adiponectin	279	−1.08 (−2.27–0.11)	.07	267	−1.21 (−2.44–0.02)	.05	202	−1.3 (−2.86–0.26)	.10

Age and biomarkers interacted with time.

[a]Adjusted for demographic characteristics (age, race, ethnicity, sex, education, clinic).

[b]Adjusted for demographic characteristics and cardiovascular disease (CVD) risk factors (hypertension, diabetes mellitus, history of heart disease (heart attack, angina pectoris, stroke, transient ischemic attack, heart failure, atrial fibrillation, deep vein thrombosis, coronary bypass surgery, balloon angioplasty, heart valve replacement, pacemaker implant, defibrillator implant), body mass index, smoking status, alcohol intake).

[c]Adjusted for demographic characteristics, CVD risk factors, and apolipoprotein E ε4 genotype.

CI = confidence interval; PTX-1 = pentraxin 3; RAGE = receptor for advanced glycation endproduct; ET-1 = endothelin-1; SAP = serum amyloid P; IL = interleukin; PAI-1 = plasminogen activator inhibitor 1.

CI = 1.00–1.19). RAGE was found to be inversely associated with cognitive decline in those without MCI, with each SD higher associated with a 11% lower hazard of decrease in 3MSE score of 5 or more (95% CI = 1.0–1.19) after adjustment for APOE-ε4. Nevertheless, all of the associations for those free of MCI were small, and none were statistically significant at the Bonferroni-corrected threshold.

The associations were generally stronger for those with MCI at baseline. Only PTX3 was statistically significantly related to cognitive decline, with 1 SD higher in biomarker associated with a 20% greater hazard of decline in 3MSE score of 5 or more (95% CI = 1.07–1.35, $P = .002$). Although the magnitude of the association remained stable, precision was attenuated with the addition of CVD risk factors and was no longer statistically significant. Higher levels of SAP tended to be associated with less cognitive decline, each SD higher of biomarker level was associated with a 25% lower risk of cognitive decline after controlling for APOE (95% CI = 0.61–0.92, $P = .01$).

Sensitivity Analyses

Biomarkers were analyzed as quartiles and log transformed, which did not change the conclusions. Biomarker interactions with APOE were small and not statistically significant and did not change results (data not shown). All biomarkers were modeled together to determine whether their combination increased predictive ability, which did not yield strong associations (data not shown). Mixed-effects regression analyses were also rerun with an APOE-ε4 indicator for missing values, and results remained consistent, except for adiponectin, which had a similar difference in rate of decline in those without baseline MCI (0.12) but remained statistically significant ($P = .001$) (data not shown). In the Cox regression analyses, interactions between biomarkers and the natural log of visit number yielded results similar to those presented in the main analysis (data not shown).

DISCUSSION

This analysis did not find strong evidence of the ability of biomarkers to predict cognitive decline in this cohort of elderly individuals. In general, the magnitude of associations detected was smaller for those without MCI at baseline than for those diagnosed with MCI. In persons with MCI, PTX3 levels at baseline tended to be associated with 1.3-point-lower cognitive scores and 0.40-point-higher

Table 3. Average Difference in Rates of Modified Mini-Mental State Examination (3MSE) Decline for a 1-Standard Deviation–Higher Baseline Biomarker Level at Midpoint of Follow-Up Stratified According to Mild Cognitive Impairment (MCI) at Baseline

	Model 1[a]			Model 2[b]			Model 3[c]		
Biomarker	N	Difference in 3MSE Decline (95% CI)	*P*-Value	N	Difference in 3MSE Decline (95% CI)	*P*-Value	N	Difference in 3MSE Decline (95% CI)	*P*-Value
No MCI at baseline									
PTX3	1,016	−0.1 (−0.17 to −0.03)	.006	982	−0.1 (−0.18 to −0.03)	.007	785	−0.07 (−0.14–0.02)	.07
RAGE	1,019	0.04 (−0.04–0.11)	.34	985	0.03 (−0.04–0.11)	.39	787	0.05 (−0.03–0.12)	.20
ET-1	1,019	−0.09 (−0.38–0.204)	.56	985	−0.03 (−0.10–0.03)	.28	787	−0.017 (−0.08–0.04)	.58
SAP	1,019	0.05 (−0.02–0.12)	.15	985	0.05 (−0.03–0.12)	.20	787	0.05 (−0.03–0.13)	.24
IL-10 (log)	1,018	0.007 (−0.05–0.07)	.81	984	0.01 (−0.05–0.07)	.82	786	−0.03 (−0.09–0.04)	.40
IL-6 (log)	1,018	0.02 (−0.05–0.08)	.60	984	0.02 (−0.05–0.08)	.57	786	0.036 (−0.03–0.1)	.28
IL-2 (log)	1,018	−0.003 (−0.07–0.07)	.94	984	−0.002 (−0.07–0.07)	.95	786	0.003 (−0.07–0.08)	.93
PAI	1,018	0.078 (0.01–0.15)	.04	984	0.08 (0.003–0.15)	.04	786	0.07 (0.00–0.15)	.05
Resistin	1,018	−0.02 (−0.10–0.06)	.64	984	−0.02 (−0.11–0.06)	.60	786	−0.11 (−0.50–0.27)	.57
Adiponectin	1,018	−0.12 (−0.19 to −0.05)	.001	984	−0.12 (−0.19 to −0.05)	.001	786	−0.08 (−0.16 to −0.01)	.03
MCI at baseline									
PTX3	279	−0.41 (−0.70 to −0.12)	.006	267	−0.38 (−0.68 to −0.07)	0.02	202	−0.4 (−0.79 to −0.02)	.04
RAGE	279	0.11 (−0.13–0.35)	.38	267	0.08 (−0.17–0.33)	.53	202	0.22 (−0.09–0.54)	.17
ET-1	279	0.01 (−0.33–0.35)	.94	267	0.05 (−0.30–0.4)	.77	202	0.19 (−0.22–0.6)	.36
SAP	279	0.36 (0.11–0.60)	.01	267	0.36 (0.10–0.61)	.01	202	0.36 (0.05–0.68)	.02
IL-10 (log)	279	−0.16 (−0.36–0.04)	.12	267	−0.17 (−0.38–0.04)	.12	202	−0.25 (−0.52–0.01)	.06
IL-6 (log)	279	−0.13 (−0.39–0.13)	.32	267	−0.12 (−0.39–0.14)	.37	202	−0.08 (−0.4–0.24)	.63
IL-2 (log)	279	−0.13 (−0.42–0.16)	.37	267	−0.12 (−0.42–0.17)	.41	202	−0.02 (−0.39–0.35)	.92
PAI	279	0.24 (−0.03–0.50)	.08	267	0.25 (−0.02–0.52)	.07	202	0.22 (−0.11–0.55)	.20
Resistin	279	0.12 (−0.07–0.30)	.21	267	0.13 (−0.06–0.31)	.19	202	0.16 (−0.06–0.38)	.16
Adiponectin	279	−0.38 (−0.68 to −0.09)	.01	267	−0.4 (−0.7 to −0.10)	.01	202	−0.37 (−0.74–0.01)	.06

Age and biomarkers interacted with time.

[a]Adjusted for demographic characteristics (age, race, ethnicity, sex, education, clinic).

[b]Adjusted for demographic characteristics and cardiovascular disease (CVD) risk factors (hypertension, diabetes mellitus, history of heart disease (heart attack, angina pectoris, stroke, transient ischemic attack, heart failure, atrial fibrillation, deep vein thrombosis, coronary bypass surgery, balloon angioplasty, heart valve replacement, pacemaker implant, defibrillator implant), body mass index, smoking status, alcohol intake).

[c]Adjusted for demographic characteristics, CVD risk factors, and apolipoprotein E ε4 genotype.

CI = confidence interval; PTX-1 = pentraxin 3; RAGE = receptor for advanced glycation endproduct; ET-1 = endothelin-1; SAP = serum amyloid P; IL = interleukin; PAI-1 = plasminogen activator inhibitor 1.

instantaneous rates of cognitive decline, but the clinical significance of this decline in score with higher PTX3 levels is probably marginal. A similarly weak relationship was found between SAP (inverse association) and adiponectin (positive association) and levels and rates of cognitive decline in individuals with MCI. For participants without MCI at baseline, all biomarkers were associated with small (≤0.5 points) differences in 3MSE scores and small differences in rates of cognitive decline (≤0.10 points). Although the association between adiponectin and 3MSE score was statistically significant in those free of MCI at baseline, the magnitude of the association was small (0.10 greater rate of decline in 3MSE score for 1 SD higher in baseline biomarker level), which was further attenuated after adjustment for APOE-ε4.

The results of the Cox regression analyses were similar to those of the mixed-effects models (Table 4). Hazard ratios were small in participants free of MCI at baseline (<10% increase or decrease in risk of cognitive decline per 1-SD-higher baseline biomarker level). Similar to the mixed-effects models, stronger associations were seen in those with MCI at baseline, and PTX was associated with a 20% greater risk of cognitive decline after adjustment for demographic characteristics. This association was statistically significant at $P < .0025$, but the *P*-value was reduced to 0.08 after adjustment for APOE-ε4. This may be partially attributed to a decrease in sample size from 279 to 202 persons resulting in reduced power and should therefore be investigated in future analyses. Similarly, SAP was found to have an inverse relationship with cognitive decline in participants with MCI at baseline. Each SD higher in SAP levels was associated with a 25% lower hazard of cognitive decline.

No other plasma biomarkers were found to be associated with cognitive function in the mixed-effects or Cox regression. Additionally, the collective predictive ability of the plasma biomarkers was not significant, nor was evidence found of interaction between any plasma biomarkers and APOE. This study yielded largely negative results, particularly in those free of MCI at baseline.

These findings should be interpreted within the context of several limitations. The 3MSE score is a commonly used indicator of cognitive decline, but if corresponding decreases in 3MSE score did not capture some cognitive decline, then the results would be biased toward the null. In addition, it is possible that biomarker levels in peripheral circulation may have limited correlation with those in the central nervous system. As is the

Table 4. Risk of Cognitive Decline (≥5 Points in Modified Mini-Mental State Examination (3MSE) Score) According to Baseline Biomarker Level Stratified According to Mild Cognitive Impairment (MCI) at Baseline

	Model 1[a]			Model 2[b]			Model 3[c]		
Biomarker	**N**	**HR (95% CI)**	***P*-Value**	**N**	**HR (95% CI)**	***P*-Value**	**N**	**HR (95% CI)**	***P*-Value**
No MCI at baseline									
PTX3	1,016	1.04 (0.95–1.14)	.43	982	1.03 (0.94–1.14)	.52	785	1.09 (1.0–1.19)	.05
RAGE	1,019	0.92 (0.83–1.01)	.08	985	0.91 (0.82–1.02)	.10	787	0.89 (0.79–1.02)	.08
ET-1	1,019	1.02 (0.94–1.1)	.68	985	1.02 (0.94–1.1)	.69	787	1.00 (0.94–1.07)	>.99
SAP	1,019	0.95 (0.86–1.05)	.29	985	0.92 (0.83–1.027)	.14	787	0.93 (0.82–1.04)	.21
IL-10 (log)	1,018	0.99 (0.91–1.07)	.78	984	0.99 (0.91–1.08)	.83	786	1.05 (0.95–1.16)	.35
IL-6 (log)	1,018	0.93 (0.85–1.02)	.14	984	0.93 (0.84–1.028)	.15	786	0.94 (0.84–1.05)	.25
IL-2 (log)	1,018	0.99 (0.91–1.08)	.79	984	0.995 (0.91–1.09)	.92	786	1.02 (0.92–1.12)	.71
PAI	1,018	0.96 (0.87–1.06)	.40	984	0.94 (0.85–1.041)	.23	786	0.94 (0.84–1.05)	.28
Resistin	1,018	0.998 (0.89–1.12)	.97	984	0.97 (0.86–1.1)	.65	786	1.03 (0.91–1.17)	.61
Adiponectin	1,018	1.09 (0.997–1.2)	.06	984	1.12 (1.02–1.229)	.02	786	1.10 (0.97–1.25)	.12
MCI at baseline									
PTX3	279	1.20 (1.07–1.35)	.002	267	1.22 (1.06–1.41)	.007	202	1.19 (0.98–1.43)	.08
RAGE	279	1.03 (0.89–1.2)	.70	267	0.995 (0.83–1.2)	.96	202	0.94 (0.75–1.16)	.55
ET-1	279	1.01 (0.83–1.23)	.89	267	0.99 (0.79–1.25)	.95	202	0.87 (0.64–1.18)	.36
SAP	279	0.82 (0.7–0.948)	.01	267	0.799 (0.68–0.94)	.01	202	0.75 (0.61–0.92)	.01
IL-10 (log)	279	1.12 (1.01–1.24)	.03	267	1.1 (0.98–1.23)	.12	202	1.12 (0.95–1.34)	.18
IL-6 (log)	279	1.03 (0.87–1.22)	.75	267	1.05 (0.88–1.26)	.57	202	0.9 (0.65–1.24)	.53
IL-2 (log)	279	1.12 (0.95–1.34)	.18	267	1.07 (0.88–1.3)	.50	202	0.99 (0.76–1.3)	.95
PAI	279	0.92 (0.78–1.09)	.35	267	0.94 (0.78–1.13)	.49	202	0.93 (0.72–1.2)	.58
Resistin	279	0.92 (0.8–1.06)	.25	267	0.89 (0.77–1.04)	.13	202	0.9 (0.77–1.05)	.17
Adiponectin	279	1.04 (0.87–1.25)	.67	267	1.07 (0.89–1.292)	.46	202	1.12 (0.89–1.41)	.33

Age and biomarkers interacted with time.

[a]Adjusted for demographic characteristics (age, race, ethnicity, sex, education, clinic).

[b]Adjusted for demographic characteristics and cardiovascular disease (CVD) risk factors (hypertension, diabetes mellitus, history of heart disease (heart attack, angina pectoris, stroke, transient ischemic attack, heart failure, atrial fibrillation, deep vein thrombosis, coronary bypass surgery, balloon angioplasty, heart valve replacement, pacemaker implant, defibrillator implant), body mass index, smoking status, alcohol intake).

[c]Adjusted for demographic characteristics, CVD risk factors, and apolipoprotein E ε4 genotype.

HR = hazard ratio; CI = confidence interval; PTX-1 = pentraxin 3; RAGE = receptor for advanced glycation endproduct; ET-1 = endothelin-1; SAP = serum amyloid P; IL = interleukin; PAI-1 = plasminogen activator inhibitor 1.

case with all clinical trials, GEMS participants are a highly selected group of individuals who volunteered to participate in clinical research for up to 7 years. The vast majority were white and highly educated. In addition, many had comorbidities, which may have reduced sensitivity to detect the outcome, particularly for weak associations. The study protocol required a rigorous examination for dementia whenever declines in 3MSE were observed. It is also possible that the study was underpowered for the number of biomarkers examined, and stratification according to MCI at baseline further reduced power. Most of the associations in persons without MCI were close to the null, which provides evidence for negative results as opposed to a lack of power, although in persons with MCI at baseline, sample sizes were considerably smaller than in those free of MCI, and there may not have been sufficient power to find associations. Additional studies are needed in more-diverse cohorts to investigate the clinical utility of determining the effect of biomarkers, particularly PTX3 and SAP, on cognitive function. The relationship between PTX3 and SAP and cognitive decline in persons with MCI should be further investigated with larger sample sizes.

These results are consistent with those of previous studies,[21] and the strengths of the study include the size of the cohort; low loss to follow-up, which minimizes survival bias; rigorous and frequent assessment of dementia; and frequent assessments of cognitive decline.

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