Journal of Alzheimer's Disease 88 (2022) 7–15
DOI 10.3233/JAD-220091
IOS Press

Short Communication

Association of Peripheral Lymphocyte Subsets with Cognitive Decline and Dementia: The Cardiovascular Health Study

Alison E. Fohner[a,b,*], Colleen M. Sitlani[a], Petra Buzkova[c], Margaret F. Doyle[d], Xiaojuan Liu[e], Joshua C. Bis[a], Annette Fitzpatrick[a,f,g], Susan R. Heckbert[a,b], Sally A. Huber[d], Lewis Kuller[h], William T. Longstreth[a,i], Matthew J. Feinstein[j], Matthew Freiberg[k], Nels C. Olson[d], Sudha Seshadri[l,m,n], Oscar Lopez[o], Michelle C. Odden[e], Russell P. Tracy[p,d], Bruce M. Psaty[a,b,q,r], Joseph A. Delaney[a,s] and James S. Floyd[a,b,q]

[a]*Cardiovascular Health Research Unit, University of Washington, Seattle, WA, USA*
[b]*Department of Epidemiology, University of Washington, Seattle, WA, USA*
[c]*Department of Biostatistics, University of Washington, Seattle, WA, USA*
[d]*Department of Pathology and Laboratory Medicine, Larner College of Medicine, University of Vermont, Burlington, VT, USA*
[e]*Department of Epidemiology and Population Health, Stanford University, Stanford, CA, USA*
[f]*Department of Family Medicine, University of Washington, Seattle, WA, USA*
[g]*Department of Global Health, University of Washington, Seattle, WA, USA*
[h]*Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA*
[i]*Department of Neurology, University of Washington, Seattle, WA, USA*
[j]*Departments of Medicine, Preventive Medicine and Pathology, Northwestern University, Evanston, IL, USA*
[k]*Department of Cardiovascular Medicine, Vanderbilt University, Nashville, TN, USA*
[l]*Glenn Biggs Institute for Alzheimer's & Neurodegenerative Diseases, University of Texas Health Science Center at San Antonio, TX, USA*
[m]*Department of Neurology, Boston University School of Medicine, Boston, MA, USA*
[n]*Framingham Heart Study, Framingham, MA, USA*
[o]*Departments of Neurology and Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA*
[p]*Department of Biochemistry, Larner College of Medicine, University of Vermont, Burlington, VT, USA*
[q]*Department of Medicine, University of Washington, Seattle, WA, USA*
[r]*Department of Health Systems and Population Health, University of Washington, Seattle, WA, USA*
[s]*College of Pharmacy, University of Manitoba, Winnipeg, MB, Canada*

Accepted 11 April 2022
Pre-press 6 May 2022

*Correspondence to: Alison E. Fohner, Department of Epidemiology, University of Washington, Box 351619, Seattle WA 98195-1619, USA. Tel.: +1 206 685 2715; E-mail: afohner@uw.edu.

ISSN 1387-2877

Abstract. Inflammatory biomarkers in plasma are associated with dementia. Thus, we examined the association of 18 types of peripheral immune cells, measured as proportions of their immune cell type, with cross-sectional measures of cognitive function, change in cognitive function over seven years, prevalent dementia, and time to death from dementia in 1,928 participants of the Cardiovascular Health Study, with mean age 80 years and 62% female. We did not identify any associations after accounting for multiple comparisons, though we identified marginal associations of peripheral regulatory T cells with cognitive decline and dementia.

Keywords: Alzheimer's disease, B cells, benton visual retention test, cognitive impairment, immune, natural killer cells, neuroinflammation, T cells

INTRODUCTION

Neuroinflammation is implicated in rapid progression of Alzheimer's disease (AD) and in altered learning and memory [1, 2]. Large scale genomic studies support the role of the immune system in dementia [3–10]. Immune cells have been identified in brain tissue and cerebrospinal fluid of people with AD, and the levels of immune cells in blood may be associated with dementias, perhaps reflecting premature immunosenescence and chronic inflammation [11–18]. For example, higher circulating proportions of natural killer (NK) cells were apparent before AD onset and the depletion of NK cells improved cognitive function in mice [19]. In humans, high NK cell activity was associated with worse cognitive performance of AD patients [14, 20, 21]. Increased numbers of CD8 + T effector memory CD45RA+(CD8 T_{EMRA}) cells and of CD4 + T effector memory CD45RA+(CD4 T_{EMRA}) cells were identified in the blood of patients with AD [12, 22, 23]. Prior studies report both decreased and increased proportions of regulatory T cells (T_{reg}) in blood of patients with AD [22–27]. Immunosenescence of T cells may promote AD by decreasing anti-amyloid antibodies, which could result in less clearance of amyloid plaques [28], or by producing high levels of pro-inflammatory cytokines and oxidative stress, which could damage neurons [29, 30]. However, many of the associations between peripheral immune cells and cognitive outcomes are based on small, cross-sectional studies. Clarifying the association of the peripheral adaptive and innate immune system with risk for cognitive decline and dementia in a large, population-based setting may illuminate underlying biological processes and lead to the development of better therapeutics.

The Cardiovascular Health Study (CHS) is a population-based, prospective cohort study that included serial cognitive evaluations, dementia adjudication, and assessment of 18 types of peripheral innate and adaptive immune cell subsets. Importantly, CHS allows evaluation of the association of circulating immune cells with both cross-sectional and longitudinal cognitive and dementia outcomes. We hypothesized *a priori* that high proportions of NK, and both CD4 + and CD8 + T_{EMRA} cells would be associated with worse prospective cross-sectional global cognition, worse cognitive decline, prevalent dementia, and shorter time to death from dementia. We hypothesized that higher proportions of T_{reg} protect against these adverse cognitive outcomes. We investigated all other available immune cell subsets as exploratory hypotheses to broadly characterize the relationship of the peripheral immune system with cognitive decline and dementia.

METHODS AND MATERIALS

Study design and approval

The CHS is a population-based, longitudinal cohort of 5,888 men and women aged 65 years or older at enrollment in 1989–93 [31]. Analytic baseline for this analysis was defined as the 1998–1999 CHS exam because this study leverages immune cell phenotype data obtained [32] from the 1998–1999 exams. Institutional review boards at the University of Washington and at each study site approved the study. All CHS participants provided written informed consent.

Immune cell measurement

Detailed methods for immune cell phenotyping and flow cytometry gating strategies have been published [32, 33]. Briefly, as part of the 1998–1999 exam, peripheral blood mononuclear cells (PBMCs) were cryopreserved. Flow cytometry was used to differentiate cell types based on surface marker expression. Cell phenotypes were expressed as proportions of larger "parent" populations, as indicated

in Table 2. Poor sample quality and technical assay errors resulted in missing data, which appear to be missing at random. IgG antibodies to cytomegalovirus (CMV) were measured in serum by enzyme immunoassay (Diamedix Corp., Miami Lakes, FL); the inter-assay coefficients of variation of CMV titer were 5.1%–6.8%.

Cognition and dementia adjudication

Global cognitive performance was assessed with the 100-point Modified Mini Mental State Exam (3MSE) for participants in 1998–1999 and was repeated among participants remaining in the study in 2005–2006. Participants who did not attend an in-person exam were contacted via telephone to complete a Telephone Interview for Cognitive Status (TICS) exam. The TICS score explains 67% of variability in 3MSE in CHS and can be used to estimate 3MSE score with a correlation of 0.82 between actual and TICS-estimated 3MSE [34, 35]. We used TICS score to estimate the 3MSE score when 3MSE was missing. TICS was used to estimate one score at the 1998–1999 exam and 194 scores at the 2005–2006 exam.

A committee of neurologists and psychiatrists used Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria to adjudicate dementia prior to 1998–1999 [36–38]. Death from dementia was ascertained on all participants through 2015 with evidence of advanced dementia prior to death without evidence of another cause [39].

Statistical analysis

For main analyses we used multiple imputation with chained equations (40 imputations) to impute all missing data, including any missing covariates, cell phenotypes, and cognitive outcomes. We imputed 20 cross-sectional cognitive scores, 996 changes in cognitive score, 402 statuses of prevalent dementia, and 19 times to death with dementia. Data were imputed in blocks of immune cells, covariates, and cognitive outcomes. Additional covariates associated with aging-related outcomes in CHS were included in the imputation process to improve estimates [40–44]. In the main imputed analyses, all participants were included in all analyses.

Due to co-linearity, we analyzed each cell phenotype separately, per standard deviation (SD) higher value. Associations between immune cell proportion per 1-SD and both cross-sectional 3MSE score and change in 3MSE between 1998–1999 and 2005–2006 were assessed using linear regression. We used Poisson regression to determine relative risk of prevalent dementia per 1-SD higher immune cell proportion. We used Cox proportional hazards regression to determine hazard ratio of death from dementia per 1-SD higher immune cell proportion. Censoring occurred at death without noted dementia or in 2015. All analyses were adjusted for age, sex, Black race, systolic blood pressure, smoking status, statin use, education, prevalent diabetes, *APOE4* allele carrier status, body mass index (BMI), CMV antibody titer, assay batch, and study site.

In exploratory analyses, we repeated all analyses stratified by sex. In sensitivity analyses, we excluded 135 participants who had experienced a stroke prior to the 1998–1999 exam. To identify potential influence of imputed missing data, we performed complete case analysis for associations between immune cell proportion and three outcomes: cross-sectional cognitive function, prevalent dementia, and time-to-death from dementia. We weighted individuals with 2005–2006 data by the inverse probability of the likelihood of having cognitive scores in 2005–2006 to perform a sensitivity analysis of the association of immune cell proportions and seven-year change in cognitive function [45].

For main endpoints, a p-value less than 0.0125 was considered significant to account for the four primary cell types assessed. All other analyses were exploratory and a p-value of 0.05 was considered significant. We conducted all analyses in RStudio (R version 3.6.3). The data that support the findings of this study are available upon reasonable request through the CHS Coordinating Center (CHS-NHLBI.org).

RESULTS

Our analysis included 1928 CHS participants with at least one peripheral immune subset measured. Table 1 presents their characteristics at analytic baseline. Cognitive evaluations were performed seven years later in a subset of 932 (48%) participants and scores declined a mean of 6.5 points (standard deviation = 9.7). Over 16 years of follow up, 272 (14%) participants died from dementia. Table 2 presents immune cells as proportions of their parent population.

Table 3 presents associations between each immune cell proportion and both cross-sectional

Table 1
Characteristics of CHS subjects with immune cell data ($n = 1928$) at analytic baseline

Variable	Mean or number	Standard deviation (SD) or %
Age, y (mean, SD)	79.7	4.4
Male (*n*, %)	732	38.0%
Black (*n*, %)	339	17.6%
Study site (*n*, %)		
North Carolina	536	27.8%
California	521	27.0%
Maryland	410	21.3%
Pittsburgh	461	23.9%
Education past grade 12 (*n*, %)	940	48.8%
Systolic blood pressure, mmHg (mean, SD)	135.2	20.5
Smoking status (*n*, %)		
Current	151	6.6%
Never	938	44.1%
Former	839	49.3%
At least one *APOE4* allele (*n*, %)	534	29.0%
BMI, kg/m^2 (mean, SD)	26.6	4.4
Prior Stroke (*n*, %)	135	7.0%
Diabetes (*n*, %)	372	19.4%
Statin user (*n*, %)	307	15.9%
CMV antibody titer, EU/mL (mean, SD)	190.3	183.6
Prevalent dementia (*n*, %)	563	36.9%
3MSE (mean, SD)	90.4	11.6

* percentages based on number of participants with data for that variable. No data were missing for age, sex, race, study site, presence of prior stroke, or statin use. The number of missing observations for the other variables are as follows: 3 for level of education, 1 for blood pressure, 25 for smoking status, 84 for *APOE4* genotype, 143 for BMI, 10 for prevalent diabetes, 189 for CMV antibody titer, 402 for prevalent dementia, and 1 for 3MSE cognitive score.

Table 2
Cellular phenotypes with their molecular description, parent population, number of samples with data (N), means and standard deviations (SD)

Cellular phenotype	Molecular description	Parent population	N	Mean	SD
Primary hypotheses					
Natural killer	CD3-CD56 + CD16+	% Lymphocytes	1,556	5.1	4.8
Treg	CD4 + CD25 + CD127–	CD4+	1,545	6.6	4.6
CD4 + TEMRA	CD4 + CD45RA + CD28-CD57+	CD4+	1,670	6.9	5.9
CD8 + TEMRA	CD8 + CD45RA + CD28-CD57+	CD8+	1,675	23.6	14.1
Exploratory hypotheses					
γδ T cells	CD3+γδ+	CD3+	1,539	5.5	4.9
B cells	CD19+	% Lymphocytes	1,556	19.7	15.9
T helper cells	CD4+	% Lymphocytes	1,673	50.1	14.5
Cytotoxic T cells	CD8+	% Lymphocytes	1,691	17.3	9.7
Th1	CD4 + CD194-CXCR3 + CD196–	CD4+	1,326	20.2	8.0
Th2	CD4 + CD194 + CXCR3-CD196–	CD4+	1,326	4.7	3.8
Th17	CD4 + CD194 + CXCR3-CD196+	CD4+	1,326	3.2	2.6
Naïve CD4 + cells	CD4 + CD45RA+	CD4+	1,690	25.8	12.6
Memory CD4 + cells	CD4 + CD45RO+	CD4+	1,690	49.7	15.5
Activated/mature CD4 + cells	CD4 + CD38+	CD4+	1,688	33.4	15.8
Naïve CD8 + cells	CD8 + CD45RA+	CD8+	1,709	42.6	16.6
Memory CD8 + cells	CD8 + CD45RO+	CD8+	1,702	30.2	14.4
Activated/mature CD8 + cells	CD8 + CD38+	CD8+	1,707	34.3	18.5
Memory B cells	CD19 + CD27+	CD19+	1,557	26.4	19.8

cognitive function and longitudinal change in cognitive function after seven years using imputed data where missing. No immune cell proportions were associated with any cognitive outcomes after accounting for multiple comparisons of the primary cell types. However, higher proportions of T_{reg} were

Table 3

Associations of lymphocyte subsets (per 1 SD) with cognitive outcomes. Analyses for cross-sectional 3MSE score and change in 3MSE score over seven years are based on multiple linear regression. Analysis of prevalent dementia is based on Poisson regression. Analysis of time to death from dementia is based on Cox proportional hazards regression. All analyses adjust for age, sex, Black race, systolic blood pressure, smoking, statin use, education, prevalent diabetes, *APOE4* carrier status, BMI, CMV antibody titer, assay batch, and study site. Participants were censored at death without dementia or in 2015. All 1928 individuals are included in each analysis. Missing data were imputed, including covariates (see Table 1), immune cells (see Table 2), and outcomes. We imputed 20 cross-sectional cognitive scores, 996 changes in cognitive score, 402 statuses of prevalent dementia, and 19 times to death with dementia. Beta values that are negative indicate lower cognitive score and a greater decline in cognition over 7 years

	Cross-sectional 3 MSE score			Change over seven years			Cross-sectional dementia			Time to dementia death		
Cellular phenotype	Beta	95% CI	*p*	Beta	95% CI	*p*	Relative Risk	95% CI	*p*	Hazard Ratio	95% CI	*p*
Natural killer	**0.06**	**–1.71, 1.84**	**0.94**	**–0.09**	**–3.90, 3.73**	**0.96**	**1.15**	**0.77, 1.70**	**0.50**	**1.16**	**0.80, 1.69**	**0.44**
Treg	**–0.52**	**–1.08, 0.03**	**0.065**	**–1.30**	**–2.36, –0.24**	**0.018**	**1.10**	**0.99, 1.22**	**0.086**	**1.13**	**0.78, 1.64**	**0.52**
CD4 + TEMRA	**–0.12**	**–0.80, 0.55**	**0.72**	**0.47**	**–0.80, 1.73**	**0.47**	**0.98**	**0.83, 1.16**	**0.82**	**1.07**	**0.76, 1.52**	**0.68**
CD8 + TEMRA	**0.05**	**–0.55, 0.65**	**0.86**	**0.24**	**–0.91, 1.39**	**0.68**	**1.03**	**0.91, 1.18**	**0.63**	**1.05**	**0.74, 1.49**	**0.79**
$\gamma\delta$ T cells	0.41	–1.81, 2.62	0.72	–0.93	–4.85, 2.99	0.64	1.00	0.66, 1.50	0.98	1.06	0.75, 1.50	0.76
B cells	0.26	–0.22, 0.73	0.29	0.08	–0.85, 1.01	0.86	0.97	0.87, 1.08	0.54	1.10	0.77, 1.57	0.60
T helper cells	–0.15	–0.33, 0.02	0.09	–0.03	–0.37, 0.31	0.86	1.01	0.97, 1.05	0.74	1.08	0.76, 1.53	0.67
Cytotoxic T cells	0.11	–0.02, 0.25	0.09	–0.07	–0.31, 0.17	0.56	1.01	0.98, 1.04	0.70	1.08	0.76, 1.54	0.67
Th1	0.14	–0.17, 0.46	0.38	–0.25	–0.87, 0.37	0.44	0.99	0.92, 1.06	0.79	1.08	0.76, 1.53	0.67
Th2	–0.12	–2.51, 2.28	0.92	1.59	–4.50, 7.68	0.61	0.99	0.57, 1.71	0.97	1.08	0.75, 1.55	0.68
Th17	0.34	–2.88, 3.56	0.84	1.51	–2.14, 5.16	0.42	0.99	0.53, 1.84	0.97	1.08	0.76, 1.53	0.68
Naïve CD4 + cells	0.12	–0.42, 0.66	0.67	0.14	–0.86, 1.15	0.78	0.90	0.79, 1.03	0.14	1.00	0.70, 1.43	0.99
Memory CD4 + cells	–0.04	–0.39, 0.30	0.81	–0.13	–0.83, 0.56	0.71	1.06	0.97, 1.15	0.19	1.01	0.71, 1.44	0.97
Activated/mature CD4 + cells	0.10	–0.45, 0.64	0.73	0.24	–0.73, 1.20	0.63	0.95	0.84, 1.08	0.45	1.09	0.77, 1.55	0.62
Naïve CD8 + cells	0.06	–0.06, 0.17	0.33	–0.13	–0.36, 0.09	0.24	0.99	0.96, 1.02	0.47	1.08	0.76, 1.53	0.66
Memory CD8 + cells	–0.25	–0.59, 0.10	0.16	0.21	–0.41, 0.82	0.51	1.04	0.96, 1.13	0.29	1.09	0.77, 1.54	0.64
Activated/mature CD8 + cells	0.10	–0.45, 0.64	0.73	0.34	–0.80, 1.48	0.56	0.97	0.85, 1.10	0.60	1.06	0.71, 1.57	0.79
Memory B cells	–0.15	–0.74, 0.45	0.63	–1.00	–2.29, 0.30	0.13	1.09	0.96, 1.23	0.21	1.16	0.81, 1.67	0.42

p-value threshold for the primary endpoints is 0.0125. Bolded cells are primary hypotheses. CI, Confidence Interval.

associated with greater decline in cognitive function over seven years if not accounting for multiple comparisons. This association was supported by suggestive associations of higher proportions of Treg with both worse cross-sectional cognitive function and higher risk of prevalent dementia. Supplementary Table 1 presents sensitivity analyses using complete case analysis for cross-sectional cognitive function (no missing data were imputed) and inverse probability weighting for change in cognitive function. These analyses were similarly null.

Additionally, no immune cell proportions were associated with prevalent dementia or time to death from dementia (Table 3). Supplementary Table 1 presents sensitivity analyses, using complete case analysis for both prevalent dementia and time to death from dementia.

We did not observe associations between immune cell subsets and any of the outcomes in analyses stratified by sex, when excluding participants with stroke prior to blood collection, or when evaluating prevalent AD specifically rather than all-cause dementia.

Exploratory analysis using principal components of the immune cell distributions did not identify significant associations.

DISCUSSION

In a large, population-based, longitudinal cohort of older adults with well-defined outcomes, we did not identify associations of peripheral immune cells with either cross-sectional or longitudinal cognitive outcomes after accounting for multiple comparisons. However, there were marginally significant associations of T_{reg} with worse cognitive decline in both imputed and weighted probability models when not accounting for multiple comparisons, and this association was supported by suggestive associations of T_{reg} with both worse cross-sectional cognitive function and prevalent dementia. Higher proportions of Treg may reflect ongoing mobilization in response to inflammation.

The immune cell subsets that we measured at a single timepoint in blood may not reflect features of the immune system most important for cognitive decline, or dynamic temporal intrapersonal variability in cell levels. Overall numbers, activity, or location of immune cells may better reflect pathology than immune cell proportions in peripheral blood. For example, all B and CD4+T cell count may be diminished in dementia, which may not be captured when evaluating proportions [13, 18]. T_{reg} and NK cells from patients with AD are reported to have altered function [14, 20, 21, 46]. Neurodegeneration may be driven by proinflammatory cytokines and chemokines produced by the immune cells [47]. For example, IL-1β, IL-6, and TNFα are thought to induce neuronal death [47]. Additionally, we may not have evaluated all relevant immune cell subphenotypes. For example, specific T_{reg} subtypes might be more associated with pathologies than proportions of T_{reg} overall [26]. Furthermore, cells may act in concert to affect cognitive decline and AD [48–50], and we have evaluated each cell type independently.

Peripheral immune cells may not reflect immune cells in the brain and cerebrospinal fluid, which may be more important for cognitive decline and dementia. The role of the immune system may vary by type of dementia [13]. NK cells may be diminished in vascular dementia, but not in AD, and the distribution of naïve and memory T cells may be altered only in AD [13]. The majority of dementia in CHS was AD, but ∼25% had vascular dementia and ∼10% had mixed dementia based on adjudicated diagnoses. Our dementia endpoint included all dementia subtypes and limiting our analysis to adjudicated AD did not affect our findings.

Other limitations of the study include large amounts of missing data and that participants with cognitive data are known to be healthier than those without cognitive data or who did not survive to the 1998–1999 exam. Survival and participation bias is especially likely to affect longitudinal analysis of cognitive decline, where participants experiencing greater cognitive decline were less likely to be re-examined in follow-up. We attempted to account for missing data through multiple imputation with chained equations and with probability weighting based on likelihood of participation in the follow up exam, but selection bias remains a concern. Nonetheless, sensitivity analyses were also null. Other sources of bias include that death from dementia is specific, but not sensitive, and we likely underestimated the number of participants who died with dementia. Immune cells were measured at only one time point, several years after cohort development. Participants must have survived and been healthy enough to participate in a blood draw during the 1998–1999 exam. These older participants may already have experienced changes in their immune system that could affect cognitive function and decline.

The role of the immune system in dementia is likely complex. While neuroinflammation is well

established with respect to AD, anti-inflammatory therapy for AD has had poor results [51]. Our findings that peripheral immune cells, measured as proportions, are not associated with cross-sectional global cognition, cognitive decline, prevalent dementia, or time to death with dementia may reflect the complexity of both the immune system and its role in AD and related dementias. Further studies are needed to clarify associations between T_{reg} and subsequent dementia and cognitive decline.

ACKNOWLEDGMENTS

This project was supported by grants HL120854 and HL135625 from the National Heart Lung and Blood Institute (NHLBI). The CHS cohort was sup ported by contracts HHSN268201200036C, HHSN 268200800007C, HHSN268201800001C, N01HC 55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, 75N 92021D00006, and grants U01HL080295 and U01HL130114 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 from the National Institute on Aging (NIA). Alison Fohner is supported by K01AG071689. A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/22-0091r1).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-220091.

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