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RESEARCH ARTICLE

Immune cell counts in cerebrospinal fluid predict cognitive function in aging and neurodegenerative disease

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Abstract

Introduction: Immune dysfunction is important in aging and neurodegeneration; lacking clinically available tools limits research translation. We tested associations of cerebral spinal fluid (CSF) monocyte-to-lymphocyte ratio (MLR)—innate immune activation surrogate—with cognition in an aging and dementia cohort, hypothesizing that elevated MLR is associated with poorer executive functioning.

Methods: CSF MLR was calculated in well-characterized, genotyped participants enrolled in studies of aging and dementia at University of California, San Francisco Memory and Aging Center (n = 199, mean age 57.5 years, SD 11.9). Linear models tested associations with episodic memory and executive function (verbal fluency, speeded set-shifting).

Results: Aging was associated with higher CSF monocyte, lower lymphocyte counts, and higher MLRs ($p < 0.001$). MLR was associated with verbal fluency ($p < 0.05$) only.

Discussion: Using clinical labs, we show an inverse association between CSF MLR and executive function in aging and dementia, supporting the utility of clinical labs in capturing associations between innate immune dysfunction and neurodegeneration.

KEYWORDS
aging, cerebrospinal fluid, cognition, executive function, inflammation, neurodegeneration

1 | BACKGROUND

An estimated 88 million Americans will be over age 65 by the year 2050 and one in 10 older Americans have dementia.[1] As we still lack understanding about basic pathomechanisms, predictive and prognostic biomarkers, and effective treatments for dementia, these figures highlight an impending public health crisis. One growing area of research is in the role of the immune system in brain aging and disease. Often, important discoveries made in research take too long to enter into the sphere of clinical care. For this study, we formulated questions based on recent research and sought to answer the questions using generalized, clinic-based laboratory tests, routinely obtained in clinical settings.

Emerging studies show that changes in the immune system are inherent to the pathophysiology of aging.[2–4] Increasingly, the immune system is implicated in the pathogenesis of neurodegenerative diseases,[2–7] and this involvement may be one of the ways aging predisposes the brain to neurodegeneration. In the blood, declines in serum lymphocyte numbers and changes in cytokine levels are seen with age.[8] Within the central nervous system (CNS), aging is associated with the development of a detrimental pro-inflammatory milieu[3] and changes in entry and exit of immune cell subtypes into the CNS.[9] Elevated serum inflammatory markers are used as indirect measures of immune activation or, depending on the context, dysfunction.[10–13] However, the lack of cellular specificity, chemical lability, and physiological variability in levels, discourages use of these secreted factors as clinical biomarkers of disease states. Immune cells, on the other hand, have the great advantage of specificity. High serum neutrophil-to-lymphocyte ratios have been reported in Alzheimer's disease (AD) and are associated with poor prognosis in amyotrophic lateral sclerosis (ALS).[11,14,15] Also, T cells isolated from cerebrospinal fluid (CSF) of individuals with AD may be implicated in AD pathologic processes.[16] CSF cells and inflammatory markers have also been associated with structural brain changes and cognitive decline in presymptomatic and early AD cases.[17] In Parkinson's disease (PD), elevated CSF lymphocyte count is associated with shorter survival.[18] Moreover, immune activation is linked to executive dysfunction.[19] Together, these support the role of immune dysregulation or activation in age-associated brain degenerative disease and cognitive dysfunction, however, most research has relied on expensive research assays not readily accessible in the clinic. The serum monocyte-to-lymphocyte ratio (MLR) is commonly used as a general measure of innate immune activation.[20] Therefore, in this study, we wanted to use the clinically available CSF MLR measure to test the hypothesis that innate immune activation, captured by this ratio, is associated with executive dysfunction in a well-characterized neurodegenerative cohort. We tested the relationship between CSF cell counts and cognition, as well as the moderating effect of disease-causing genetic mutations on this relationship.

By analyzing standard CSF cell counts as markers of immune system dysfunction, we take a first step toward translating findings from high accuracy, low availability research methods, to low sensitivity, generalizable clinical labs that all patients with CSF studies obtain in clinic. We hypothesize that higher MLR, representing innate immune activation, will be associated with worse cognition, with specificity for executive function.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors conducted a review of the literature using PubMed and available posters, presentations, and abstracts. While there is a growing interest in the role of inflammation in the initiation and propagation of neurodegeneration, clinical assays are limited. Moreover, knowledge about changes in cellular profiles in cerebrospinal fluid (CSF) with age, disease, and disease outcomes is lacking. The relevant articles have been appropriately cited.
2. **Interpretation**: We describe a large sample of CSF cell profiles in a diverse aging and neurodegenerative cohort, establish the utility of clinically available tests as proxies of inflammation, and we correlate CSF cell profiles with cognitive measures to begin to bridge the gap between research and clinic.
3. **Future directions**: Future directions include correlating CSF profiles directly with research assays of inflammation to further validate this approach. Additionally, we hope to correlate CSF profiles with patient outcomes in neurodegenerative disorders and relate this to underlying disease mechanisms.

2 | METHODS

2.1 | Participants

The sample for this study consisted of all research participants at the University of California, San Francisco, Memory and Aging Center enrolled in longitudinal natural history research studies of aging, AD, and frontotemporal dementia (FTD) with University of California, San Francisco (UCSF) Institutional Review Board approval and available clinical measures of interest. Data included in this analysis were collected from January 2016 to December 2018. All research participants who completed a clinical assessment, nursing assessment, and lumbar puncture during the study period were included in the analyses. Within the framework of parent studies, after written informed consent was obtained, participants underwent neurological, neuropsychological, and functional assessments including informant interviews as described elsewhere.[21] Participants underwent structural imaging with brain MRI, peripheral blood, and CSF collection. Clinical diagnosis was determined by a multidisciplinary consensus conference using published diagnostic criteria for behavioral variant frontotemporal dementia (bvFTD),[22] non-fluent/agrammatic variant of a primary progressive aphasia (nfvPPA),[23] semantic variant primary progressive aphasia (svPPA),[23] logopenic variant primary progressive aphasia (lvPPA),[23] corticobasal syndrome (CBS),[24] progressive supranuclear palsy (PSP),[25,26] mild cognitive impairment (MCI),[27] AD,[28] or was deemed clinically normal. All samples with complete clinical data and CSF specimens were included in this study. Available clinical data used in models include demographic information, detailed history, physical and neurological examination, comprehensive neuropsychological assessments, and functional rating scales described previously.[21] Additional data include genetic mutation status (carrier vs. non-carrier) for common disease-causing genes including *MAPT*, *GRN*, *C9orf72*, and *apolipoprotein E (APOE)* genotyping. All genetic testing was performed in the same laboratory at the University of California Los Angeles using standardized methods previously described.[21] Individual genetic

mutations were not included to protect patient privacy. When clinical data were conflicting or absent, a chart review was performed by a board-certified neurologist (A.S., F.E.) to determine the best clinical diagnosis. Participants were excluded if no CSF data were available or if clinical data were incomplete for variables needed for the statistical models testing primary hypotheses. If data from multiple time points existed, data from the closest time point to the CSF collection were used. For participants with multiple associated entries, any entries that were flagged by clinicians during data quality review were removed and subsequently, a single entry per participant for analysis using a selection criterion for the entry with (1) the highest Clinical Dementia Rating (CDR) Box Sum score and (2) the highest MLR value was selected for use in statistical models.

2.2 | CSF samples

Research participants underwent lumbar punctures as part of routine research protocols. Lumbar punctures were performed in the morning after an overnight fast with a 24-gauge Sprotte needle in the L3/4 or L4/5 space. Spinal fluid was collected in a polypropylene tube and analyzed at the UCSF Clinical Laboratories, Mission Bay Hematology. Analyses included appearance, glucose, protein, white and red blood cell counts, and percentage lymphocytes, monocytes, and neutrophils. Analyses were performed using a hemocytometer for cell count and Wright stained cytocentrifuge preparation for differential at the UCSF Clinical Laboratory. Cell differentials were performed on concentrated samples and expressed as 0×10^6/L. Unusual results were flagged and reviewed by a pathologist. CSF variables in this analysis included CSF monocyte and lymphocyte counts as well as the MLR, which was calculated by dividing absolute monocyte count by absolute lymphocyte count.

2.3 | Cognitive measures

For the purposes of this analysis, we focused on aspects of cognition that have demonstrated sensitivity to typical brain aging and AD and FTLD-related neurodegenerative disease, including processing speed, executive functioning, and episodic memory.[28–31] Primary outcomes of interest included measures of executive functioning via verbal fluency (number of D words, L words, and F words per 60 seconds) and speeded set-shifting performance (number of correct lines/second on the Modified Trail Making Test), as described in detail elsewhere.[31–33] The Short Form of the California Verbal Learning Test, Second Edition (CVLT-II), was used to assess episodic memory performance (number of words freely recalled after a 10-min delay).[34]

2.4 | Statistical analyses

To investigate the relationship between MLR and cognitive outcome measures, we fit linear models using the open-source programming language R1.[35,36] The models adjusted for genetic factors of disease including carrier status of disease-causing genetic mutations and *APOE* risk genotype, age, gender, education, and CDR Box Sum outcome as covariates. Specifically, we first fit models with the following formula to investigate possible interaction between MLR and each genetic factor of disease (Eq. 1):

$$Outcome = MLR * genetic\ factors\ of\ disease + Age + Gender + Education + CDR\_BoxSum \quad (1)$$

where the asterisk "*" in the equation represents modeling the main effects as well as the interaction between MLR and the genetic factor of disease. Data were prepared for modeling using tidyverse[37] for data frame manipulation. Linear models were fit using the "lm" function in the statspackage1. Type III analysis of variance (ANOVA) was performed on the fit linear models by the "Anova" function from the cars[36] package to observe the significance of interaction (i.e., between MLR and the genetic factor of disease) and main effect terms (i.e., MLR and genetic factor of disease). After determining that the interaction terms were insignificant across all models, we removed the interaction terms and treated the genetic factors of disease as a covariate to improve the power of our analysis (Eq. 2):

$$Outcome = MLR * genetic\ factors\ of\ disease + Age + Gender + Education + CDR\_BoxSum. \quad (2)$$

Type III ANOVA was performed on the new set of fit linear models for the significance of the relationship between MLR on the outcome

TABLE 1 Demographics

Participants	N = 199
Age, mean (SD) years	57.5 (11.9)
Sex, n	
Female	103
Male	96
Education, mean (SD) years	16.2 (2.5)
Race/ethnicity	89.4% White
Gene mutation carrier status, n	
APOE4	51
Genetic Carrier	58
Unknown	44
Clinical diagnosis, n	
Clinically normal	60
MCI	31
Alzheimer's disease[a]	46
FTD[b]	51
Other[c]	11
CDR Box Sum score, mean (SD)	2.8 (3.2)

[a]AD includes lvPPA (8) and PCA (7).
[b]FTD includes bvFTD (29), nfvPPA (9), svPPA (3), PSP (6), and CBS (4).
[c]Other includes TAND (6), ALS (3), PD (1), and CTE (1).

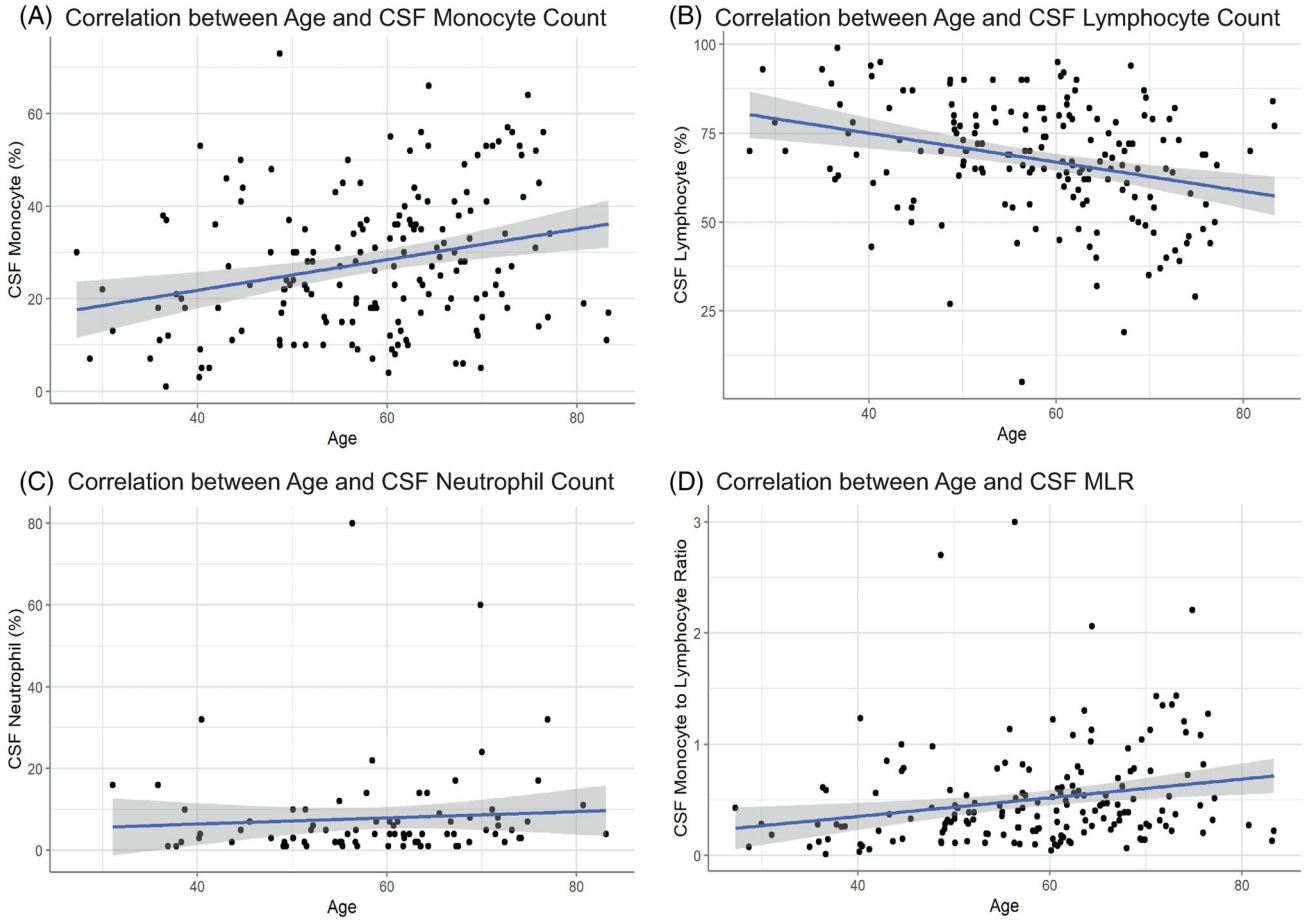

FIGURE 1 Correlation between age and cerebrospinal fluid (CSF) cell counts. Correlation between subject age and CSF cell populations. Older subjects exhibited (A) a positive correlation with CSF monocyte population (n = 173; $p < 0.001$) and (B) a negative correlation with CSF lymphocyte population (n = 173; $p < 0.0001$). (C) There was no significant correlation between subject age and CSF neutrophil population (n = 83). There was a positive correlation between age and the (D) CSF monocyte-to-lymphocyte ratio (n = 173; $p < 0.01$).

measures. Data visualization was performed utilizing the ggplot4R package.[38] Plots of the relationship between MLR and outcome measures show the line generated by the fitted linear models as generated with Eq. (2).

Statistical significance was defined as $p < 0.05$, and statistical trends were defined as $0.05 < p < 0.10$.

3 | RESULTS

3.1 | Demographics

The final analyses included a total of 199 participants. Participant characteristics are shown in Table 1. Mean age was 58 (SD 12, 27-83 years). Mean CDR Box Sum score was 3 (SD 3, 0-16) (Figure S1). The majority of the sample was composed of white older adults with high level of education (Table 1). Clinical diagnoses, described in Table 1, include: clinically normal; FTD, including bvFTD, CBS, PSP, nfvPPA, svPPA; AD, including lvPPA, and posterior cortical atrophy (PCA); MCI; and other, including PD, tuberous sclerosis complex associated neurocognitive disorder (TAND), ALS, and chronic traumatic encephalopathy (CTE).

Linear regression models including all participants found that CSF monocyte count was positively correlated with age ($p = 0.0005$; standardized $\beta_{age} = 0.26$; adjusted R2 = 0.064) (Figure 1A). Conversely, CSF lymphocyte count was negatively correlated with age ($p = 0.0001$; standardized $\beta_{age} = -0.30$; adjusted R2 = 0.084) (Figure 1B). CSF MLR was positively correlated with age ($p = 0.004$; standardized $\beta_{age} = 0.22$; adjusted R2 = 0.043) (Figure 1D). There was no significant correlation between CSF neutrophil count and age (Figure 1C) nor was there a relationship between CSF monocyte, lymphocyte, or neutrophil counts and sex (Figure 2). There was no effect of genetic status on CSF cell populations (Figure 3).

3.2 | Immune activation and cognition

To investigate the association of MLR to cognition, linear regression was performed while controlling for age, sex, education, clinical impairment (CDR Box Sum score), and *APOE* risk genotype or carrier status

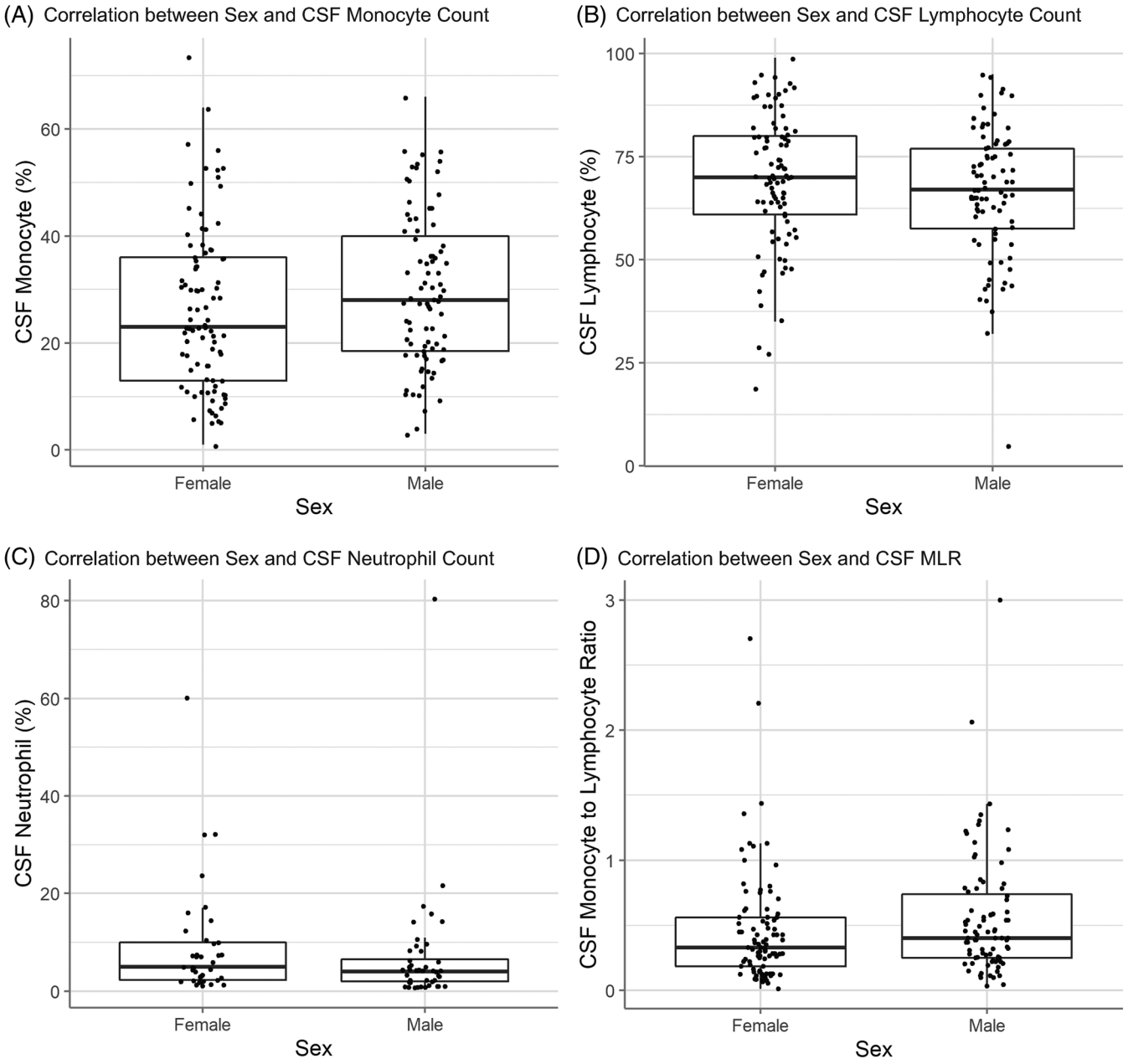

FIGURE 2 Correlation between sex and cerebrospinal fluid (CSF) cell counts. Correlation between sex and CSF cell populations. No significant correlation was observed between subject sex and (A) CSF monocyte population (n = 173), (B) CSF lymphocyte population (n = 173), or (C) CSF neutrophil population (n = 82). There was not a significant correlation between subject sex and the (D) CSF monocyte-to-lymphocyte ratio (n = 173).

as covariates. The analysis revealed that MLR was significantly associated with verbal fluency, for all letters tested (D, L, and F words), when controlling for either *APOE* risk genotype (D words $p = 0.04$; L words $p = 0.02$; F words $p = 0.05$) or carrier status (D words $p = 0.03$; L words $p = 0.01$; F words $p = 0.05$) (Figure 4). Mean verbal fluency scores were 11.25 (SD = 6.28), 11.69 (SD = 5.76), and 11.54 (SD = 5.47) for D words, F words, and L words, respectively (Figure 2). In contrast, similar linear models did not reveal any significant relationship between MLR and Modified Trail Making Test or episodic memory.

4 | DISCUSSION

Despite advances in understanding of the pathogenesis in neurodegenerative disorders, the field still lacks easily accessible diagnostic and predictive biomarkers. Inflammation and immune activation are implicated in brain aging across neurodegenerative diseases.[4,5,13,32,39–45] In this study, we leverage readily available, standard clinical laboratory data and use them as surrogate measures of immune activation to explore the association between immune activation and clinical impairment in aging, autosomal dominant genetic forms of FTLD

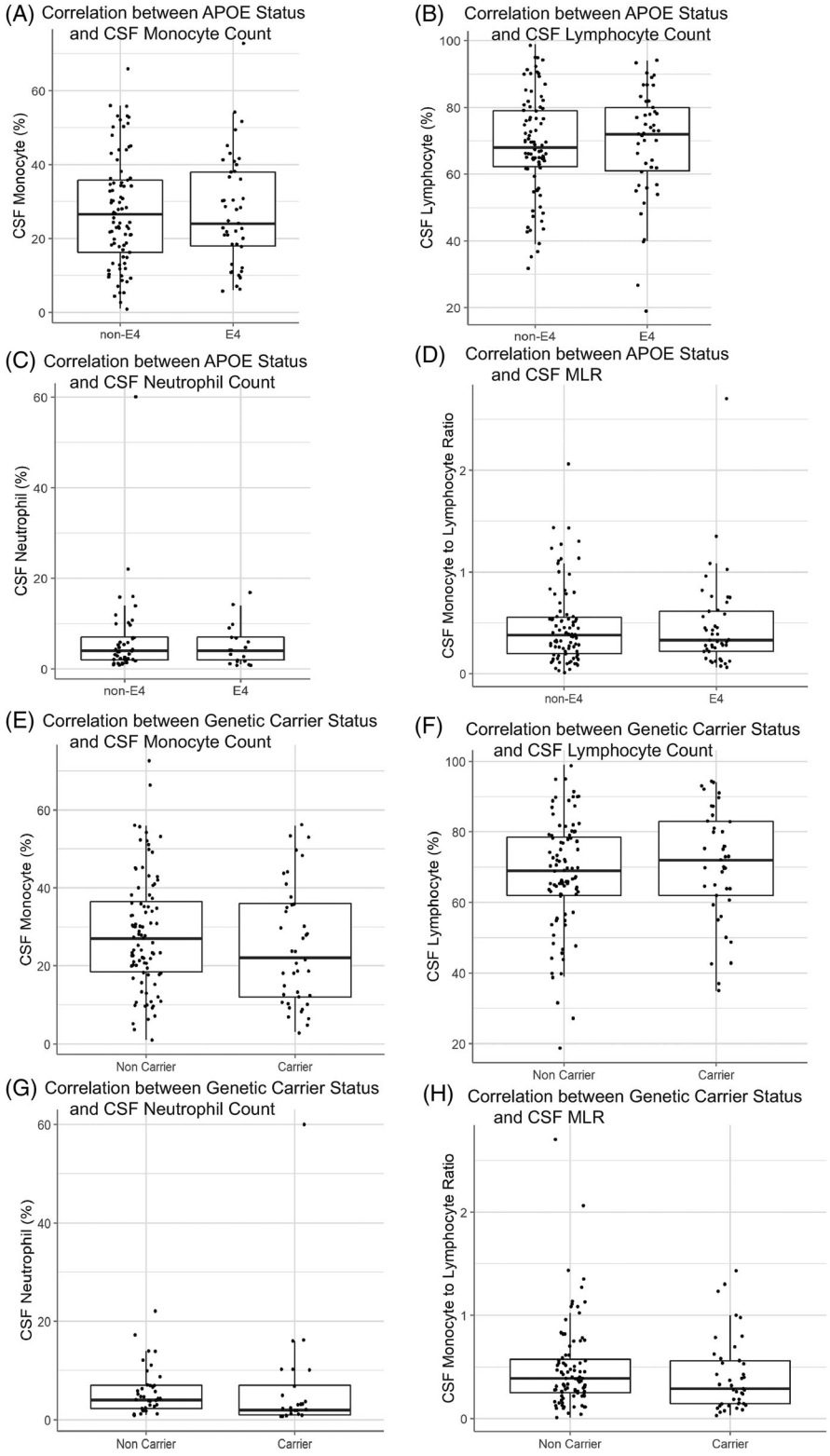

FIGURE 3 Correlation between genetic status and cerebrospinal fluid (CSF) cell counts. Correlation between *apolipoprotein E4 (APOE4)* genetic status and CSF cell populations (A-D). No significant correlation was observed between the *APOE4* genetic status and (A) CSF monocyte population (n = 131), (B) CSF lymphocyte population (n = 131), or (C) CSF neutrophil population (n = 62). There was not a significant correlation between the *APOE4* genetic status and the (D) CSF monocyte-to-lymphocyte ratio (n = 131). (E-H) Correlation between genetic status for monogenic disease genes (carrier vs. non-carrier) and CSF cell populations. No significant correlation was observed between the presence of monogenic disease mutations and (E) CSF monocyte population (n = 132), (F) CSF lymphocyte population (n = 132), or (G) CSF neutrophil population (n = 63). There was not a significant correlation between the presence of monogenic disease mutations and the (H) CSF monocyte-to-lymphocyte ratio (n = 132).

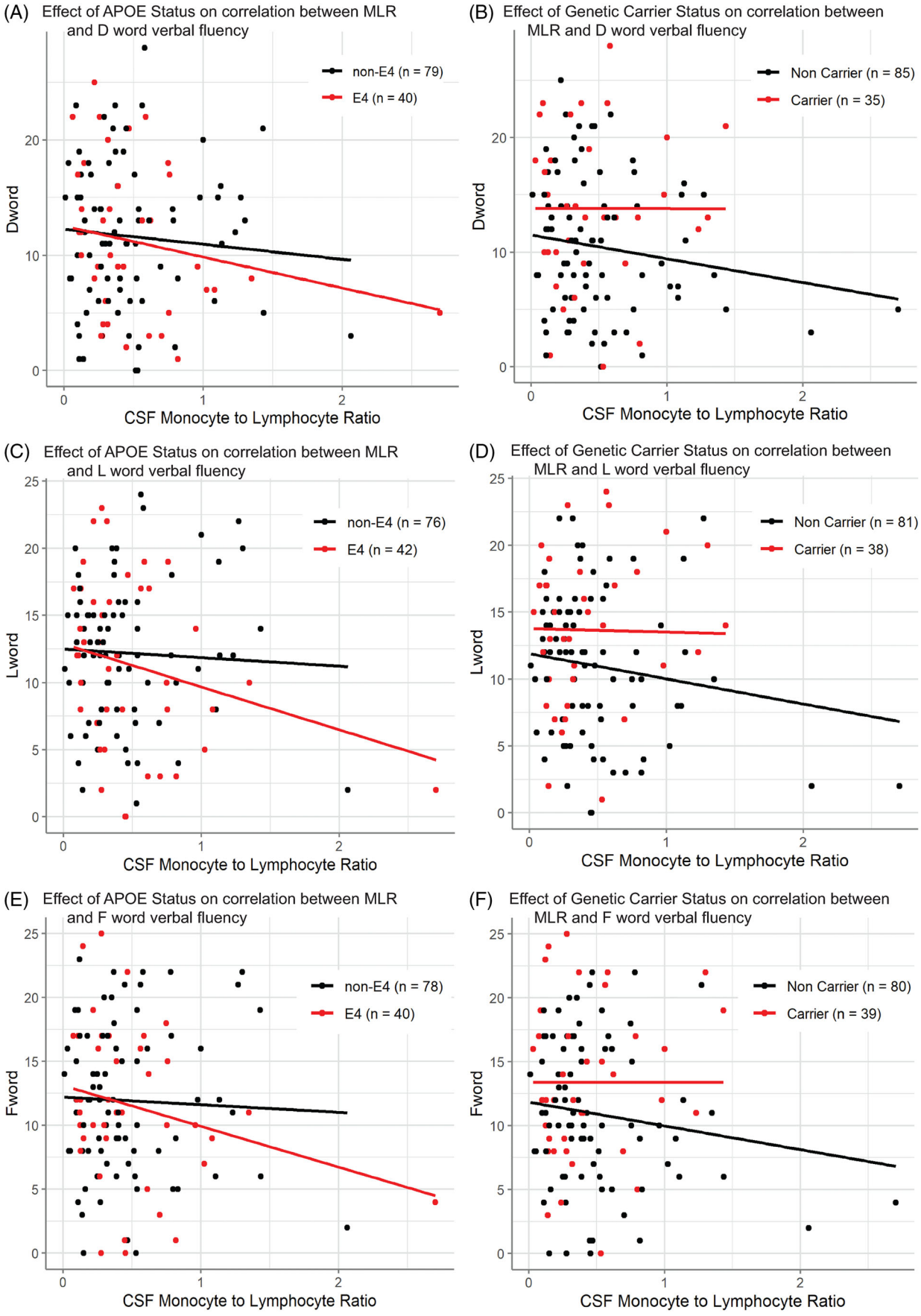

FIGURE 4 Correlation between cerebrospinal fluid (CSF) monocyte-to-lymphocyte ratio (MLR) and measures of executive function. (A,B) Correlation between CSF monocyte-to-lymphocyte ratio and performance on [D Word] cognitive test after controlling for age, sex, education, and

and sporadic AD, with and without *APOE4* genotype. We found that executive function—specifically verbal generativity performance—but not episodic memory, was negatively associated with the CSF MLR (Figure 5).

CSF pleocytosis has been observed in many different diseases including human immunodeficiency virus, multiple sclerosis, and PD.[12,14,18,46] These diseases may share certain cellular pathogenesis involving the immune system and may portend disease outcome.[12,18] Several studies have investigated serum monocyte or neutrophil-to-lymphocyte ratios as clues to the role of the immune system in cerebrovascular disease, AD, and ALS.[14,15,46] Direct assays of the CSF in neurodegenerative diseases provides a close albeit indirect look at intracerebral disease processes. While it is not possible to comment on causative, versus reactive, based on correlated models, CSF cell counts readily available in clinical labs, suggest that there is involvement of immune system across a diversity of neurodegenerative disease categories. More cells in the brain compartment, and higher counts in CSF could reflect higher entry, lower exit, or both.

Interestingly, drops in circulating lymphocytes in the blood and changes in the immune system composition is one of the hallmarks of aging.[8] Immunosenescence is an emerging mechanism invoked in the neurodegenerative disease process.[7] While this phenomenon has been well studied in the periphery, despite mounting evidence of the role of immune dysregulation in neurodegenerative diseases, this has not been well studied in humans, using CSF cells. We use CSF from a well-characterized sample of participants across the aging and neurodegenerative disease spectrum to demonstrate that even simple clinical labs, with limited sensitivity and coarse specificity, provide support for innate immune involvement in neurodegenerative disease. In the first study of its kind, our findings indicate that, similar to peripheral blood, CSF lymphocyte levels may decline with age.

Across two main cognitive domains investigated in this study—executive function and episodic memory—executive function, specifically verbal generativity, seems most sensitive to changes in the immune state within our mixed cohort. Consistent with this, mouse models show that inflammation has a selective impact on executive functions while sparing memory.[19] Additionally, this finding is supported by observations from other neurological diseases where executive dysfunction is prominent, including both primary autoimmune conditions such as multiple sclerosis and autoimmune encephalitis, as well as other neurodegenerative disorders such as vascular-related cognitive impairment.[6,10,12,47,48] Many studies have similarly found an association with peripheral markers of inflammation and executive function broadly as well as verbal fluency specifically, although none have reported this finding within the CSF.[49–53] Moreover, it has also been demonstrated that immune activation alters brain connectivity specifically in the executive network while sparing the default mode network associated with memory as well as the salience network associated with emotional regulation.[54,55] This finding may support network vulnerability and neuroanatomical correlations between areas of the executive network vasculature most exposed to peripheral influences. However, a retrospective study in a sample of convenience has limitations and potential sample biases that are not known and accounted for in analyses. Therefore, future prospective studies would be better suited to confirm and further explore test specificity of various cognitive domains with CNS immune dysfunction. Inclusion of imaging and more specific disease state biomarkers, such as A/T/N for AD[56] may help further clarify causal relationships between immune activation and neurodegeneration, at least in AD.

The most important limitation of this study is that we had to rely on a sample of convenience and cross-sectional data, limiting the interpretation of our findings in relation to disease course and causal inferences. Use of a sample of convenience also meant that our sample lacked diversity, with mainly whites from high socioeconomic environments. We hope to extend this study in the future, by including diverse populations, larger sample sizes, and incorporating measures of vascular and brain barrier function, A/T/N biomarkers, and longitudinal assessments to begin to differentiate causal from reactive immune responses in neurodegenerative diseases and the effect of genetic mutations on this relationship.

CDR Box Sum score as covariates. Additionally, the effect of (A) *apolipoprotein E4 (APOE4)* (n = 119) or (B) monogenic disease carrier (n = 120) statuses were also included (red: genetic mutation; black: no genetic mutation). Importantly, neither *APOE4* nor monogenic disease carrier status had a significant interaction with CSF monocyte-to-lymphocyte ratio on [DWord] performance. The plotted lines show the best fit for the corresponding model's prediction of each subject. There was a significant negative correlation between CSF monocyte-to-lymphocyte ratio and [DWord] performance when *APOE4* status is included as a covariate ($p < 0.05$) and when monogenic disease carrier status was included as a covariate ($p < 0.05$). (C,D) Correlation between CSF monocyte-to-lymphocyte ratio and performance on [LWord] cognitive test after controlling for age, sex, education, and CDR Box Sum score as covariates. Additionally, the effect of (C) *APOE4* (n = 118) or (D) monogenic disease carrier (n = 119) statuses were also included (red: genetic mutation; black: no genetic mutation). Importantly, neither *APOE4* nor monogenic disease carrier status had a significant interaction with CSF monocyte-to-lymphocyte ratio on [LWord] performance. The plotted lines show the best fit for the corresponding model's prediction of each subject. There was a significant negative correlation between CSF monocyte-to-lymphocyte ratio and [LWord] performance when *APOE4* status is included as a covariate ($p < 0.05$) and when monogenic disease carrier status was included as a covariate ($p < 0.05$). (E,F) Correlation between CSF monocyte-to-lymphocyte ratio and performance on [FWord] cognitive test after controlling for age, sex, education, and CDR Box Sum score as covariates. Additionally, the effect of (E) *APOE4* (n = 118) or (F) monogenic disease carrier (n = 119) statuses were also included (red: genetic mutation; black: no genetic mutation). Importantly, neither *APOE4* nor monogenic disease carrier status had a significant interaction with CSF monocyte-to-lymphocyte ratio on [FWord] performance. The plotted lines show the best fit for the corresponding model's prediction of each subject. There was a significant negative correlation between CSF monocyte-to-lymphocyte ratio and [FWord] performance when *APOE4* status is included as a covariate ($p < 0.05$) and when monogenic disease carrier status was included as a covariate ($p < 0.05$).

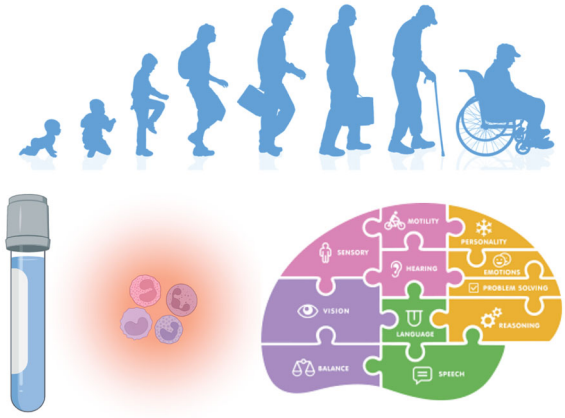

FIGURE 5 Graphical summary. Immune cell counts in cerebrospinal fluid (CSF) correlate with measures of executive function in aging and neurodegeneration.

The field lacks clinically reliable biomarkers for immune activation in neurodegeneration. Here we use clinical labs to test the association of innate immune dysfunction with cognitive impairment in aging, neurodegenerative disease, FTLD, and AD. We describe age-related differences in cell counts, which fit with previously described peripheral blood changes.[8] We also provide data showing a unique cognitive profile of executive dysfunction—specifically impaired phonemic fluency—associated with immune system activation in both genetic and sporadic forms of neurodegenerative diseases. This suggests the possibility of common cellular immune pathways in brain degeneration. In light of the phenotypic diversity of immune cells across coarse categorical buckets, such as mononuclear cells, lymphocytes, and neutrophils, future investigations will need more in-depth characterization of cells. This study provides support for the value of investigating CNS immune cells across neurodegenerative disease categories and linking back findings to labs that can be implemented in clinic and translated to improving clinical classification, prognostications, and ultimately therapeutics.

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CONFLICT OF INTEREST

This article was partially prepared while Dr. Snyder was employed at UCSF. The opinions expressed in this article are the authors' own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States Government. Drs. Snyder, Chou, and Lindbergh and Ms. Grant have no relevant disclosures. Dr. Elahi serves as associate editor for the Alzheimer's & Dementia Journal. Dr. Kramer has received royalties from Pearson, Inc. in addition to the grants to the institution listed above. Dr. Miller is the recipient of grants/contracts P30AG062422, P01AG019724, R01AG057234 from the National Institutes of Health/NIA. As an additional disclosure, he serves as Medical Director for the John Douglas French Foundation; Scientific Director for the Tau Consortium; Director/Medical Advisory Board of the Larry L. Hillblom Foundation; Scientific Advisory Board Member for the National Institute for Health Research Cambridge Biomedical Research Centre and its subunit, the Biomedical Research Unit in Dementia (UK) and Board Member for the American Brain Foundation (ABF). Author disclosures are available in the supporting information.

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