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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.

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METHODS: CSF MLR was calculated in well-characterized, genotyped participants enrolled in studies of aging and dementia at UCSF 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

Inflammation; Cognition; Executive Function; Cerebrospinal Fluid; Aging; Neurodegeneration

1. Background

An estimated 88 million Americans will be over age 65 by the year 2050 and one in ten older Americans have dementia¹. 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 aging²⁻⁴. Increasingly, the immune system is implicated in the pathogenesis of neurodegenerative diseases²⁻⁷, 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 age⁸. Within the central nervous system, aging is associated with the development of a detrimental pro-inflammatory milieu³ and changes in entry and exit of immune cell subtypes into the CNS⁹. Elevated serum inflammatory markers are used as indirect measures of immune activation or, depending on context, dysfunction¹⁰⁻¹³. 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 CSF of individuals with AD may be implicated in AD pathologic processes¹⁶. CSF cells and inflammatory markers have also been associated with structural brain changes and cognitive decline in presymptomatic and early AD cases¹⁷. In Parkinson disease, elevated CSF lymphocyte count is associated with shorter survival¹⁸. Moreover, immune activation is linked to executive dysfunction¹⁹. 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 is commonly used as a general measure of innate immune activation²⁰. 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 cerebrospinal fluid 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.

2. Methods:

2.1 Participants:

The sample for this study consisted of all research participants enrolled in longitudinal natural history research studies at the University of California, San Francisco, Memory and Aging Center, enrolled in studies of aging, Alzheimer's disease (AD), and frontotemporal dementia (FTD) with UCSF IRB approval and available clinical measures of interest included in analyses. 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 elsewhere²¹. Participants underwent structural imaging with brain MRI, peripheral blood, and cerebrospinal fluid collection. Clinical diagnosis was determined by multidisciplinary consensus conference using published diagnostic criteria for behavioral variant frontotemporal dementia (bvFTD)²², non-fluent/agrammatic variant of 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, Alzheimer's disease (AD)²⁸, or were deemed clinically normal. All samples with complete clinical data and cerebrospinal fluid 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 previously²¹. Additional data includes genetic mutation status (carrier vs. non-carrier) for common disease-causing genes including *MAPT*, *GRN*, *C9orf72*, and *APOE* genotyping. All genetic testing was performed in the same laboratory at the University of California Los Angeles using standardized methods previously described²¹. Individual genetic mutations are not included to protect patient privacy. When clinical data were conflicting or absent, chart review was performed by a board-certified neurologist (AS, FE) to determine best clinical diagnosis. Participants were

excluded if no CSF data was available or if clinical data was 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 was used. For participants with multiple associated entries, any entries that were flagged by clinicians during data quality review were removed. Subsequently a single entry per participant for analysis using a selection criterion for the entry with (1) the highest CDR box sum score and (2) highest MLR value were selected for use in statistical models.

2.2 Cerebrospinal fluid samples:

Research participants underwent lumbar puncture 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 $0 \times 10^6/L$. 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 FTL-related neurodegenerative disease, including processing speed, executive functioning, and episodic memory²⁸⁻³¹. 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 elsewhere³¹⁻³³. 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-minute 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 R^{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 (Equation 1):

$$\text{Outcome} = \text{MLR} * \text{genetic factors of disease} + \text{Age} + \text{Gender} + \text{Education} + \text{CDR_BoxSum} \quad (\text{Eq 1})$$

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 was prepared for modeling using tidyverse³⁷ 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 cars³⁶ 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 (Equation 2):

$$\text{Outcome} = \text{MLR} + \text{genetic factors of disease} + \text{Age} + \text{Gender} + \text{Education} + \text{CDR_BoxSum} \quad (\text{Eq 2})$$

Type III ANOVA was performed on the new set of fit linear models for the significance of the relationship between MLR on the outcome measures. Data visualization was performed utilizing the ggplot4R package³⁸. Plots of the relationship between MLR and outcome measures show the line generated by the fitted linear models as generated with Equation 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 sum of boxes was 3 (SD 3, 0–16) (Supp. Fig.1). 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; frontotemporal dementia (FTD), including behavioral variant FTD (bvFTD), corticobasal syndrome (CBS), progressive supranuclear palsy (PSP), nonfluent variant primary progressive aphasia (nfvPPA), semantic variant primary progressive aphasia (svPPA); Alzheimer’s disease, including logopenic variant primary progressive aphasia (lvPPA), and posterior cortical atrophy (PCA); mild cognitive impairment (MCI); and other, including Parkinson disease (PD), tuberous sclerosis complex associated neurocognitive disorder (TAND), amyotrophic lateral sclerosis (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 $\beta_{\text{age}} = 0.26$; adjusted $R^2 = 0.064$) (Fig 1A). Conversely, CSF lymphocyte count was negatively correlated with age ($p=0.0001$; standardized $\beta_{\text{age}} = -0.30$; adjusted $R^2 = 0.084$) (Fig 1B). CSF MLR was positively correlated with age ($p=0.004$; standardized $\beta_{\text{age}} = 0.22$; adjusted $R^2 = 0.043$) (Fig 1D). There was no significant correlation between CSF neutrophil count and age (Fig 1C) nor was there a relationship between CSF monocyte, lymphocyte, or neutrophil counts and sex (Fig 2). There was no effect of genetic status on CSF cell populations (Fig 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 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 $p=0.05$) (Fig 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 (Supp. Fig. 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 and sporadic Alzheimer's disease, 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 (Fig 5).

Cerebrospinal fluid pleocytosis has been observed in many different diseases including human immunodeficiency virus, multiple sclerosis, and Parkinson disease^{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, Alzheimer's disease and ALS^{14,15,46}. Direct assays of the CSF in neurodegenerative diseases provides an indirect, close 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 aging⁸. Immunosenescence is an emerging mechanism invoked in the neurodegenerative disease process⁷. 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 cerebrospinal fluid 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 memory⁴⁷. 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^{6,10,12,48,49}. Many studies have similarly found an association with peripheral markers of inflammation and executive function broadly as well as verbal fluency specifically, though none have reported this finding within the CSF^{50–54}. 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^{55,56}. 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 AD⁵⁷ 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.

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, FTLN, and AD. We describe age-related differences in cell counts, which fit with previously described peripheral blood changes⁸. 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. Although future investigations need higher depth in characterization of cells, in light of the phenotypic diversity of immune cells across coarse categorical buckets, such as mononuclear cells, lymphocytes, and neutrophils. This study provide 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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 the neurodegeneration, clinical assays are limited. Moreover, knowledge about changes in cellular profiles in 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.

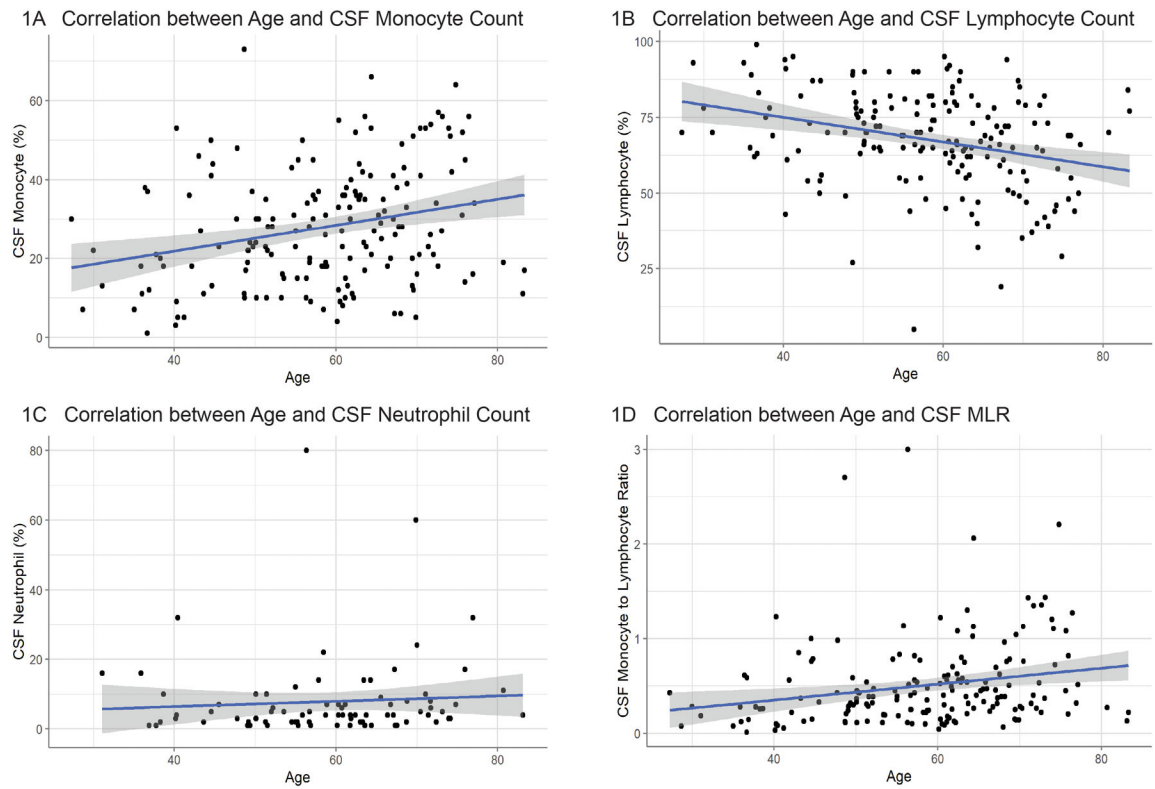


Figure 1: Correlation between Age and CSF Cell Counts

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; $p < 0.01$).

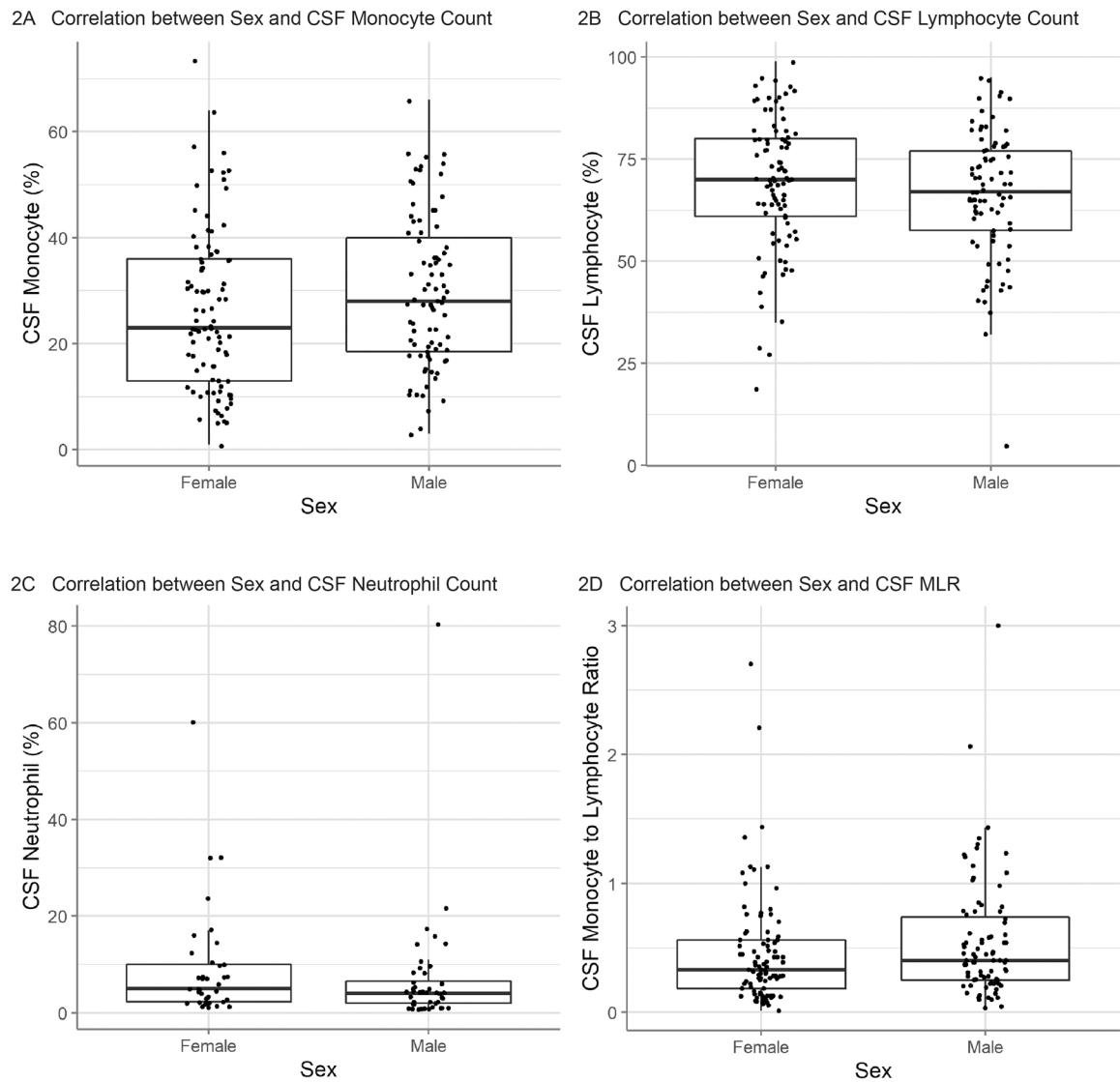


Figure 2: Correlation between Sex and CSF Cell Counts

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).

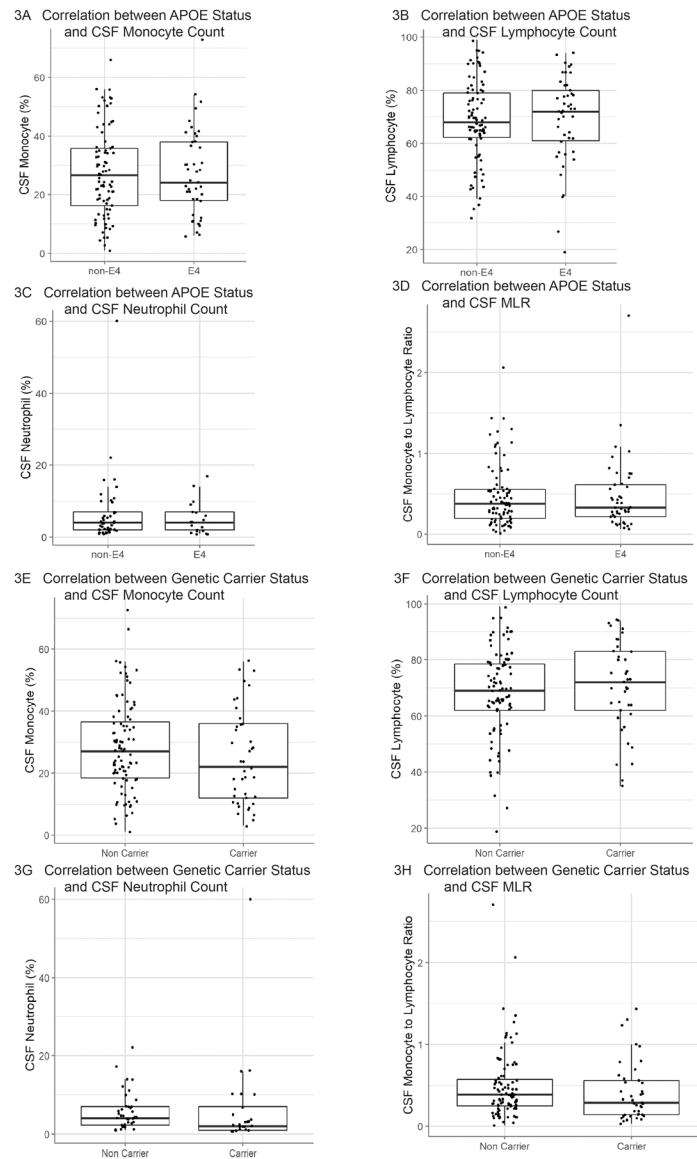


Figure 3: Correlation between Genetic Status and CSF Cell Counts

Correlation between 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).

Correlation between genetic status for monogenic disease genes (carrier vs non-carrier) and CSF cell populations (E-H). 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).

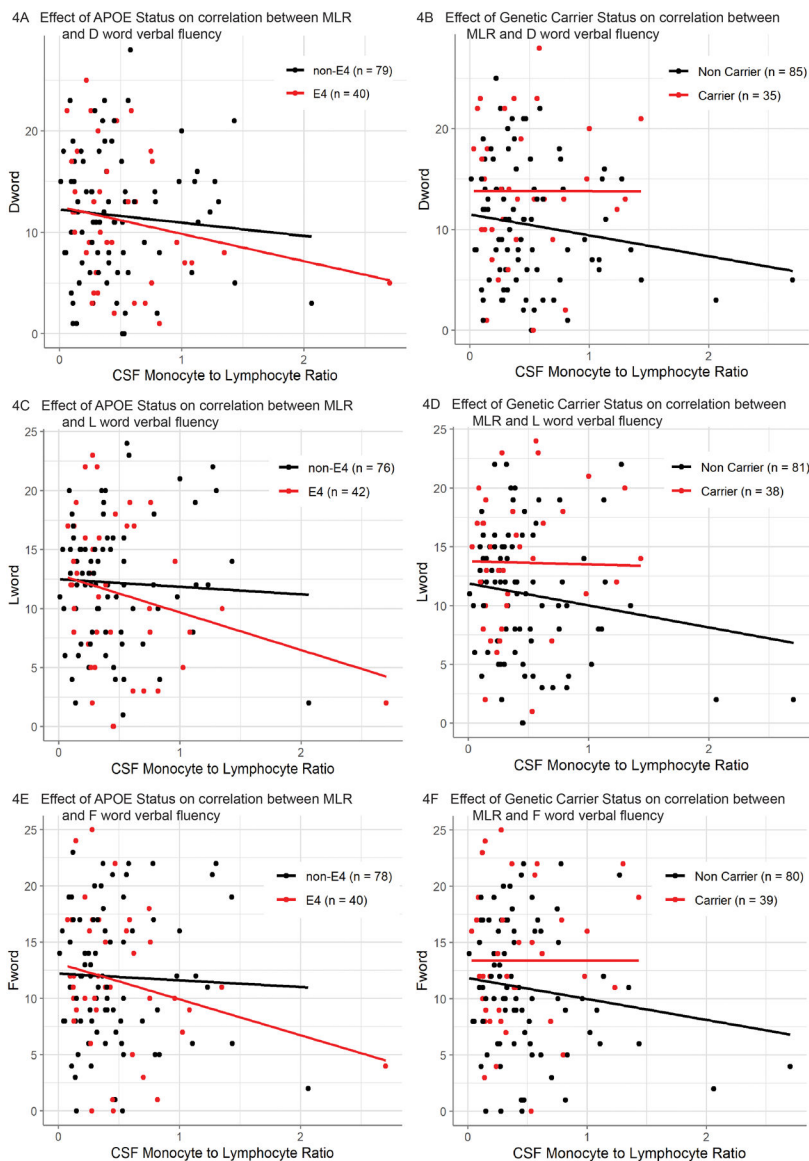


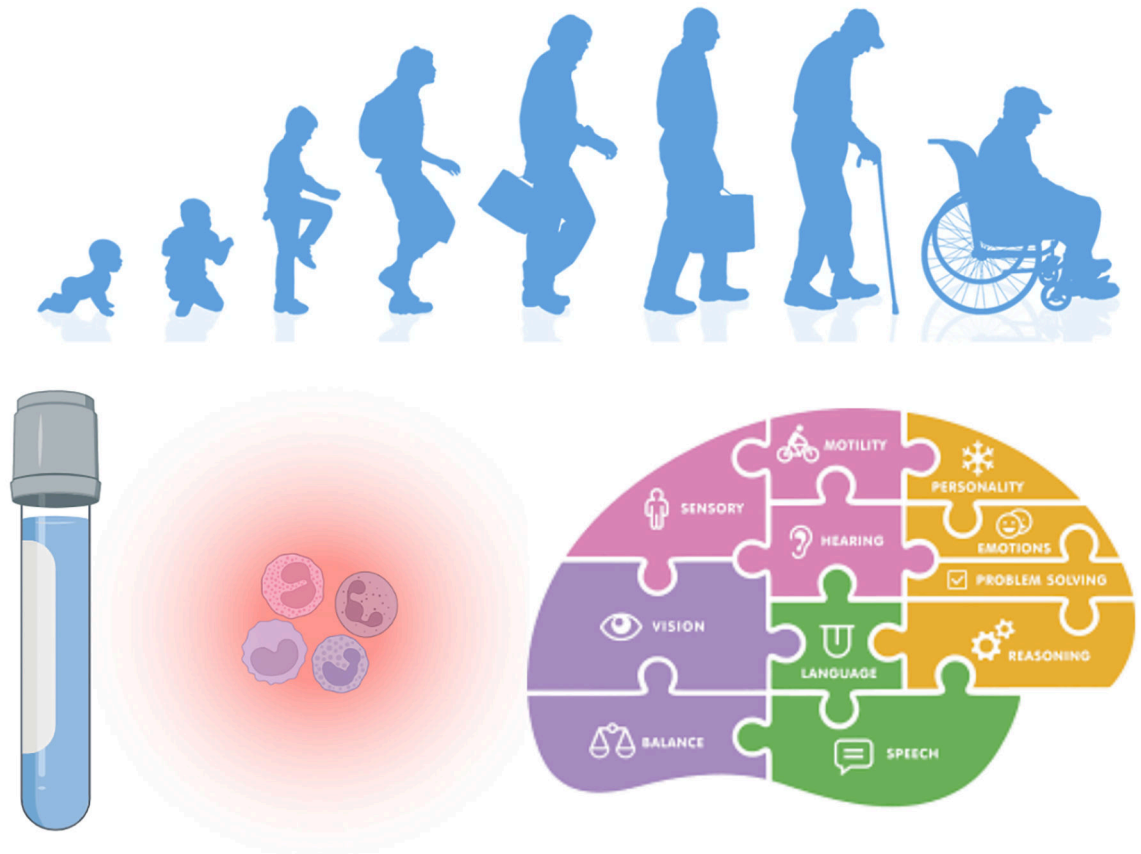
Figure 4: Correlation between CSF MLR and Measures of Executive Function

Fig [4A-B]. Correlation between CSF monocyte-to-lymphocyte ratio and performance on [D Word] cognitive test after controlling for age, sex, education, and CDR Box Sum score as covariates. Additionally, the effect of (A) 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 [D Word] 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 [D Word] performance when ApoE4 status is included as a covariate ($p < 0.05$) and when monogenic disease carrier status was included as a covariate ($p < 0.05$).

Fig [4C-D]. Correlation between CSF monocyte-to-lymphocyte ratio and performance on [L Word] 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 [L Word] 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 [L Word] performance when ApoE4 status is included as a covariate ($p < 0.05$) and when monogenic disease carrier status was included as a covariate ($p < 0.05$).

Fig [4E-F]. Correlation between CSF monocyte-to-lymphocyte ratio and performance on [F Word] 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 [F Word] 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 [F Word] performance when ApoE4 status is included as a covariate ($p < 0.05$) and when monogenic disease carrier status was included as a covariate ($p < 0.05$).



Simple CSF Measures of Immune Dysfunction Correlate with Executive Function Across Aging and Neurodegeneration

Figure 5: Graphical Summary

Immune cell counts in CSF correlate with measures of executive function in aging and neurodegeneration.

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	APO E4	51
	Genetic Carrier	58
	Unknown	44
Clinical Diagnosis, n	Clinically Normal	60
	MCI	31
	Alzheimer's Disease *	46
	FTD †	51
	Other ‡	11
CDR Sum of Boxes, mean (SD)	2.8 (3.2)	

* AD includes lvPPA (8) and PCA (7)

† FTD includes bvFTD (29), nvPPA (9), svPPA (3), PSP (6) and CBS (4)

‡ Other includes TAND (6), ALS (3), PD (1) and CTE (1)