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Association of a multiplex immune marker panel with incident cognitive impairment and dementia: The Northern Manhattan Study

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ABSTRACT

Objective: To determine whether a panel of immune markers adds significant information to known correlates of risk of dementia and cognitive impairment.
Background: The impact of immune mechanisms on dementia risk is incompletely characterized.
Design/methods: A subsample of the Northern Manhattan Study, a prospective cohort study in the racially/ethnically diverse population of New York City, underwent comprehensive neuropsychological testing up to three times, at approximately 5-year intervals. Cognitive outcomes were adjudicated as no cognitive impairment, mild cognitive impairment (MCI), or dementia. Immune markers were assessed using a multiplex immunoassay on plasma samples collected at the time of the first neuropsychological test. Least absolute shrinkage and selection operator (LASSO) techniques were employed to yield a panel of immune markers linearly related to the outcome of dementia/MCI vs. no cognitive impairment. Nested logistic regression models were run to determine the independent association of the immune marker panel with dementia/MCI after adjusting for other predictors of risk.
Results: Among 1179 participants (mean age 70.0 ± 8.9 years, 60% women, 68% Hispanic), immune markers improved model fit above demographic and vascular risk factors (p-value for likelihood ratio test <0.0001) as correlates of MCI/dementia. Individual immune markers found to be associated with dementia/MCI were C-X-C Motif Chemokine Ligand 9 (CXCL9) and C-C Motif Chemokine Ligand 2 (CCL2). The effect of the immune markers was comparable to traditional risk factors, with CCL2 (per SD) having almost the same effect as 1 year of aging and CXCL9 (per SD) showing approximately twice this magnitude.
Conclusion: Immune markers are associated with cognitive decline and dementia outcomes in a multi-ethnic cohort. More work is needed to further characterize these associations and determine therapeutic strategies. (Funded by the National Institute of Health/National Institute of Neurological Disorders and Stroke; grant number R01 29993 (Sacco/Elkind)).

1. Introduction

In response to insult, injury or microbial exposures, the brain mounts a response of innate and adaptive immune elements, known as neuroinflammation (Milatovic et al., 2017; Ransohoff et al., 2015). This response is characterized by glial activation and production of immune molecules. Accompanying this is the increased presence of pro-inflammatory cytokines and chemokines which have been shown to play critical roles in many neurodegenerative diseases, most prominently Alzheimer's dementia (AD), but including other dementia forms as well. Notably, as previous studies have shown, the role of peripheral innate immune mechanisms in the development of all-cause dementia (ACD), in particular AD and vascular dementia (VD), is considerable. As elaborated upon by Zhang et al., increased presence of peripheral innate

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immunity in a large UK prospective cohort, measured in the form of certain indices such as neutrophil-lymphocyte ratio, was associated with an increased risk of all-cause dementia, AD, and VD. Conversely, the potentially alleviative role played by adaptive immunity here, measured in the form of lymphocyte count and lymphocyte-monocyte ratio, on these clinical entities was demonstrated by an inversely proportional relationship between the described markers and ACD, VD. Of note, age-stratified analyses in this study revealed a more prominent role for innate immunity in younger age groups and that of adaptive immunity in older age groups, suggesting a dynamic relationship between peripheral immunity and dementia entities (Zhang et al., 2022). The specific immunological pathways evoking such pathology have yet to be fully elucidated, however. These pathways, moreover, may contribute to response to injury and to further damage.

We hypothesized that plasma levels of 60 immune markers may positively relate to later neurocognitive outcomes and associate with mild cognitive impairment (MCI) and/or dementia and provide further insight into the contribution of neuroinflammation. This hypothesis was explored in a population-based cohort study, the Northern Manhattan Study (NOMAS), a multiethnic urban sample of individuals living in New York City. Of note, dementia as a broader entity was chosen as the endpoint for this study as a clinically relevant diagnosis.

2. Methods

2.1. Study population

The study population consisted of a subcohort of the NOMAS sample. NOMAS is a racially/ethnically diverse prospective cohort with 3298 stroke-free participants enrolled between 1993 and 2001. Participants were identified by random-digit dialing and those eligible for inclusion were individuals who had been residing in northern Manhattan for $\geq$3 months in a household with a telephone, no prior stroke history, and age $\geq$40 years at the time of enrollment. From this initial sample, we recruited 1091 participants and 199 unrelated household members (total sample 1290) for a total of 1290 stroke-free participants recruited to undergo brain MRI and neuropsychological (NP) testing from 2003 to 2008. Exclusion criteria included cognitive impairment precluding informed consent, age <50 years, and an unwillingness or inability to undergo MRI. Detailed neuropsychological assessments were conducted, and blood samples were collected; methods of recruitment and characteristics of this sample have been described previously (Wright et al., 2009). The study was approved by the Columbia University and University of Miami Institutional Review Boards, and all participants provided informed consent. The study was funded by the National Institute of Health/National Institute of Neurological Disorders and Stroke; grant number R01 29993 (Sacco/Elkind).

2.2. Baseline evaluation

Both information on demographic variables and a comprehensive evaluation of risk factors were a part of the study enrollment process, assessed through in-person interviews by trained bilingual staff, as described previously (Wright et al., 2009). More specifically, these are predictors that have been previously demonstrated to be associated with cognitive impairment in other studies and particularly in prior studies in the NOMAS cohort. Historical predictors were adapted from the BRFSS questionnaire used by the CDC. Race-ethnicity was determined by self-identification (White non-Hispanic, Black non-Hispanic, Hispanic). Insurance status was defined as no insurance/Medicaid versus private insurance/Medicare. Educational achievement in terms of years of schooling and degree achieved were self-reported. Physical activity was defined dichotomously, and smoking was categorized as current (within the past year), former, or never smoker of cigarettes, cigars, or pipes. Hypertension and diabetes were assessed using standardized questionnaires adapted from the Behavioral Risk Factor Surveillance System (Centers and For Disease Control and Prevention, 1993). Hypertension was defined either as participant self-report of hypertension, blood pressure measurement of 140/90 mm Hg or greater, or use of anti-hypertensive medication. Diabetes was defined either as participant self-report of diabetes, fasting glucose of 126 mg/dL or greater, or use of insulin or oral anti-diabetic medications.

2.3. Cognitive and functional assessment

Participants in the NOMAS MRI sub-study were seen at three different timepoints for assessment of cognitive status and function. Visit 1 was at the time of initial recruitment at the time of MRI, visit 2 occurred at a mean of 5.0 $\pm$ 0.6 years post MRI, and visit 3 occurred at a mean of 7.0 $\pm$ 1.2 years after visit 2.

Cognitive status determination for visit 2 has been described previously (Wright et al., 2021). In the same manner, we adjudicated outcomes of dementia and mild cognitive impairment for visits 1 and 3. Briefly, an algorithm segregated participants based on the results of the NP battery into those requiring adjudication and those rated as cognitively normal, using criteria described previously (Wright et al., 2021). Those requiring adjudication were evaluated by a team of neuropsychologists and neurologists with dementia expertise, using all available information from participant visits and interviews.

The data available included neuropsychological tests, participant's self-reported clinical dementia rating at visit 1, Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) at visit 2, and Functional Activities Questionnaire (FAQ) at visit 3 (by an informant), and depression diagnoses derived from self-reported depression, use of medications to treat depression or CES-D score >15.

Among participants who did not come back for visit 2 or 3, we reviewed self-reported diagnoses of dementia or cognitive problems by an informant, use of medications to treat dementia, or cognitive performance and diagnoses derived from the annual telephone interview or obtained with the Telephone Interview for Cognitive Status (TICS) questionnaire applied annually during follow up. Lastly, we reviewed medical records at our hospital where >60% of our population is followed, or external records from primary care providers or other hospitals obtained as part of adjudication of vascular events, for any mention of dementia.

2.4. Multiplex immunoassay assessment

Assays of 60 immune molecules were obtained in 1179 participants from stored fasting plasma drawn at the time of the cognitive assessment using a customized, highly multiplexed, magnetic bead-based immunoassay, as previously described (Hornig et al., 2015, 2016; Che et al., 2022; Elkind et al., 2021). Plasma samples to be assayed for immune marker measurements were collected on the same day as neuropsychological testing for 87% of the cohort (mean [$\pm$SD] distribution of interval between blood collection and neuropsychological testing, 2.9 min $\pm$ 96 min). Briefly, the assay included molecules prominent in immune and inflammatory response profiles and other molecules critical to intercellular and endocrine communication. Plasma samples were coded, randomized, and assayed in duplicate, and median fluorescence intensities (MFI) measurements were acquired. Pre-determined quality control standards (serial cytokine standards, bead counts, intra- and inter-assay coefficients of variation) were met within and across assay plates, as done previously (Elkind et al., 2021). Averaged MFI values for each of the 60 cytokines were used in final analyses rather than absolute concentrations to minimize biases associated with censoring of data outside of the serial standard curves (Breen et al., 2015, 2016).

Immune markers were first log base 2 transformed ($\log_2$) to reduce skewness and then standardized before analyses to minimize potential biases relating to differences in the scaling of variables and to enhance comparability. Additionally, as these markers are often highly correlated within individuals, all possible two-way interactions (n = 1770)

were also analyzed. Data reduction was performed using Least Absolute Shrinkage and Selection Operator (LASSO) technique, yielding a subset of immune markers and interactions linearly related to the outcome.

2.5. Statistical analyses

Distributions of baseline demographic characteristics and vascular risk factors in the study cohort were reported using descriptive statistics, using mean and standard deviation for continuous variables and proportions and frequencies for categorical variables. The cognitive outcome was dementia or MCI, and normal cognition was considered the reference category. We first performed LASSO procedures to select a subset of immune markers linearly predicting cognitive outcome among individual immune markers and two-way interactions, using 5-fold cross validation based on the sum of squared error criterion. Then, to examine the effect that LASSO-selected markers, as a whole, display with the cognitive outcome in addition to traditional predictors of cognition (age, sex, education, race/ethnicity, socioeconomic status and traditional vascular risk factors), a series of nested logistic regression models was examined. Namely, Model 1 consisted of traditional risk factors only; Model 2 consisted of traditional risk factors and LASSO-selected immune markers (i.e., the full model); Model 3 consisted of the immune markers only; and Model 4 consisted of age only. Comparisons of fit across nested models were assessed using Likelihood Ratio Tests (LRTs), with p-values <0.05 indicating statistical significance for improvement of fit. Goodness-of-fit was also assessed using R-squared (R^2) to estimate the improvement of variance in cognitive outcome explained by the addition of immune markers to the model. Finally, sensitivity analyses were performed using conditional logistic regression models to assess for consistency of results after accounting for different individual follow-up times. All analyses were performed using SAS version 9.4 (SAS Institute Inc. Cary, NC) and R version 3.4.

3. Results

3.1. Description of cohort

There were 1179 participants for whom immune marker data was available (Table 1). Mean age at visit 1 was 70.0 ± 8.9 years, 60% were women and 67% were Hispanic. The mean education level was 10 ± 5 years. The burden of diabetes was 28%; several other cardiovascular risk factors were present as well.

Of the 1179 individuals with immune marker data, longitudinal cognitive diagnoses were available in 1177 participants, as follows.

- Visit 1: 79 diagnosed with dementia;
- Visit 2: 44 diagnosed with dementia;
- Visit 3: 36 diagnosed with dementia.

Overall, 768 (65.3%) remained cognitively normal, 250 (21.2%) developed MCI, and 159 (13.5%) developed dementia; the mean follow-up time was 8.2 (±3.9) years. MCI and dementia were collapsed into one category for further analysis, as in prior analyses in this cohort (Wright et al., 2021).

Table 1
Description of cohort.

Age at visit 1, years, mean (±SD)		70 (±8.9)
Men, n (%)		473 (40.1%)
Years of Education, mean (±SD)		9.7 (±5.1)
Race-ethnicity, n (%)	Hispanic	785 (66.6%)
	Non-Hispanic Black	198 (16.8%)
	Non-Hispanic White	171 (14.5%)
Medical Insurance, n (%)	Medicaid or no Insurance	560 (47.5%)
	Medicare or Private Insurance	619 (52.5%)
Current Smokers, n (%)		182 (15.4%)
Moderate or heavy physical activity, n (%)		119 (10.2%)
Diabetes, n (%)		324 (27.5%)
Systolic blood pressure in mm Hg, mean (±SD)		137 (±17.6)
Diastolic blood pressure in mm Hg, mean (±SD)		78 (±9.6)
High-density lipoprotein, mg/dl, mean (±SD)		53.3 (±16.9)
Low-density lipoprotein, mg/dl, mean (±SD)		115.6 (±35.1)
eGFR, mean (±SD)		73.6 (±18.0)
APOE4, n (%)	Non-carrier	798 (67.7%)
	Carrier	264 (22.4%)
	Missing	117 (9.9%)
Total		1179

eGFR = estimated glomerular fraction rate; SD = standard deviation.

3.2. Association of LASSO-selected immune markers with cognitive outcome

Collectively, the inclusion of LASSO-selected immune markers into the model was significantly associated with improved fit over a model that consisted of age and other traditional vascular risk factors (p-value for LRT <0.0001) (see Table 2). The proportion of the variance for cognitive outcome explained by the LASSO-selected immune markers collectively was slightly greater than that explained by age, suggesting that these immune variables explained roughly the same amount of variance as that of a year of aging did in our models. Approximately 6.2% of the variance in the outcome was explained by the model containing traditional vascular risk factors, with increase to 9.2% with the addition of immune markers, representing an increase of 47% in relative terms (and 3% in absolute terms).

3.3. Sensitivity analyses

In a sensitivity analysis using conditional logistic regression accounting for the individual variation in follow-up times, we found results consistent with the main analyses; inclusion of the LASSO-selected immune markers led to greater fit over the reduced model limited to traditional vascular risk factors (p-value for LRT = 0.0009).

Another sensitivity analysis incorporating APOE4 status, a predictor of dementia, revealed that those with APOE4 had 1.6 times greater odds of dementia or MCI than those without APOE4 (OR = 1.61 [1.18–2.20]), results consistent with previous findings in the literature (Stephenson et al., 2018). Upon addition of immune markers to a model containing both vascular risk factors and APOE4, the model fit improved from 7.5% to 10.5%, a relative increase of 40% and absolute increase of 3%.

Finally, a third sensitivity analysis was done wherein individuals with dementia were compared to a control group comprised of those with MCI/no cognitive impairment, given that MCI may revert and/or

Table 2
Impact of immune markers and other risk factors with dementia and mild cognitive impairment.

Model		adjusted R^2
Model 1[€]	risk factors[a] only	0.0624
Model 2[€]	immune markers[b] + risk factors[a]	0.0917
Model 3	immune markers[b] only	0.0418
Model 4	age alone	0.0344

€ $p < 0.0001$ for likelihood ratio test comparing two nested models of Model 1 and Model 2.

[a] Demographic and risk factors: Age at MRI scan, sex, race-ethnicity, education, medical insurance, physical activity, smoking status, diabetes, systolic blood pressure, diastolic blood pressure, high-density lipoprotein, low-density lipoprotein, and estimated glomerular filtration rate.

[b] Immune markers and their two-way interactions selected from least absolute shrinkage and selection operator.

not progress over long periods of time. This revealed that a model consisting of immune markers and vascular risk factors explained approximately 23.8% of the variance in dementia outcomes, compared to a value of approximately 12.7% in a model with only vascular risk factors, an increase of 11.1% in absolute terms and approximately 87% in relative terms.

3.4. Specific immune markers predicting cognitive outcomes

Several immune markers and two-way interaction terms were found to be associated with dementia/MCI. The two individual markers C-C motif chemokine ligand 2 (CCL2) and C-X-C motif chemokine ligand 9 (CXCL9) were selected in the LASSO analysis. In addition to this, seven two-way interaction terms between various immune markers, were also selected in the LASSO analysis; these did not include CCL2 or CXCL9. Markers included in these interactions were interleukin 1-beta, interleukin 6, leptin, vascular endothelial growth factor, and nerve growth factor (see Table 3).

Table 3
Associations of immune markers with outcome of dementia/mild cognitive impairment.

Risk Factor	P value	Odds Ratio	95% Confidence Interval of the Odds Ratio	
CCL2	0.17	1.05	0.91	1.21
CXCL9	0.09	1.11	0.97	1.29
sFasL*CCL3	0.19	0.87	0.70	1.07
CCL3*VEGFD	0.07	0.87	0.74	1.03
CCL3*TNFb	0.29	0.92	0.78	1.10
IL6*Leptin	0.01	1.27	1.07	1.51
IL31*PDGFBB	0.21	0.95	0.81	1.11
IL1RA*bNGF	0.37	0.89	0.78	1.01
CCL5*IFNg	0.46	0.76	0.63	0.93
Per year of age at time of Neuropsychological testing visit 1	<0.001	1.06	1.04	1.08
Education level (in years)	0.12	1.011	0.98	1.05
Race				
White non-Hispanic		Ref	–	–
Black non-Hispanic	0.14	1.40	0.86	2.29
Hispanic	0.47	1.49	0.91	2.45
Gender				
Female		Ref	–	–
Male	0.90	0.88	0.656	1.178
Insurance				
Private or Medicare		Ref	–	–
Medicaid or no insurance	0.11	1.60	1.17	2.17
Moderate-heavy physical activity (referent none to minimal)	0.76	0.87	0.53	1.41
Current smoker (referent never and past smoker)	0.09	1.43	0.99	2.06
Diabetes mellitus	0.12	1.22	0.91	1.64
Diastolic blood pressure, per SD	0.89	0.89	0.76	1.05
Systolic blood pressure, per SD	0.48	1.08	0.92	1.26
High-density lipoprotein, per SD	0.17	0.92	0.79	1.07
Low-density lipoprotein, per SD	0.57	1.01	0.88	1.15
Estimated glomerular filtration rate, per SD	0.23	1.00	0.87	1.15

CCL2 = C-C Motif Chemokine Ligand 2; CXCL9 = C-X-C Motif Chemokine Ligand 9; sFasL: soluble Fas ligand; CCL3 = C-C Motif Chemokine Ligand 3; VEGFD = Vascular Endothelial Growth Factor D; TNFb = Tumor Necrosis Factor b; IL6 = Interleukin 6; IL31 = Interleukin 31; PDFGBB = Platelet-Derived Growth Factor BB; IL1RA = Interleukin 1 Receptor antagonist; bNGF = Beta Nerve Growth Factor; CCL5 = C-C Motif Chemokine Ligand 5; IFNg = Interferon Gamma; SD = standard deviation.

3.5. Magnitude of immune marker effects

The magnitude of effect of some of the individual immune markers was on the order of that shown by traditional vascular risk factors. For example, the magnitude of effect of CCL2 on dementia/MCI (OR per SD increase = 1.05 [0.91–1.21]) was approximately equal to that of one year of age, one of the most important predictors of cognitive decline and dementia (OR per year = 1.06 [1.04–1.08]). The effect of CXCL9 on cognitive outcome (OR per SD = 1.11, [0.97–1.29]) had a magnitude approximately double that of one year of aging. For CXCL9, the magnitude of effect (OR per SD = 1.11, [0.97–1.29]) was greater than the effect of systolic blood pressure (SBP) (OR = 1.08, [0.92–1.26]). The effects of each of these immune markers was greater than that of low density lipoprotein cholesterol (OR per SD = 1.01, [0.88–1.15]).

4. Discussion

Our results revealed that plasma levels of a panel of immune markers help explain variance in dementia or MCI outcomes in an ethnically diverse population-based cohort, and suggest a role for inflammation in cognitive decline (Yang et al., 2020). Additionally, these immune markers add information even after consideration of traditional vascular risk factors. The impact of the immune panel, while modest in absolute terms, was similar to that of aging, the most important risk factor for incident cognitive impairment. LASSO techniques were incorporated for data reduction, with the final panel selected by LASSO consisting of both individual markers and a number of two-way interaction terms.

The magnitude of effect of the final selected immune markers and two-way interactions was comparable to traditional risk factors. For example, magnitudes of CCL2 and CXCL9 were shown to be approximately equivalent to or even greater than the classical risk factors of aging, systolic blood pressure, and LDL cholesterol. Furthermore, when comparing the model with only traditional vascular risk factors to a model that additionally incorporated LASSO-selected immune markers, the latter improved the R^2 of the model by almost 50% in relative terms, though the absolute increase was small.

These results suggest that the incorporation of immune markers provides additional information regarding the development of cognitive decline and dementia. Moreover, given the wide variety of immune markers and interaction terms in the analysis, it appears unlikely that there is a singular immune pathway, but rather a complex array of mechanisms through which immune function may have an impact on the maintenance of cognitive function. Notably, however, the model fit in absolute terms was relatively low – 10.5% when incorporating immune markers as well as APOE4 – indicating a need for further research to elucidate the pathophysiologic mechanisms underlying the development of dementia.

4.1. Immune markers and dementia

The role of immune mechanisms in the development of dementia complexes such as AD have been discussed in the literature (Lutshumba et al., 2021; Cao and Zheng, 2018). Importantly, this elaboration of immune mechanisms also explores the molecular mechanisms through which peripheral immune activation can influence central nervous system (CNS) pathology, a repudiation of earlier hypotheses that peripheral markers were bystanders. Previous work has shown that dysregulation of inflammatory markers is indeed associated with AD and all-cause dementia (Bettcher et al., 2021). Moreover, high levels of peripheral immune markers in mid-late life have also been associated with cognitive decline in general, not just in dementia complexes (Bettcher et al., 2021). It is possible that entry of inflammatory compounds into the CNS occurs at the capillary venules whereas cell migration is seen at post-capillary venules, controlled in part by cytokines and chemokines (Stephenson et al., 2018).

The presence of peripheral immune markers has also been shown to

display important interactions with other correlates of dementia and cognitive decline. In the present study, the addition of machine learning-informed immune markers to models including only traditional risk factors improved model fit by 3%, in absolute terms, a notable increase. This appears to be in line with previous work demonstrating that presence of inflammation in the setting of metabolic syndrome, a cluster of vascular risk factors, increases the risk of cognitive impairment; crucially, individuals with metabolic syndrome and low peripheral measures of inflammation did not exhibit increased risk (Yaffe et al., 2004). This indicates that immune markers likely interact significantly with traditional vascular risk factors in augmenting the risk of cognitive impairment.

Chronic inflammatory states have also been found to interact with the presence of the APOE4 gene, a well-known risk factor for AD. The significance of APOE4 sensitivity analyses in our study yielded results consistent with relationships previously well-described. For example, previous work has shown that longstanding elevations in peripheral immune markers are associated with earlier AD onset in those with the APOE4 gene, a prominent interaction element (Tao et al., 2018).

4.2. Specific chemokines

CXCL9 is an immune molecule that features prominently in the Th1 proinflammatory immune response. CXCL9, like other CXC chemokines, increases with CNS infection and inflammation and has in particular been shown to be increased in AD brains in humans (Galimberti et al., 2003). Further information regarding the effects of CXCL9 may be assessed through analyzing the role of its receptor, CXCR3, in the brain. Studies have shown increased CXCR3 expression in pertinent brain regions in individuals with AD (Zhou et al., 2019). Additionally, CXCR3 deficiency in transgenic APP/PS1 murine models of AD was shown to facilitate microglial Aβ uptake, which was associated with improved maze performance (Zhou et al., 2019). This indicates that CXCR3 may potentially be a therapeutic target for AD intervention, but further studies are needed.

In the brain, a significant aspect of the neuroinflammation hypothesized to underlie AD is thought to be driven by CCL2-recruited mononuclear phagocytes such as monocytes and microglia (Hickman et al., 2012). Importantly, CCL2 has been shown in animal models to be correlative, not causative, with AD. Monocyte recruitment leads to their accumulation at perivascular plaques, suggesting that they may play a role in plaque clearance (Hickman et al., 2012). Evidence to support this also comes from the observations that decreased CNS levels of CCR2, the receptor for CCL2, is associated with higher brain Aβ plaque levels and that increased monocyte recruitment into the brain leads to delayed AD progression (Hickman et al., 2012; Ben-Yehuda et al., 2021). This role of monocyte recruitment is of particular importance in the early stages of AD with initial plaque clearance (Westin et al., 2012). In general, the CCL2-CCR2 axis has been shown to play a role in the mitigation of amyloid-related pathologies and tauopathies (Ben-Yehuda et al., 2021).

4.3. Interleukins, growth factors, and related immune molecules

Other molecules found to be associated with dementia/MCI in our models included interleukins, related immune and vascular inflammatory molecules, and the adipokine/hormone, leptin. These factors were shown to be present in LASSO-selected interaction terms, indicating that interplay of various immune, vascular inflammatory and endocrine mechanisms may be important in the development of dementia and MCI. Our results revealed the presence of IL6 in several interactions. This is in line with prior work demonstrating the importance of this molecule in AD pathogenesis (Lyra et al., 2021). Another important molecular correlate in our study was IL-1RA, which serves as an endogenous inhibitor of IL-1β (Wikipedia). IL-1β plays a prominent role in acute neuroinflammatory processes *in vivo*. Regarding AD specifically, increased IL-1 expression has been seen in reactive microglia surrounding amyloid plaques (Wikipedia). However, some findings contrary to this paradigm have also been observed, namely in that intracerebral lipopolysaccharide injection, which stimulates IL-1β production, has led to decreased plaque burden, indicating a potential beneficial effect of neuroinflammation, possibly through microglial phagocytosis of Aβ plaques (Wikipedia). Leptin is the most prominent member of the adipokine family and is involved in the interface between the immune system and metabolism, being increased in obesity. Population studies have shown that low body weight in late-life has been associated with increased risk of developing cognitive decline and AD. In the brain, findings suggest that leptin deficiency plays a role in the development of declining cognitive and memory functions (McGuire and Ishii, 2016).

Growth factors, including vascular endothelial growth factor (VEGF) isotypes, are important signaling proteins in the growth and maintenance of, among others, neural cells (Mahoney et al., 2021). Studies have shown that higher prefrontal cortex expression of VEGF B is associated with decreased cognitive trajectory, measured here by a global cognitive composite score comprised of 17 tests across several domains of cognition; lower cognition scores at the last clinical visit before death; and increased Aβ and tau burden (Mahoney et al., 2021). However, VEGF generally appears to have a neuroprotective effect (Storkebaum and Carmeliet, 2004). Nerve growth factor (NGF) plays an important role in the survival and maintenance of cholinergic neurons (Nabeshima, 1995) and imbalance in NGF pathways in basal forebrain cholinergic neurons has been shown to occur in AD brains (Amadoro et al., 2021). Indeed, to this end, NGF therapy has been tested in clinical trials for AD (Eu et al., 2021).

4.4. Clinical relevance

Due to the potentially modifiable nature of inflammation, our results may have clinical implications. For example, novel therapeutics used in AD include non-steroidal anti-inflammatory drugs (NSAIDs) and anti-herpetic medications. NSAIDs have shown limited success thus far, with extended results from the ADAPT trial indicating that NSAIDs have some success in asymptomatic stages but adverse effects in later stages. This was assessed by evaluating T-tau, $A\beta_{1\text{-}42}$, and their ratio (Breitner et al., 2011; Barnes and Yaffe, 2011). With regards to anti-herpetic medications, these have shown efficacy in lowering the risk of dementia in individuals affected by HSV infections, prompting the need for further research into this mechanism of action (Tzeng et al., 2018). Additionally, acetylcholinesterase inhibitors have been shown to decrease IFN-γ, TNFα, IL1β, and IL6 secretion; this shows parallels with the aforementioned importance of NGF in maintaining cholinergic neurons[37].

Given that several of our markers have a magnitude of effect comparable to those of traditional vascular risk factors, it is pertinent to mention the impact that these have on the prevalence of dementia. Based on our modeling, the magnitude of effect of CXCL9 and CCL2 on dementia outcome almost equal those posed by the presence of diabetes and hypertension, with the effect of CXCL9 larger in magnitude than one standard deviation greater SBP. It has been estimated that the population attributable risk (PAR) of DM for AD is 2% globally and 3% in the US (Barnes and Yaffe, 2011) and that for mid-life hypertension approximately 5% globally. The effects of late-life hypertension on dementia are inconsistent (Barnes and Yaffe, 2011). Finally, the PAR for smoking on AD is considerable, being approximately 14% globally and 11% in the US. (Barnes and Yaffe, 2011) These numbers indicate that dementia and cognitive aging are complex, multifactorial processes, and that while several risk factors may contribute to risk, it is unlikely that a single risk factor, whether traditional or immune-related, will alone explain a large proportion of cases.

There are limitations to our study. Firstly, as both exposure and outcomes were assessed in a retrospective manner, while albeit a longitudinal design, causal interpretations may be limited; additionally,

given collection of exposure data at a singular timepoint, this also means that mean effects over time are analyzed and insight into the effects of short-term changes in immune marker levels is less robust. However, the NOMAS cohort is followed over time and the cognitive status of individuals is regularly assessed for the generation of longitudinal data. Secondly, specific dementia subtype information was not available, limiting understanding of more specific biological mechanisms; however, we note that it is quite common for multiple dementia types to co-occur, making an overall diagnosis of dementia most clinically relevant. Thirdly, immune markers were assessed in the blood only and not in the CSF, though blood biomarkers have been shown to correlate with CNS inflammation (Elkind et al., 2021). Fourthly, though a multiplexed assay of 60 immune markers was used, other markers not assessed here may be predictive. Similarly, imaging findings such as MRI biomarkers or PET assessment of amyloid, tau, or microglial activation were not included as outcomes in this analysis, although future efforts are underway to collect and analyze those data. However, the use of cognitive test scores is likely more clinically relevant and informative of patient status. Finally, confirmatory analyses should be undertaken in other cohorts.

There are strengths to our study as well. The NOMAS cohort is diverse and includes Black and Hispanic participants, groups often excluded from dementia research. Demographic and vascular risk factors associated with dementia were also well considered, especially in comparative models. Finally, our multiplex immune assay included a large number of molecules that may contribute to cognitive decline.

5. Conclusion

In conclusion, the present study assessed the association of LASSO-selected immune markers, based on data from multiplexed immunoassays of plasma specimens, with development of MCI and dementia in a diverse, elderly longitudinal cohort. Results indicated that these immune markers may hold additional explanatory power when compared to that afforded by traditional risk factors. These hint at potential therapeutic pathways, though further research is needed.

CRediT authorship contribution statement

Mohammad Abdurrehman Sheikh: Writing – review & editing, Writing – original draft. **Michelle P. Moon:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis. **Clinton B. Wright:** Writing – review & editing, Supervision. **Jose Gutierrez:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Investigation, Data curation. **Minghua Liu:** Formal analysis. **Tatjana Rundek:** Writing – review & editing, Supervision. **Ken Cheung:** Writing – review & editing, Supervision. **Mady Hornig:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization. **Mitchell S.V. Elkind:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

There are no conflict of interest among all authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2024.100937.

Data availability

Data will be made available on request.

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