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RESEARCH ARTICLE

Brain atrophy and white matter hyperintensities are independently associated with plasma neurofilament light chain in an Asian cohort of cognitively impaired patients with concomitant cerebral small vessel disease

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Abstract

Introduction: Plasma neurofilament light chain (NfL) is a potential biomarker for neurodegeneration in Alzheimer's disease (AD), ischemic stroke, and non-dementia cohorts with cerebral small vessel disease (CSVD). However, studies of AD in populations with high prevalence of concomitant CSVD to evaluate associations of brain atrophy, CSVD, and amyloid beta (Aβ) burden on plasma NfL are lacking.

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Methods: Associations were tested between plasma NfL and brain Aβ, medial temporal lobe atrophy (MTA) as well as neuroimaging features of CSVD, including white matter hyperintensities (WMH), lacunes, and cerebral microbleeds.

Results: We found that participants with either MTA (defined as MTA score ≥2; neurodegeneration [N]+WMH−) or WMH (cut-off for log-transformed WMH volume at 50th percentile; N−WMH+) manifested increased plasma NfL levels. Participants with both pathologies (N+WMH+) showed the highest NfL compared to N+WMH−, N−WMH+, and N−WMH− individuals.

Discussion: Plasma NfL has potential utility in stratifying individual and combined contributions of AD pathology and CSVD to cognitive impairment.

KEYWORDS

Alzheimer's disease, amyloid beta, biomarkers, brain atrophy, cerebrovascular disease, neurofilament light chain, non-Alzheimer's pathophysiology, plasma

1 | INTRODUCTION

Alzheimer's disease (AD) is characterized by abnormal accumulation of amyloid beta (Aβ) plaques and neurofibrillary tangles (NFTs), as well as progressive neuronal loss, culminating in brain atrophy and clinical symptoms. In addition, cerebral small vessel disease (CSVD), including white matter (WM) lesions, lacunes, and cerebral microbleeds, not only contributes directly to vascular dementia (VaD), but is also frequently observed in AD brains[1–4] where it interacts with AD pathophysiology processes in an additive or synergistic manner to exacerbate cognitive decline.[2,5–7] Importantly, the prevalence of concomitant AD and CSVD may be higher in non-White populations such as those in Asia, with consequent implications for prevention, diagnostic, and treatment strategies.[8–10] Currently, positron emission tomography (PET) and magnetic resonance imaging (MRI) are used to detect and monitor the progression of AD and CSVD pathologies. However, there are constraints for both techniques such as invasiveness and high cost, which limit their widespread use. By contrast, sensitive blood-based biomarkers with comparable performance could be more readily and widely adopted.[11]

Neurofilament light chain (NfL), a cytoskeletal protein primarily found in the axoplasm of neurons, is leaked into the periphery under various pathophysiological conditions such as neuroaxonal injury.[12] Cerebrospinal fluid (CSF) and blood NfL levels are increased in patients with neurodegenerative diseases including AD, and associated with MRI markers of brain atrophy, supporting its role as a biomarker of neuroaxonal degeneration or loss.[12–15] Additionally, CSF NfL levels were elevated in VaD patients and were associated with white matter hyperintensities (WMH),[12,16–18] a neuroimaging marker of WM lesions. Furthermore, studies on cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and ischemic stroke patients, as well as non-demented elderly with CSVD, revealed significant association between blood NfL, as measured by the ultrasensitive single molecule array (Simoa) platform, and neuroimaging CSVD markers.[4,19–26] However, more recent studies of cognitively impaired subjects reported an absence of associations between blood NfL and WMH, likely due to the exclusion of patients with substantial vascular burden.[13,14] Therefore, there is an unmet need to delineate the possible individual and combined effects of brain atrophy, CSVD, and amyloid burden on plasma NfL in populations with a high prevalence of mixed pathologies (brain atrophy, CSVD, and Aβ burden).[4]

In this study, we measured plasma NfL in a Singaporean memory clinic cohort known to have a relatively high prevalence of concomitant amyloid and CSVD[8,10,27] and studied its associations with neuroimaging measurements of brain atrophy, CSVD, and Aβ burden.

2 | MATERIALS AND METHODS

2.1 | Study population

From April 2016 to 2019, 217 participants were recruited from the National University Hospital memory clinic and community in Singapore. Control subjects were defined as having no objective cognitive impairment (NCI) based on formal neuropsychological assessments. Clinical diagnoses of cognitive impairment no dementia (CIND), AD dementia, and VaD were made as previously described.[27] Participants provided detailed medical histories and underwent physical, clinical, and neuroimaging examinations and a neuropsychological battery consisting of seven cognitive domains[27,28] (see Table S1 in supporting information for component tests of each domain). Apolipoprotein E (*APOE*) ε4 status was as previously described.[29] Among these 217 participants, 208 participants had plasma NfL measurements available and were thus included in this study. Approval for the study was obtained from the Singapore National Healthcare Group Domain-Specific Review Board (2018/00996, 2015/00406, and 2015/00441). Written informed consent was obtained for all participants prior to recruitment.

2.2 | Plasma neurofilament light chain measurements

Non-fasting blood was collected into tubes containing ethylenediaminetetraacetic acid as anticoagulant and centrifuged at 2000*g* for 10 minutes at 4°C. Plasma (approximately 2 mL) was extracted, mixed well, and aliquoted in 0.2 mL aliquots that were stored in polypropylene tubes at −80°C until use. On average, blood samples were collected within 9 months (standard deviation [SD] = 8 months, maximum time 35 months) and 2 months (SD = 5 months, maximum time 31 months) of MRI and amyloid PET scans, respectively. Plasma NfL was measured blinded to clinical information at the Sahlgrenska Academy at University of Gothenburg, Sweden on the Simoa HD-1 platform (Quanterix), using commercially available kits (Quanterix).

2.3 | Neuroimaging

2.3.1 | MRI markers of brain atrophy and cerebrovascular disease

MRI scans were performed on a 3T Siemens Magnetom Trio Tim scanner, using a 32-channel head coil, at Clinical Imaging Research Centre (CIRC) at the National University of Singapore (NUS). The sequences included T1-weighted, fluid-attenuated inversion recovery (FLAIR), T2-weighted, and susceptibility-weighted imaging (SWI) sequences as previously described.[30]

Detailed description of the neuroimaging measures of WMH, hippocampal volume, and intracranial volume is provided in supporting information S1(a). Presence of elevated WMH (WMH+) was defined at the cut-off of 50th percentile (median) of log-transformed WMH volume.

The description for the visual gradings of lacunes and cerebral microbleeds (CMBs) is found in supporting information S1(b). Medial temporal lobe atrophy (MTA) was graded on the Scheltens' scale, and the presence of significant MTA or neurodegeneration (N+) was defined by a score of ≥2.[31,32]

2.3.2 | Amyloid PET acquisition and quantification

PET imaging for brain amyloid burden was conducted at CIRC NUS using either the [^{11}C]Pittsburgh compound B (PiB; n = 178) or [^{18}F]Flutafuranol (n = 30) amyloid tracer radioligands, as previously described.[27] A comprehensive description of the amyloid PET measurement is provided in supporting information S1(c).

2.4 | Statistical analyses

Statistical analyses were performed using SPSS version 26 (IBM SPSS) and R statistical software.[33] Group comparisons of continuous demographic variables were performed using one-way analysis of variance (ANOVA) with Bonferroni post hoc tests for normally distributed data, and non-parametric Kruskal–Wallis test with Dunn's procedure for skewed distributed data. Chi-square tests were used for categorical variables. Correlation analyses were performed using Spearman's rank correlations. WMH volumes and plasma NfL levels were logarithmically transformed due to the skewed distribution for further analyses. We first assessed the association between each neuroimaging measure (MTA scores, WMH volume, lacune counts, CMB counts, or Aβ standardized uptake volume ratio [SUVR]) with blood NfL using separate linear regression models. All neuroimaging measures were treated as continuous variables in the regression analyses, with each regression model adjusted for age and sex. Next, we studied the independent contribution of each imaging marker to blood NfL. For this, neuroimaging measures that were significant in the aforementioned regression analyses (all except Aβ SUVR), were entered simultaneously as independent variables in a multiple regression model, adjusting for age and sex. To determine the possible interactions between MTA scores and WMH volume on blood NfL, the cross-product term MTA x WMH was added. Subgroup analyses were also performed for the cognitively impaired participants (CIND, AD, and VaD). Outcome measures for the regression analyses were reported as mean differences (β) with the 95% confidence intervals (CI). Differences in plasma NfL among diagnostic groups, as well as groups stratified by N and WMH status, were assessed using univariate general linear model, adjusted for covariates, and post hoc least significant difference tests for pairwise group

RESEARCH IN CONTEXT

1. **Systematic Review**: Studies of Alzheimer's disease (AD) have reported significant associations between blood neurofilament light chain (NfL) and neuroimaging measures of neurodegeneration. Additionally, blood NfL has also been correlated with neuroimaging markers of cerebral small vessel disease (CSVD), such as white matter hyperintensities (WMH), in ischemic stroke and elderly subjects without dementia. However, while there is increasing recognition that CSVD also contribute to neurodegeneration, the status of blood NfL of AD patients in populations with high prevalence of concomitant CSVD is at present unknown.
2. **Interpretation**: Using a Singapore-based cohort of cognitively impaired patients with concomitant CSVD, we found that while individuals with either medial temporal lobe atrophy (neurodegeneration [N]+WMH−) or WMH (N−WMH+) manifested increased plasma NfL levels, participants with both pathologies (N+WMH+) showed the highest NfL.
3. **Future Directions**: The potential utility of plasma NfL in stratifying individual and combined contributions of AD pathology and CSVD to cognitive impairment should be further assessed.

comparisons. Diagnostic performance was assessed using area under the receiver-operating characteristic curve (AUC). AUC and 95% CIs were computed using DeLong's method with the pROC package. Cut-off, sensitivity, and specificity values, as well as the respective 95% CIs, were determined by maximizing the Youden index and bootstrap procedures with 2000 iterations,[34,35] with the cutpointr package. Results were considered significant at $P < 0.05$.

3 | RESULTS

3.1 | Participant characteristics

Demographic data, neuroimaging, and plasma NfL measurements are shown in Table 1. For brain atrophy, CIND, AD dementia, and VaD subjects had significantly higher MTA scores (median [interquartile range (IQR)] = 1 [1], 2 [2] and 2 [1], respectively) and lower hippocampal volume (mean [standard deviation (SD)] = 6.3 [1.1] mL, 5.1 [0.9] mL, and 6.1 [0.8] mL, respectively) compared to NCI (median [IQR] MTA score = 1 [0] and mean [SD] hippocampal volume = 7.2 [0.8] mL; $P < 0.05$ for all comparisons). For CSVD, CIND, AD dementia, and VaD had significantly higher WMH volumes (median [IQR] = 4.1 [13] mL, 5.5 [11] mL, and 14.6 [18] mL, respectively) compared to NCI (median [IQR] = 1.4 [4] mL; $P < 0.05$ for all comparisons). AD patients showed the highest PiB-PET SUVR values (median [IQR] = 1.9 [0.8]) among the diagnostic groups (median [IQR] = 1.1 [0.2] to 1.2 [0.4] for other diagnostic groups; $P < 0.05$ for comparisons to AD). Plasma NfL correlated with age (rho = 0.375; $P < 0.001$) and sex (median [IQR] = 28.8 [18] pg/mL for male vs. 24.4 [13] pg/mL for female; $P = 0.020$), but not with *APOE* ε4 genotype (median [IQR] = 26.9 [17] pg/mL in carriers vs. 24.6 [14] pg/mL in noncarriers; $P = 0.49$). Plasma NfL was significantly increased in the presence of diabetes ($P = 0.001$; Figure S1 in supporting information). As expected, plasma NfL was negatively associated with cognitive performance (all $P < 0.05$; Table S2a and S2b in supporting information).

Plasma NfL was significantly increased in AD dementia (median [IQR] = 28.4 [18] pg/mL) and VaD (median [IQR] = 33.5 [29] pg/mL) patients compared to controls (median [IQR] = 20.0 [12] pg/mL; $P \leq 0.001$ for both comparisons using Kruskal–Wallis test with post hoc Dunn's tests; Table 1). The significance remains after adjustment for age and sex ($P < 0.001$ for both comparisons using univariate general linear model and post hoc least significant difference tests; Figure S2 in supporting information). There was no significant difference between CIND and NCI (Table 1 and Figure S2).

3.2 | Medial temporal lobe atrophy and white matter hyperintensities are independently associated with plasma NfL

Correlations between plasma NfL and each neuroimaging variable are shown in Table S3a in supporting information. All neuroimaging variables (rho = 0.239 to 0.379; all $P \leq 0.001$), except brain amyloid burden (rho = 0.086; $P = 0.26$), correlated with plasma NfL. Next, linear regression models were separately performed for each neuroimaging marker, adjusting for age and sex (Table S3b). In agreement with the correlation results, only MTA scores, WMH volume, CMBs, and lacune counts were associated with plasma NfL ($\beta = 0.076, 0.121, 0.004$, and 0.022, respectively; all $P \leq 0.007$). PiB-PET SUVR was not associated with plasma NfL ($\beta = 0.040$, $P = 0.27$).

To assess the independent associations of the neuroimaging markers on plasma NfL, MTA, WMH, lacunes, and CMBs were entered simultaneously as independent variables into a linear regression model. In this combined regression model (Table 2), after adjustment for age and sex, MTA scores and WMH volume were independently associated with plasma NfL, both in the combined cohort (MTA: $\beta = 0.062$; $P = 0.001$ and WMH: $\beta = 0.082$; $P = 0.009$) and cognitively impaired subgroup (MTA: $\beta = 0.047$; $P = 0.017$ and WMH: $\beta = 0.085$; $P = 0.011$). The independent effects of CMBs and lacunes counts did not reach statistical significance (CMBs: $\beta = 0.002$; $P = 0.14$ and lacunes: $\beta = 0.005$; $P = 0.57$).

In a subset of participants ($n = 177$) with hippocampal volume available, we repeated the analyses above with hippocampal volume instead of MTA (Tables S4 and S5 in supporting information). Similar to our aforementioned findings, hippocampal volume and WMH volume were independently associated with plasma NfL in the multiple regression model, both in the combined cohort (hippocampal volume: $\beta = -0.051$; $P < 0.001$ and WMH: $\beta = 0.092$; $P = 0.003$) and cognitively impaired subgroup (hippocampal volume: $\beta = -0.044$; $P = 0.002$ and WMH: $\beta = 0.097$; $P = 0.003$). The congruity of results obtained using both semi-quantitative MTA scores and quantitative hippocampal volume measurements further supports associations between markers of neurodegeneration and elevated plasma NfL.

3.3 | Significant interaction between MTA scores and WMH volume on plasma NfL

A significant interaction was observed between MTA scores and WMH volume on plasma NfL (Table 3; $P = 0.037$ and 0.042 for interaction term in all subjects and cognitively impaired subgroup, respectively). To further examine this interaction, we assessed the association between MTA and WMH on plasma NfL within tertiles of NfL (Table S6 in supporting information). A significant interaction MTA x WMH, $P = 0.033$, was observed only among individuals in the highest tertile of NfL.

3.4 | Plasma NfL differences in groups stratified by neurodegeneration and white matter hyperintensities status

Demographic data, neuroimaging, and plasma NfL measurements of the groups stratified by N and WMH status are shown in Table S7 in supporting information. In the entire cohort, after adjustment for age and sex, plasma NfL levels were increased in N+WMH− and N−WMH+ groups compared to N−WMH− group ($P = 0.037$ and 0.007, respectively; Figure 1, Figure S3a, Table S8a and S8b in supporting information). This corroborates the regression results, which

TABLE 1 Demographic and clinical characteristics

	NCI	CIND	AD	VaD	*P*-value
Maximum *n*	43	99	44	22	
Demographic factors					
Age, yr, mean (SD)	74 (6)	76 (6)	77 (8)	75 (9)	0.352
Female, *n* (%)	27 (63)	50 (51)	34 (77)[b]	8 (36)[c]	**0.004**
Education, yr, mean (SD)	10 (5)	8 (5)	6 (5)[a,b]	5 (4)[a]	**<0.001**
APOE ε4 carrier, *n* (%)	9 (21)	26 (26)	18 (41)	6 (27)	0.187
Vascular risk factors					
Hypertension, *n* (%)	29 (67)	73 (74)	33 (75)	20 (91)	0.232
Hyperlipidemia, *n* (%)	33 (77)	72 (73)	27 (61)	18 (82)	0.258
Diabetes, *n* (%)	7 (16)	32 (32)	10 (23)	8 (36)	0.163
Smoker, *n* (%)	3 (7)	4 (4)	0 (0)	3 (14)	0.088
Intracranial volume, mL, median (IQR)	1054 (158)	1078 (164)	1049 (112)	1074 (177)	0.640
Brain atrophy					
Medial temporal lobe atrophy scores, median (IQR)	1 (0)	1 (1)[a]	2(2)[a,b]	2(1)[a]	**<0.001**
Hippocampal volume, mL, mean (SD)	7.2 (0.8)	6.3 (1.1)[a]	5.1 (0.9)[a,b]	6.1 (0.8)[a,c]	**<0.001**
N+, *n* (%)	6 (14)	40 (40)[a]	30 (68)[a,b]	13 (59)[a]	**<0.001**
Cerebral small vessel disease					
White matter hyperintensities (WMH) volume, mL, median (IQR)	1.4 (4)	4.1 (13)[a]	5.5 (11)[a]	14.6 (18)[a]	**<0.001**
WMH+, *n* (%)	12 (28)	51 (52)	26 (61)[a]	14 (67)[a]	**0.005**
Cerebral microbleeds count, median (IQR)	0 (2)	0 (1)	1 (3)	1 (6)	0.184
Lacunes count, median (IQR)	0 (0)	0 (1)	0 (0)	2 (3)[a,b,c]	**<0.001**
Brain amyloid burden					
PiB-PET SUVR, median (IQR)	1.1 (0.2)	1.2 (0.4)	1.9 (0.8)[a,b]	1.1 (0.3)[c]	**<0.001**
Plasma NfL, pg/mL, median (IQR)	20.0 (12)	24.6 (15)	28.4 (18)[a]	33.5 (29)[a,b]	**<0.001**

Notes: *P*-values are derived from chi-square tests for categorial variables, and from one-way analysis of variance with post hoc Bonferroni test or Kruskal–Wallis test with post hoc Dunn's procedure and Bonferroni correction for multiple comparisons, for normally distributed or skewed continuous variables, respectively. N+ was defined by medial temporal atrophy score ≥2. For WMH+, the cut-off for log-transformed WMH volume was at the 50th percentile. Diabetes status was available for 207 participants. Intracranial volume data was available for 178 participants. Hippocampal volume was available for 177 participants. WMH volume data were available for 206 participants. CMB count data were available for 178 participants. PiB-PET SUVR data were available for 177 participants.

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; CIND, cognitive impairment no dementia; CMB, cerebral microbleed; IQR, interquartile range; N, neurodegeneration; NCI, no cognitive impairment; NfL, neurofilament light chain; PET, positron emission tomography; PiB, Pittsburgh compound B; SD, standard deviation; SUVR, standardized uptake value ratio; VaD, vascular dementia; WMH, white matter hyperintensities.

[a]Significantly different from NCI.

[b]Significantly different from CIND.

[c]Significantly different from AD.

indicated the independent effects of MTA scores and WMH volume on plasma NfL. Notably, the NfL levels were highest in the presence of both pathologies, namely N+WMH+, compared to the other groups ($P \leq 0.003$ compared to N−WMH−, N−WMH+, and N+WMH−, respectively; Figure 1 and Figure S3a). Similar trends were noted after further adjustment for cognitive status (two-level variable: NCI vs. CIND + dementia), except that the difference between N+WMH− and N+WMH+ groups was at the margin of statistical significance ($P = 0.054$; Figure S3b and Table S8c). We have also adjusted for cognitive status as a three-level variable (NCI vs. CIND vs. dementia; Figure S3c and Table S8d). Plasma NfL remained significantly higher in N−WMH+ and N+WMH+ compared to N−WMH−. The N+WMH+ group had increased NfL levels compared to N+WMH−, but not with N−WMH+.

To test the utility of plasma NfL in distinguishing between subjects with and without neurodegeneration and/or WMH pathology status, we applied a receiver operating characteristic curve analysis (Table 4). The highest AUC of 0.798 was obtained for distinguishing between N+WMH+ and N−WMH− groups, at a cut-off of 26.2 pg/mL for plasma NfL levels. Plasma NfL also detected individuals with at least one pathology (N−WMH+, N+WMH−, and N+WMH+ vs. N−WMH−) with an AUC of 0.715, at a lower cut-off of 19.3 pg/mL for plasma NfL levels. Similar AUC was obtained for detecting individuals with both pathologies (N+WMH+ vs. N−WMH+, N+WMH−, and N−WMH−),

TABLE 2 Independent associations of neuroimaging markers with plasma NfL

	Plasma NfL[a] (outcome)					
	All subjects ($n = 178$)			Cognitively Impaired ($n = 151$)		
Neuroimaging markers	**β coefficient**	**95% CI**	***P*-value**	**β coefficient**	**95% CI**	***P*-value**
Model 1						
Medial temporal lobe atrophy scores	0.085	**(0.049, 0.121)**	**<0.001**	0.064	**(0.024, 0.104)**	**0.002**
White matter hyperintensities volume[a]	0.102	**(0.037, 0.166)**	**0.002**	0.097	**(0.029, 0.165)**	**0.006**
Cerebral microbleeds count	0.000	(−0.002, 0.003)	0.711	0.000	(−0.002, 0.003)	0.811
Lacunes count	0.004	(−0.014, 0.021)	0.686	0.004	(−0.014, 0.022)	0.680
Model 2						
Medial temporal lobe atrophy scores	0.062	**(0.028, 0.097)**	**0.001**	0.047	**(0.009, 0.086)**	**0.017**
White matter hyperintensities volume[a]	0.082	**(0.021, 0.142)**	**0.009**	0.085	**(0.020, 0.150)**	**0.011**
Cerebral microbleeds count	0.002	(−0.001, 0.004)	0.137	0.002	(−0.001, 0.004)	0.184
Lacunes count	0.005	(−0.012, 0.021)	0.574	0.003	(−0.014, 0.020)	0.722

Notes: Analysis performed in a subcohort of 178 individuals in which all neuroimaging measures were available. To assess the independent contribution of each imaging-based brain atrophy (MTA scores) or CSVD marker (WMH, CMBs, and lacunes) on plasma NfL, all the neuroimaging markers were entered simultaneously as independent variables in the linear regression model (Model 1), and further adjusted for age and sex (Model 2), as stated below. β, mean difference.
Model 1: Independent variables: MTA scores, WMH volume, CMB count, and lacunes count.
Model 2: Independent variables: MTA scores, WMH volume, CMB count, lacunes count, age, and sex.
Interpretation:
MTA scores: For every unit increase in MTA scores, the $\log_{10}$ [plasma NfL] will increase by β unit.
WMH volume: For every 1% increase in WMH volume, the [plasma NfL] will increase by β%.
[a]Log-transformed.
Abbreviations: CI, confidence interval; CMB, cerebral microbleed; CSVD, cerebral small vessel disease; MTA, medial temporal lobe atrophy; NfL, neurofilament light chain; WMH, white matter hyperintensities.

TABLE 3 Association of brain atrophy and WMH burden with plasma NfL in a model with the interaction term medial temporal lobe atrophy (MTA) score x WMH

	Plasma NfL[a] (outcome)					
	All subjects ($n = 206$)			Cognitively impaired ($n = 163$)		
Neuroimaging markers	**β coefficient**	**95% CI**	***P*-value**	**β coefficient**	**95% CI**	***P*-value**
Model 1 (without interaction term)						
MTA score	0.063	**(0.030, 0.096)**	**<0.001**	0.049	**(0.011, 0.088)**	**0.012**
WMH volume[a] (WMH)	0.097	**(0.045, 0.149)**	**<0.001**	0.099	**(0.039, 0.158)**	**0.001**
Model 2 (with interaction term MTA x WMH)						
MTA score	0.113	**(0.056, 0.171)**	**<0.001**	0.114	**(0.041, 0.187)**	**0.002**
WMH volume[a] (WMH)	0.211	**(0.092, 0.330)**	**0.001**	0.240	**(0.092, 0.388)**	**0.002**

Notes: Analysis performed in a subcohort of 206 individuals in which MTA scores and WMH volume data were available. In Model 1, both MTA and WMH were entered simultaneously as independent variables in the linear regression models and adjusted for age and sex. In Model 2, the significant interaction term MTA x WMH ($P = 0.037$ and 0.042 in all subjects and cognitively impaired subjects, respectively) was added. β, mean difference.
Model 1: Independent variables: MTA scores, WMH volume, age, and sex.
Model 2: Independent variables: MTA scores, WMH volume, MTA x WMH, age, and sex.
Interpretation:
MTA scores: For every unit increase in MTA scores, the $\log_{10}$ [plasma NfL] will increase by β unit.
WMH volume: For every 1% increase in WMH volume, the [plasma NfL] will increase by β%.
[a]Log-transformed.
Abbreviations: CI, confidence interval; MTA, medial temporal lobe atrophy; NfL, neurofilament light chain; WMH, white matter hyperintensities.

TABLE 4 AUROC analyses of plasma NfL

	AUC (95% CI)	Cut-off in pg/mL (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
N−WMH− vs. N+WMH−, N−WMH+, and N+WMH+ (at least 1 pathology)	0.715 (0.641, 0.789)	19.3 (18.9, 37.1)	82.4% (33.6%, 88.7%)	51.6% (43.9%, 96.7%)
N−WMH−, N+WMH−, and N−WMH+ vs. N+WMH+ (2 pathologies)	0.715 (0.631, 0.800)	29.0 (26.3, 31.2)	70.8% (61.7%, 89.5%)	71.5% (56.8%, 78.4%)

Notes: AUC and 95% CI were derived from DeLong test. Cut-off, sensitivity and specificity, along with respective 95% CI, were determined by maximizing the Youden index and bootstrap procedures of 2000 iterations. N+ was defined by medial temporal atrophy score ≥2. For WMH+, the cut-off for log transformed WMH volume was at the 50th percentile.

Abbreviations: AUC, area under the curve; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; N, neurodegeneration; NfL, neurofilament light chain; WMH, white matter hyperintensities.

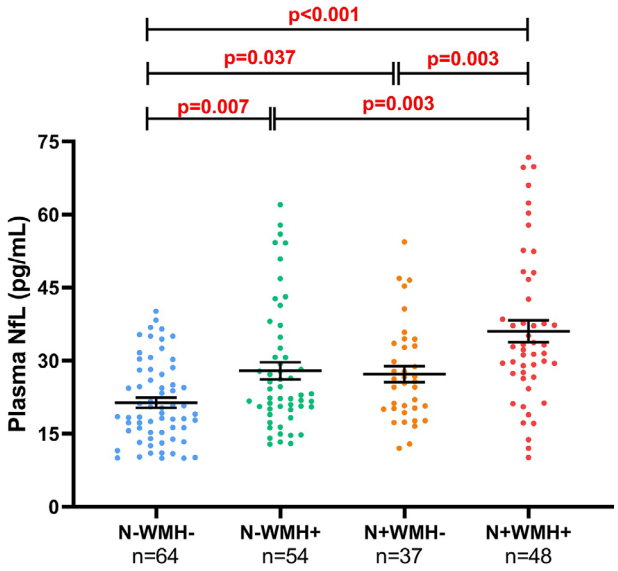

FIGURE 1 Plasma neurofilament light chain (NfL) across groups stratified by neurodegeneration (N) and white matter hyperintensities (WMH) status in 203 participants. N+ was defined by medial temporal atrophy score ≥2. For WMH+, the cut-off for log transformed WMH volume was at the 50th percentile. The graph shows the unadjusted mean and standard error mean (SEM). The group differences were assessed with univariate general linear model using log-transformed plasma NfL levels, adjusted for age and sex, with *P*-values representing least significant difference tests (post hoc) for pairwise group comparisons. For better visualization of the differences among the groups, three outlying participants (one from N−WMH+, two from N+WMH−) were excluded from the graph and analysis (although the comparisons above remained significant even after inclusion of the three individuals, see Figure S2a in supporting information). Compared to N−WMH−, the partial eta squared for N−WMH+, N+WMH−, and N+WMH+ are 0.036, 0.022, and 0.138, respectively

but at a higher cut-off of 28.9 pg/mL. These results suggest that specific appropriate cut-offs for plasma NfL may be useful in identifying individuals with mixed pathologies in the brain.

4 | DISCUSSION

Using data from a Singaporean cohort across the cognitive spectrum (NCI, CIND, AD, and VaD) with comprehensive neuroimaging assessments, we explored the associations of brain atrophy, AD, and CSVD pathologies with plasma NfL. Our main findings include: (1) plasma NfL increases in both AD dementia and VaD patients; (2) independent, positive associations between brain atrophy/WMH burden and plasma NfL; and (3) significant increases of plasma NfL in the N+WMH− and N−WMH+ groups compared to the N−WMH− group. Additionally, plasma NfL levels were found to be highest in the presence of both pathologies (N+WMH+). Taken together, our results suggest that elevated plasma NfL may be reflective of changes in vascular disease pathology (specifically the presence and progression of WM lesions, see Figure S4 in supporting information), as well as neurodegenerative changes (such as medial temporal atrophy). The further elevation of plasma NfL in the N+WMH+ group implies that NfL may have utility in identifying individuals with mixed pathologies in the brain with the use of appropriate cut-offs (Table 4).

Our findings of significant associations between plasma NfL and the individual MRI markers of brain atrophy and CSVD (Tables S3 and S4) are corroborated by previous studies.[4,13,14,19–26] Importantly, in a combined regression model, we showed that MTA and WMH were independently associated with plasma NfL (Table 2). To the best of our knowledge, this is the first Asian-based study of peripheral NfL in both AD and vascular cognitive impairment. This has important bearing on the potential utility of NfL as a biomarker for clinically relevant outcomes in populations with significant baseline CSVD burden. This is contrasted with Western cohorts in which neurodegenerative changes seem to be the dominant driver of plasma NfL increase.[13,14] Furthermore, with increasing appreciation that CVSD and other vascular pathologies also manifest with concomitant neurodegeneration, these results also indicate that plasma NfL could reflect both non-vascular and vascular pathological correlates of neurodegeneration in mixed disease. In the former, neuroaxonal loss may be associated with accumulation of protein aggregates (e.g., NFTs) in neurodegenerative diseases.[13,14,36,37] In the latter, active CSVD leads to reduced cerebral blood flow and chronic hypoperfusion of WM, culminating in neuroinflammation, demyelination, and axonal loss (WM lesions),[38–40] and likely resulting in prolonged NfL release into the periphery. Overall, our results corroborate the general consensus that blood NfL reflects the severity of neuroaxonal damage, which may be caused by diverse pathological processes.

Besides AD, serum NfL was reported to be elevated in ischemic small vessel stroke patients during both the acute phase and 6 months post-stroke.[23,41,42] The prolonged release of NfL into the blood after acute neuronal injury may be due to active progression of CSVD, which may in turn predict recurrent stroke/death[25] or new vascular lesions[23,41] at follow-up. In line with this, studies on non-demented elderly demonstrated associations between higher baseline plasma NfL and incident stroke[43] or longitudinal progression of WMH.[22] However, these studies could not rule out the contribution of underlying neurodegenerative processes that may influence blood NfL levels. In this regard, we showed that plasma NfL levels were significantly elevated in elderly participants with higher WMH burden, even in the absence of significant brain atrophy (e.g., N−WMH+). The elevated plasma NfL in these N−WMH+ individuals could be due to expanding WM lesions, supporting the potential utility of plasma NfL as a prognostic marker for active, progressive CSVD.

There is evidence suggesting potential associations between plasma NfL and diabetes status or blood glucose levels,[44–46] potentially through diabetic neuropathy, which is associated with increased plasma NfL concentration.[46] Here, we reported significant plasma NfL increases in participants with diabetes (Figure S1). Therefore, in addition to age and sex, we included diabetes status as a covariate in regression analyses (Tables S5, S9, and Figure S3d in supporting information). Briefly, further adjustments for diabetes status did not change the significance of our results, except for the comparison between N+WMH− ($n = 39$) and N+WMH+ ($n = 48$), which was approaching statistical significance ($P = 0.077$). This suggests that our observations were independent of diabetes status.

Blood NfL has been proposed as a screening tool to identify individuals with ongoing neurodegeneration in neurodegenerative disorders.[15,47] Based on current findings, we further propose that NfL may be useful in elderly populations with high prevalence of mixed pathologies, with the highest cut-off (e.g., 29.0 pg/mL) indicating both vascular and degenerative changes, and intermediate cut-off (e.g., 19.3 pg/mL) indicating at least one pathology. Future studies of biomarker utility may include blood NfL as part of a multi-marker panel, along with other putative blood AD biomarkers[11] in stratifying risks of AD dementia and/or vascular cognitive impairment.

Our study's strengths include the use of comprehensive neuropsychological assessments to properly diagnose cognitive impairment and dementia, as well as the availability of well-established neuroimaging measurements for amyloid pathology, medial temporal atrophy, and a range of CSVD. Furthermore, we have considered possible confounding effects of demographic characteristics and vascular risk factors that may affect the results. However, several limitations are also apparent. First, we do not yet have PET or CSF measurements for tau, and therefore could not determine the associations between these parameters and plasma NfL. The current state of knowledge regarding associations between brain amyloid and plasma NfL is ambiguous, with some recent studies showing significant associations[13,48] but not others[49,50] (including the present one). Instead, the other major neuropathological hallmark of AD, namely NFT, may be more closely related with neuronal loss and consequently plasma NfL.[51,52] Next, we recognize that the maximum interval between blood collection and MRI is substantial (35 months). However, because only a small proportion (≈6%) has an interval of more than 24 months, the conclusions from this study remain conceivable. In this study, the 50th percentile (median) cut-off for WMH volume was performed to classify the participants into "low" and "high" WMH load. This binarization approach has previously been adopted by us and others for WMH volume as well as other biomarkers.[5–7] We understand that the exact cut-off values may differ across cohorts depending on the severity and prevalence of WMH burden, and the use of median cut-offs may therefore not be generalizable. However, because there is no current consensus on established cut-offs for WMH volume, we propose that a median cut-off remains an unbiased method for dichotomization to observe any potential differences between the groups. While differences in plasma NfL across groups stratified by N and WMH status were statistically significant, the magnitude of differences was small, thus warranting further validation in bigger independent cohorts. The cross-sectional design of this study does not allow for the examination of the temporal associations between plasma NfL and the progression of brain atrophy as well as WMH burden, necessitating follow-up longitudinal studies. Given that the blood–brain barrier (BBB) may be compromised in dementia,[53] future study may explore the contributing effects of BBB permeability on plasma NfL alterations. Finally, plasma NfL cannot differentiate between central and peripheral neuroaxonal injury, which is a problem inherent to the general expression of this protein in both central and peripheral nerves.[54]

In conclusion, in elderly populations with high prevalence of CSVD burden, plasma NfL may reflect both neurodegenerative and vascular pathologies (specifically WMH). Plasma NfL should be further assessed for its potential utility in stratifying independent and combined contributions of AD and CSVD in patients with cognitive impairment.

AUTHOR CONTRIBUTIONS

Drs Lai and Chen have full access to all of the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. Concept and design: Schöll, Zetterberg, Blennow, Chen, and Lai. Acquisition, analysis, or interpretation of data: all authors. Drafting of the manuscript: Chong and Lai. Critical revision of the manuscript for important intellectual content: all authors. Statistical analyses: Chong, Hilal, Chen, and Lai. Supervision: Chen and Lai.

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CONFLICTS OF INTEREST

Dr. Zetterberg has served on scientific advisory boards for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). Dr. Blennow has served as a consultant, on advisory boards, or on data-monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. Drs. Chong, Hilal, Ashton, Karikari, Reihac, Vrooman, Schöll, Chen, and Lai declare that they have no competing interests. Author disclosures are available in the supporting information.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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