Serum Neurofilament Light Chain Is Associated with Incident Lacunes in Progressive Cerebral Small Vessel Disease

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Background and Purpose Serum neurofilament light (NfL)-chain is a circulating marker for neuroaxonal injury and is also associated with severity of cerebral small vessel disease (SVD) cross-sectionally. Here we explored the association of serum-NfL with imaging and cognitive measures in SVD longitudinally.

Methods From 503 subjects with SVD, baseline and follow-up magnetic resonance imaging (MRI) was available for 264 participants (follow-up 8.7±0.2 years). Baseline serum-NfL was measured by an ultrasensitive single-molecule-assay. SVD-MRI-markers including white matter hyperintensity (WMH)-volume, mean diffusivity (MD), lacunes, and microbleeds were assessed at both timepoints. Cognitive testing was performed in 336 participants, including SVD-related domains as well as global cognition and memory. Associations with NfL were assessed using linear regression analyses and analysis of covariance (ANCOVA).

Results Serum-NfL was associated with baseline WMH-volume, MD-values and presence of lacunes and microbleeds. SVD-related MRI- and cognitive measures showed progression during follow-up. NfL-levels were associated with future MRI-markers of SVD, including WMH, MD and lacunes. For the latter, this association was independent of baseline lacunes. Furthermore, NfL was associated with incident lacunes during follow-up (P=0.040). NfL-levels were associated with future SVD-related cognitive impairment (processing speed: β=-0.159; 95% confidence interval [CI], -0.242 to -0.068; P=0.001; executive function β=-0.095; 95% CI, -0.170 to -0.007; P=0.033), adjusted for age, sex, education, and depression. Dementia-risk increased with higher NfL-levels (hazard ratio, 5.0; 95% CI, 2.6 to 9.4; P<0.001), however not after adjusting for age.

Conclusions Longitudinally, serum-NfL is associated with markers of SVD, especially with incident lacunes, and future cognitive impairment affecting various domains. NfL may potentially serve as an additional marker for disease monitoring and outcome in SVD, potentially capturing both vascular and neurodegenerative processes in the elderly.

Keywords Stroke; Dementia; Small vessel diseases; Neurofilament; Magnetic resonance imaging; Biomarkers
Introduzione

La neurofilament light (NFL) è una parte del neurofilamento e dei processi patologici che causano danni neuroaxonal, rilasciando NFL nell’ambiente extracellulare, inclusi il sangue periferico. Pertanto, dato il suo contenuto e specificità per neuroni, NFL è un marcatore ematico emergente per danni neuroaxonali in vari disturbi neurologici che affollano l’anziano, incluse le neurodegenerative e le malattie del piccolo vaso cerebrale (SVD).\(^1\) SVD è una condizione frequente nell’anziano e un importante fattore di rischio del demenza.\(^4\) Diagnosi SVD è basata sulla misurazione clinica e radiologica, inclusi le iperintensità di materiale bianco (WMH), lacune, e microsangue.\(^5\) Tuttavia, esistono limitazioni potenziali del MRI nell’anziano e la sua disponibilità. Inoltre, altri marcatori accessibili, come il sangue basato sui marcatori riflettono le condizioni sottostanti, sono garantiti.

Recentemente, abbiamo osservato elevati livelli di NFL nel siero di individui con SVD confrontati con controlli sani e cross-sectionally troviamo una relazione tra i livelli di NFL e la severità di SVD-MRI marcatori, in particolare con la massa bianca strutturale del tessuto del cervello organizzati da diffusione tridimensionale (MD) da imageria diffusione-tensori.\(^1\) Anche, livelli di NFL erano fortemente legati con l’impegnamento di processamento del giro per dimensione nella SVD.\(^1\)\(^6\) Pertanto, livelli di NFL possono potenzialmente servire come marcatori di progressione di SVD, ma studi longitudinali per accertare questo sono mancanti. Qui, quindi abbiamo valutato la associazione di livelli di siero NFL con i marcatori futuri MRI di SVD e cognizione compromessa e demenza in un grande numero di pazienti con SVD sporadica.

Metodi

Popolazione Studi

Lo Studio delle Dignità Diffusione Tensoriale e Radiografia Magnetica Radboud University Nijmegen (RUN DMC) è uno studio prospettico della popolazione di 503 non-dementati anziani con SVD, indagando i rischi e le conseguenze cliniche di SVD. Il protocollo dettagliato è stato pubblicato precedentemente.\(^7\) I dati che supportano i risultati di questo studio sono disponibili al disponente autorizzato su richiesta ragionata.

Informazioni sull’anziano in demenza all’analisi follow-up erano disponibili per tutti i 503 partecipanti. Di tutti i partecipanti, 336 hanno svolto test cognitivi ripetuti alla base (2006) e follow-up (2015), con un follow-up medio di 8.7±0.2 anni. Di questi, 264 partecipanti completarono MRI al follow-up.\(^8\) I rischi vasculari (Tabella 1) sono stati descritti precedentemente.\(^1\)\(^7\) L’ipertensione veniva considerata presente se il paziente stava assumendo farmaci antitensionali. La demenza veniva definita basandosi sull’uso di farmaci antitensionali e raccomandazioni alle basi.

Neurofilament-analysis and MRI

Dettagli riguardanti il protocollo di laboratorio e radiografico sono stati pubblicati precedentemente.\(^7\) In breve, per analisi NFL tutti gli campioni furono analizzati su un singolo macchinario (Simoa HD-1, Quanterix, Lexington, MA, USA) in Basel. Utilizzavamo l’anticondimento monoclonale (mAB) 47.3, e il biotinilato di dittione mAB 2:1 (UmanDiagnostics, Umeå, Sweden),\(^9\) trasferito sull’impianto Simoa. Il bovine lyophilized NFL è stato ottenuto da UmanDiagnostics. I campioni di calibrazione campioni di calibrazione variavano dal 0 al 2,000 pg/mL. Intra- e inter-assay variabilità dell’analisi del campione erano inferiori al 20%, rispettivamente. La sensibilità analitica era 0.32 pg/mL. Tutti i campioni produssero segnali al di sopra della sensibilità analitica dell’analisi.

MRI images were acquired at three time points on 1.5-Tesla MRIs, in which only the two MRI assessments from baseline and final follow-up were used in the present study. The following measures were assessed at both time-points: total brain volume, gray/white matter volume, WMH-volume, MD, fractional anisotropy (FA), lacunes, and microsangue (Supplementary Table 1). WMH-volumes were calculated as the sum of all voxels designated as WMH multiplied by the voxel volume in mL; WMH-volumes were checked for segmentation-errors by one trained rater, lacunes, and microsangue were rated manually on fluid-attenuated inversion recovery (FLAIR)/T1-weighted and T2*-weighted MRI by two trained raters, all blinded for clinical data. Inter-/intra-rater reliability were excellent.\(^9\) The segmented WMH maps were resampled and registered to the white matter maps in order to obtain WMH maps and normal-apparing white matter (NAWM; e.g., white matter not containing WMH) maps.

Cerebro microsangue vennero esaminati su T2*-weighted gradient echo sequence with same protocol parameters for all time points (time repetition=800 ms, time echo=26 ms, voxel size 1.3x1.0x5.0 mm, interslice gap 1.0 mm). We used the same head coils at all three time points. The intra- and inter-rater variability vennero ben con weighted kappa’s of 0.87 and 0.95 for lacunes and 0.85 and 0.86 for microsangue, calculated in
10% of the scans. Volumes (mL) were corrected for interscan differences in intracranial volume (ICV) and normalized to baseline ICV. Diffusion-weighted-images were denoised and corrected. Diffusion-tensor and scalar parameters (MD/FA), were calculated using DTIFIT from FSL’s FDT toolbox. The segmented WMH maps were resampled and registered to the white matter maps in order to obtain WMH maps and NAWM (e.g., white matter not containing WMH) maps.

Median time between blood-sampling and baseline MRI-scan was 14 days (mean±standard deviation [SD], 19±32 days). Samples were aliquoted in polypropylene vials and stored deep-frozen until analysis (performed 2018).

**Dementia status**

Dementia case finding was described previously. In short, dementia was diagnosed after examination at the Radboud Alzheimer Center or by expert panel consensus diagnosis. Age and education were considered for interpretation, next to interference with daily living, confirmed by family/caregivers. In total, 65 out of 503 participants were diagnosed with dementia during follow-up.

**Cognitive function**

Cognitive performance was measured using an extensive neuropsychological test battery, as has been described previously. In short, the test battery included the Mini-Mental State Examination (MMSE), Rey Auditory Verbal Learning Test (RAVLT), Rey Complex Figure Task (RCFT), verbal fluency, Paper-Pencil Memory Scanning Task (PPMST), Stroop Color Word Test (short form), Symbol Digit Substitution Task (SDST), and Verbal Series Attention Test (VSAT). To account for possible learning effects, parallel versions of the RAVLT, RCFT, and verbal fluency task were used for the follow-up assessment. Raw scores of all time-points were transformed into z-scores based on the mean±SD of the overall study population at baseline. We calculated speed-accuracy trade-off (SAT) scores where appropriate. Cognitive decline over time was calculated for each subject individually, by subtracting baseline scores from the follow-up scores. We calculated compound scores for global cognitive func-

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**Table 1. Baseline characteristics**

| Characteristic                        | All participants (n=503) | Study population cognition (n=336) | Study population imaging (n=264) |
|---------------------------------------|--------------------------|-----------------------------------|---------------------------------|
| **Demographics**                      |                          |                                   |                                 |
| Age (yr)                              | 65.7±8.8                 | 63.3±8.1                          | 62.3±7.7                        |
| Male sex                              | 284 (56.5)               | 195 (58.0)                        | 157 (59.5)                      |
| MMSE score                            | 28.1±1.6                 | 28.5±1.4                          | 28.6±1.3                        |
| Education (yr)                        | 9.8±1.8                  | 10.0±1.6                          | 10.1±1.5                        |
| Depressive symptoms                   | 12.4±9.3                 | 12.3±9.0                          | 12.1±8.9                        |
| **Vascular risk factors**             |                          |                                   |                                 |
| Hypertension                          | 369 (73.4)               | 233 (69.3)                        | 182 (68.9)                      |
| Diabetes                              | 75 (14.9)                | 37 (11.0)                         | 27 (10.2)                       |
| Hypercholesterolemia                  | 237 (47.1)               | 149 (44.3)                        | 113 (42.8)                      |
| Smoking, ever                         | 353 (70.2)               | 238 (70.8)                        | 186 (70.5)                      |
| Alcohol (glasses/wk)                  | 7.9±9.3                  | 8.1±9.1                           | 8.2±9.0                         |
| Body mass index (kg/m²)               | 27.1±4.1                 | 27.1±4.0                          | 27.2±4.1                        |
| **MRI-markers**                       |                          |                                   |                                 |
| Total brain volume (mL)               | 1,060.9±80.1             | 1,082.8±71.2                      | 1086.4±71.1                     |
| Grey matter volume (mL)               | 606.2±52.6               | 617.8±48.1                        | 620.8±49.1                      |
| White matter volume (mL)              | 454.7±46.0               | 465.1±48.1                        | 465.6±39.3                      |
| WMH volume (mL)                       | 3.6 (1.2–11.4)           | 2.6 (0.9–7.6)                     | 2.2 (0.8–5.8)                   |
| Lacunes                               | 132 (26.2)               | 74 (22.0)                         | 54 (20.5)                       |
| Microbleeds                           | 83 (16.5)                | 46 (13.7)                         | 36 (13.6)                       |
| Serum NfL (pg/mL)                     | 53.4 (38.1–77.7)         | 47.2 (35.0–68.8)                  | 44.4 (33.0–62.0)                |

Values are presented as mean±standard deviation, number (%), or median (interquartile range). MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; WMH, white matter hyperintensity; NfL, neurofilament light. *8 missing; †5 missing; ‡4 missing.
tion, memory, processing speed, and executive function. We calculated the cognitive index as a compound score for global cognitive function, using the mean of the z-scores of all tests from the neuropsychological test battery. Z-scores for both baseline and follow-up were calculated using the mean±SD of the baseline tests. Higher z-scores always indicate a better performance. Change in cognitive functioning for separate cognitive domains was calculated within-subject, by subtracting the baseline domain compound score from the follow-up domain compound score.

Memory was measured using the two-letter and three-letter subtasks of the PPMST and the immediate and delayed recall of the RAVLT and the RCFT. Processing speed was calculated as the mean of the z-scores of the one-letter subtask of the PPMST, the reading and color naming tasks of the stroop test and the SDST. Executive function was measured using the verbal fluency task, the interference score of the stroop test, which was calculated by dividing the color-word task by the mean of the reading and color naming tasks of the stroop test, and the VSAT.

Statistics

Statistical analyses were performed using SPSS Statistics version 20 (IBM Co., Armonk, NY, USA). Serum-NfL and WMH-volumes were log-transformed because of skewedness. Associations between baseline NFL (independent variable) and MRI-markers (dependent variables) were analyzed by linear regression analyses. Associations between NFL and (incident) lacunes and microbleeds were calculated by analysis of covariance (ANCOVA). Analyses for lacunes and microbleeds were performed based on (1) presence or absence of the respective lesion; (2) quantitative categorization according to the number of the respective lesion into four groups: 0, 1, 2 to 4, 5 or more.

We adjusted for age, sex, hypertension, and the respective variable at baseline. For cognitive measures we performed linear regression analyses. Cox-proportional-hazard analyses were performed for association between NFL-levels and development of dementia.

Results

Baseline characteristics and analysis

Baseline characteristics are presented in Table 1. Participants for whom follow-up MRI and/or cognitive testing was available were slightly younger and had less severe MRI-markers of SVD than the overall RUN DMC-cohort. Median level of serum NFL in the two subgroups did not significantly differ from the overall cohort.

We adjusted for age, sex, hypertension, and the respective variable at baseline. For cognitive measures we performed linear regression analyses. Cox-proportional-hazard analyses were performed for association between NFL-levels and development of dementia.

Table 2. Associations between NFL levels and MRI markers of SVD in patients with follow-up

| Variable          | Baseline MRI markers | Future MRI markers |
|-------------------|----------------------|--------------------|
|                   | β (95% CI)           | P                  | β (95% CI)       | P                  |
| MRI markers       |                      |                    |                   |                    |
| Total brain volume| -0.086 (-0.207 to 0.014) | 0.087               | -0.071 (-0.183 to 0.025) | 0.136             |
| Gray matter volume| -0.061 (-0.184 to 0.047) | 0.241               | -0.028 (-0.144 to 0.082) | 0.587             |
| White matter volume| -0.079 (-0.221 to 0.043) | 0.187               | -0.093 (-0.229 to 0.021) | 0.103             |
| WMH volume        | 0.212 (0.106 to 0.370) | <0.001             | 0.173 (0.062 to 0.327) | 0.004             |
| Mean diffusivity  | 0.142 (0.045 to 0.284) | 0.007               | 0.165 (0.048 to 0.334) | 0.009             |
| Fractional Anisotropy| -0.084 (-0.236 to 0.048) | 0.195               | -0.089 (-0.247 to 0.049) | 0.188             |

Associations are presented as standardized betas with 95% CIs, analyzed by linear regression analyses adjusted for age and sex. NFL levels and WMH volumes were log-transformed.

NfL, neurofilament light; MRI, magnetic resonance imaging; SVD, small vessel disease; CI, confidence interval; WMH, white matter hyperintensity.

Table 3. Serum NFL levels at baseline in SVD patients with or without lacunes and microbleeds

| Variable | Lacunes | Microbleeds |
|----------|---------|-------------|
|          | Absent  | Present     | P       | Absent  | Present | P       |
| Baseline | 42.7 (31.9–58.5) | 55.7 (43.1–85.5) | <0.001 | 44.0 (32.6–59.8) | 56.9 (41.2–82.2) | 0.002 |
| Follow-up| 41.2 (31.6–55.4) | 55.3 (42.4–78.5) | <0.001 | 43.1 (32.3–59.3) | 50.1 (40.1–73.5) | 0.549 |
| Incidence| 41.3 (31.8–55.9) | 57.2 (42.9–82.4) | <0.001 | 43.5 (32.6–60.2) | 50.7 (41.2–71.5) | 0.921 |

Values are presented as median (interquartile range; NFL levels in pg/mL) for participants with and without (incident) lacunes or microbleeds. Differences are calculated by analysis of covariance (ANCOVA) with Bonferroni correction, adjusted for age and sex.

NfL, neurofilament light; SVD, small vessel disease.
els were associated with WMH-volume, MD, lacunes, and microbleeds at baseline in the subgroup with follow-up MRI (Tables 2 and 3). Association with lacunes and microbleeds at baseline remained significant when categorizing the lesions according to their numbers (lacunes: β=0.36, P<0.001; microbleeds: β=0.16, P=0.013).

NfL levels were significantly higher in subjects with lacunes (mean 122.3 pg/mL) versus without lacunes (59.7 pg/mL; P<0.0001). NfL levels were associated with processing speed (P<0.001), cognitive index (P=0.001), and memory function (P=0.016) at baseline for the subgroup with cognitive follow-up, adjusted for age, sex, education, and depression (Table 4).

Changes of MRI and cognitive measures over time
Changes in MRI-markers of SVD and cognitive performance over time are shown in the Supplementary Table 1: SVD-related MRI-markers showed progression during follow-up (P<0.0001). This was also the case for cognitive performance. No difference of progression regarding both, MRI-measures and cognition, was observed in subjects with or without antithrombotic medication.

Association of NfL with MRI-markers at follow-up
Serum NfL levels were associated with WMH-volume (β=0.173; 95% confidence interval [CI], 0.062 to 0.327; P=0.004) and MD (β=0.165; 95% CI, 0.048 to 0.334; P=0.009) at follow-up, after adjustment for age and sex (Table 2). This association was however not significant after additional adjustment for the respective baseline MRI-measure. We did not observe an association of NfL with changes of WMH-volume or MD over time.

NfL levels were associated with lacune number at follow-up and incident lacunes during the follow-up period after adjustment for age and sex (P<0.001) (Table 3). This was also the case when categorizing lacunes according to their number (β=0.32, P<0.001). The association with incident lacunes remained significant after adjustment for baseline lacunes, also in the quantitative categorization (P=0.040).

Association of NfL with cognition and dementia at follow-up
NfL was associated with cognitive index (P=0.001), memory (P=0.003) as well as processing speed (P=0.001) and—in contrast to baseline—also with executive function (P=0.033) at follow-up, independent of age, sex, educational level, and depression (Table 4). This association was, however, not independent of the respective cognitive measure at baseline. NfL levels were higher in participants who developed dementia during follow-up (n=65; NfL 74.8 pg/mL) versus those without dementia (n=438; 50.1 pg/mL; P<0.001). However, this difference disappeared when adjusting for age. The risk of developing dementia was increased with higher NfL levels (hazard ratio [HR], 5.0; 95% CI, 2.6 to 9.4; P<0.001), but again significance was lost after adjusting for age (HR, 1.6; 95% CI, 0.6 to 4.1; P=0.312).

Discussion
In subgroups from the RUN DMC-study with a 9-year follow-up, we here found serum NfL to be associated with incident lacunes in cerebral SVD, thus indicating disease progression. Recent studies have identified other biomarkers, such as markers of inflammation and hemostasis to be associated with imaging markers of SVD progression. Serum NfL may be of additional value as a blood based marker of SVD progression, given its association with both, imaging lesion-burden and cognitive performance.

Serum NfL was significantly higher in subjects with versus those without lacunes at baseline and was related to the presence and number of lacunes at follow-up as well as with the occurrence of incident lacunes during follow-up, independent of baseline lacune number.

### Table 4. Associations between baseline NfL levels and cognitive performance in patients with follow-up

| Variable           | Baseline cognition | Future cognition |
|--------------------|--------------------|-----------------|
|                    | β (95% CI)         | P               | β (95% CI) | P       |
| Cognitive performance |                   |                 |             |         |
| Cognitive index    | −0.160 (−0.184 to −0.045) | 0.001           | −0.145 (−0.206 to −0.057) | 0.001 |
| Memory             | −0.130 (−0.169 to −0.018) | 0.016           | −0.136 (−0.212 to −0.045) | 0.003 |
| Processing speed   | −0.204 (−0.262 to −0.090) | <0.001          | −0.159 (−0.242 to −0.068) | 0.001 |
| Executive function | −0.080 (−0.137 to 0.012) | 0.101           | −0.095 (−0.170 to −0.007) | 0.033 |

Associations between NfL levels and cognitive performance for all participants who completed repeated cognitive assessments (n=336), presented as standardized betas with 95% CIs, analyzed by linear regression analyses. NfL levels were log-transformed. P-values, adjusted for age, sex, education, and depressive symptoms. NfL, neurofilament light; CI, confidence interval.
Besides WMH, lacunes are a hallmark of SVD and the occurrence of lacunes is known to be of clinical relevance in SVD.\textsuperscript{16} Silent lacunes are associated with mild neuropsychological deficits in subjects with first-ever lacunar stroke.\textsuperscript{17} Lacunes occur in a temporospatial relation to WMH, appearing later and mostly at the edge of WMH.\textsuperscript{18} Our finding is in line with this course of tissue damage in SVD and with previous reports on NfL being sensitive to active SVD\textsuperscript{2} and on NfL predicting lacunar cavitation in small subcortical infarcts.\textsuperscript{19} Serum NfL is a circulating marker for neuroaxonal damage, thus reflecting altered structural integrity of axons and therefore a rather severe disease state. In SVD it is known that a higher baseline severity is associated with a more progressive course.\textsuperscript{9} Our results indicate that serum NfL represents this disease severity in SVD, indicating an active and potentially progressive course of the disease associated with the occurrence of incident lacunes.

NfL was furthermore associated with WMH volume and MD at follow-up, independent of age and sex. However, this association was not independent of the respective imaging measure at baseline. The latter is overall not surprising, given the strong cross-sectional association of NfL with lesion burden at baseline,\textsuperscript{1} indicating that NfL reflects disease severity captured by MRI. Serum NfL may thus be regarded as an additional marker to the MRI markers also capturing the extent of pathology related to SVD.

We found NfL to be associated with processing speed, independent of age and after adjustment for MD, which is an underlying substrate of vascular cognitive impairment in SVD. NfL thus also seems to be a sensitive marker of vascular neuroaxonal damage, reflecting the disease process underlying cognitive impairment in SVD. However, again, the association was not observed when adjusting for the respective cognitive test at baseline. Therefore, NfL cannot be regarded as an independent, but rather an additional marker of cognitive impairment, in line with our findings for WMH and MD.

Independently of relevant variables, including age, education, and depression, NfL was associated with future cognitive impairment in the group with follow-up, including processing speed, the domain most affected in SVD,\textsuperscript{16} and—in contrast to baseline—also executive function, which is also known to be an affected domain in SVD,\textsuperscript{20} therefore potentially indicating disease progression. The broader association with other domains, including global cognitive index and memory performance may suggest that NfL overall does not only reflect vascular, but also neurodegenerative pathologies associated with aging, as also previously shown in other studies.\textsuperscript{21-24} A recent study from our cohort showed that memory decline in elderly with SVD is best explained by the interaction of white matter damage and hippocampal volume, suggesting that memory decline observed in subjects with SVD is a heterogeneous process.\textsuperscript{11} However, in our study we did not identify serum NfL to be an independent marker of cognitive decline. Therefore, further studies in independent cohorts are needed to further address role of NfL as a potential predictor of future cognitive deterioration. Importantly, our results indicate that—when studying the role of NfL in neurodegenerative disease—concomitant cerebral SVD should be taken into account.

Limitations of our study are the lack of NfL-values at follow-up, thus not allowing to study the temporal course of NfL-levels. Different MRI-scanning/sequences at the timepoints potentially caused segmentation-differences. However, this is unlikely based on previous validation by repeating analyses for all time-periods within the RUN DMC-cohort.\textsuperscript{25} Finally, given the long-term follow-up, some—especially more affected participants—could not complete the follow-up. However, this attrition bias most likely results in underestimation of our findings, having potentially missed more severe lesion burden.

The strengths are the large cohort and longitudinal design over approximately 9 years and the comprehensive and standardized clinical and imaging work-up. Measurements of NfL was performed at an experienced centre using a very sensitive state-of-the-art Simoa assay.

**Conclusions**

Serum NfL was associated with future MRI measures and especially the occurrence of incident lacunes in SVD and may potentially be of additional value for disease monitoring and outcome, reflecting disease severity as well as progression in SVD. There was an independent association of NfL also with future cognitive impairment related to SVD. The overall association with a broader spectrum of cognitive impairment may indicate that NfL reflects both vascular and neurodegenerative processes in the elderly.

**Supplementary materials**

Supplementary materials related to this article can be found online at https://doi.org/10.5853/jos.2019.02845.

**Disclosure**

The authors have no financial conflicts of interest.
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**Supplementary Table 1.** MRI markers and cognitive performance over time

| Variable                                           | Baseline         | Follow-up        | P     |
|----------------------------------------------------|------------------|------------------|-------|
| MRI markers (n=264)                                |                  |                  |       |
| Total brain volume (mL)                            | 1,086.4±71.1     | 1,043.6±80.8     | <0.001|
| Grey matter volume (mL)                            | 620.8±49.1       | 599.2±51.7       | <0.001|
| White matter volume (mL)                           | 465.6±39.3       | 444.4±46.0       | <0.001|
| WMH volume (mL)                                    | 2.2 (0.8–5.8)    | 4.6 (2.0–11.4)   | <0.001|
| Lacunes                                            | 54 (20.5)        | 83 (31.4)        | <0.001|
| Microbleeds                                        | 36 (13.6)        | 66 (25.0)        | <0.001|
| NAWM mean diffusivity (10⁻³ mm²/sec)               | 0.85±0.04        | 0.85±0.07        | 0.999 |
| Cognitive performance (n=336)                      |                  |                  |       |
| Cognitive index                                    | 0.19±0.68        | −0.15±0.85       | <0.001|
| Memory                                             | 0.17±0.67        | −0.07±0.89       | <0.001|
| Processing speed                                   | 0.21±0.82        | −0.27±0.92       | <0.001|
| Executive function                                 | 0.16±0.73        | −0.14±0.87       | <0.001|

Values are presented as mean±standard deviation, median (interquartile range), or number (%). For cognitive performance z-scores based on the mean and standard deviation of the overall study population at baseline were used. Significant differences were calculated by repeated measures analysis of variance (ANOVA) for normally distributed variables and nonparametric tests.

MRI, magnetic resonance imaging; WMH, white matter hyperintensity; NAWM, normal appearing white matter.