Structural network changes in cerebral small vessel disease

Anil M. Tuladhar, MD, PhD[1]*; Jonathan Tay[9]; Esther M.C. van Leijsen, PhD[1]; Andrew Lawrence, PhD[2]; Ingeborg W.M. van Uden, MD, PhD[1]; Mayra I. Bergkamp, MSc[1]; Helena M. van der Holst, MD, PhD[3]; Roy P.C. Kessels, PhD[5,6]; David G. Norris, PhD[7,8]; Hugh S. Markus, MD FMED Sci[9]; Frank-Erik de Leeuw, MD, PhD[1]

[1] Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Donders Center for Medical Neuroscience, Radboud University Medical Center, Nijmegen, The Netherlands
[2] Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingkom
[3] Department of Neurology, Jeroen Bosch Ziekenhuis, 's-Hertogenbosch, The Netherlands
[4] Radboud University Medical Center, Diagnostic Image Analysis Group, Department of Radiology and Nuclear Medicine, Nijmegen, the Netherlands
[5] Department of Medical Psychology & Radboud Alzheimer Center, Radboud University Medical Center, Nijmegen, The Netherlands
[6] Radboud University, Donders Institute for Brain, Cognition and Behaviour, Centre for Cognition, Nijmegen, The Netherlands
[7] Radboud University, Donders Institute for Brain, Cognition and Behaviour, Centre for Cognitive Neuroimaging, Nijmegen, the Netherlands
[8] Erwin L. Hahn Institute for Magnetic Resonance Imaging, University of Duisburg-Essen, Essen, Germany
[9]Stroke Research Group Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK

*Corresponding author: Anil Man Tuladhar
Anil.Tuladhar@radboudumc.nl
Radboudumc, Department of Neurology
PO-box 9101, HP 935, 6500HB Nijmegen, The Netherlands

Keywords:
Cerebral small vessel disease; Structural neuroimaging; Diffusion Tensor Imaging; Cognitive decline; Mortality

Number of references: 45
Word count: 4409

Funding statement
Dr. Tuladhar is supported by a junior staff member grant of the Dutch Heart Foundation (grant number 2016 T044). Hugh Markus is supported by an NIHR Senior Investigator award. Prof. Dr. de Leeuw is supported by a clinical established investigator grant of the Dutch Heart Foundation (grant number 2014 T060), and by a VIDI innovational grant from The Netherlands Organisation for Health Research and Development (ZonMw grant 016.126.351).

Competing of Interests: The authors have no conflicts of interest.

ABSTRACT

Objectives To investigate whether longitudinal structural network efficiency is associated with cognitive decline and whether baseline network efficiency predicts mortality in cerebral small vessel disease (SVD).

Methods A prospective, single-center cohort consisting of 277 non-demented individuals with SVD was conducted. In 2011 and 2015, all participants were scanned with MRI and underwent neuropsychological assessment. We computed network properties using graph-theory from probabilistic tractography and calculated changes in psychomotor speed and overall cognitive index. Multiple linear regressions were performed, while adjusting for potential confounders. We divided the group into mild-to-moderate WMH and severe WMH group based on median split on WMH volume.

Results The decline in global efficiency was significantly associated with a decline in psychomotor speed in the group with severe WMH (β=0.18, p=0.03) and a trend with change in cognitive index (β=0.14, p=0.068), which diminished after adjusting for imaging markers for SVD. Baseline global efficiency was associated with all-cause mortality (HR per decrease of 1 SD 0.43, 95% CI 0.23–0.80, p = 0.008, C-statistic 0.76).

Conclusions Disruption of the network efficiency, a metric assessing the efficiency of network information transfer, plays an important role in explaining cognitive decline in SVD, which was however not independent of imaging markers of SVD. Furthermore, baseline network efficiency predicts risk of mortality in SVD that may reflect the global health status of the brain in SVD. This emphasises the importance of structural network analysis in the context of SVD research and the use of network measures as surrogate markers in research setting.

INTRODUCTION

Cerebral small vessel disease (SVD) is a common finding on brain imaging in older adults and plays a pivotal role in the development of cognitive impairment. Imaging markers of SVD constitute white matter hyperintensities (WMH), lacunes of presumed vascular origin, microbleeds[1] and impaired white matter microstructural integrity as assessed by diffusion tensor imaging (DTI).[2] These markers are associated with cognitive decline,[3,4] dementia[3,5] and death,[6] though the underlying mechanisms of how SVD affects these clinical outcomes remain incompletely understood.

A potential hypothesis is that SVD disrupts white matter tracts and thereby affects the underlying structural connectivity within the large-scale brain network. Structural networks can be derived from diffusion tensor imaging (DTI) followed by tractography in which the white matter tracts are represented as the underlying connections between brain regions. In previous cross-sectional studies, measures of network disruption at the structural level were more strongly associated with cognitive performance at baseline[7-9] and mediate at least in part the associations between the conventional MRI markers of SVD and cognitive impairment in SVD. As yet, little is known about the longitudinal change of structural network efficiency and its association with cognitive decline.

In a recent large systemic review and meta-analysis, individual neuroimaging markers of SVD (i.e., WMH, lacunes and microbleeds) were associated with increased risk of mortality, showing the clinical significance of these markers.[10] More importantly, the combined MRI markers of SVD may be more predictive of increased risk of mortality.[11] As structural network efficiency have been shown to mediate the effects of these conventional markers of SVD on functional outcome, they may better capture the cumulative effects of these markers,[9] and therefore be a stronger predictor of mortality. However, to date, it is not known whether this measure predicts functional status (mortality) in SVD participants.

In this study, we had two objectives. First, we computed structural networks based on DTI data and deterministic tractography to prospectively investigate the change in organizational properties of these structural networks in SVD, and how this is associated with cognitive decline. Second, we tested whether baseline network efficiency is predictive for all-cause mortality. We hypothesise that change in network efficiency is associated with change in cognitive performance, independently of the conventional MRI markers of SVD and that baseline network efficiency is associated with an increased risk of mortality.

Materials and methods

Study population

The study is a part of Radboud University Nijmegen Diffusion tensor and Magnetic resonance Cohort (RUN DMC) study[12] that prospectively investigates risk factors and clinical consequences of SVD. Participants were included if they had WMH and/or lacunar infarcts on MRI. Baseline data collection occurred in 2006, with two follow-ups occurring in 2011 and 2015. Due to a scanner upgrade between baseline and first follow-up, we only included participants with data from both 2011 and 2015. Of the 503 participants enrolled at baseline in 2006, 104 were lost to follow-up, deceased, or refused to participate in the follow-up assessment, 99 were excluded due to issues with the acquisition or processing of the MRI data or not having participated in the follow-up MRI assessment, and 23 individuals were excluded who developed dementia during the study period. This brought the final sample used in our study to 277. For the longitudinal analyses, we excluded an additional 50 participants, because patients were deceased at third follow-up or no follow-up MRI assessment could be performed (Figure 1). The study received ethical approval from the Medical Ethics Committee Arnhem-Nijmegen, and all participants signed an informed consent form.

Standard protocol approvals, registrations, and patient consents

All participants signed an informed consent form. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study (ID: 2005/256).

Cognitive assessment

Extensive neuropsychological evaluation included among others the Mini-Mental State Examination, Rey Auditory Verbal Learning Test (RAVLT),[13] Rey Complex Figure Task (RCFT),[14] Paper-Pencil Memory Scanning Task (PPMST),[15] an adapted version of the Stroop Color-Word Test,[16] Symbol Digit Substitution Task (SDST),[16] verbal fluency[16] and Verbal Series Attention Test (VSAT).[17,18] To account for possible learning effects, parallel versions have been used, where possible. Raw scores of all time-points were transformed into z-scores based on the mean and standard deviation (SD) of the baseline study population. We calculated Speed–Accuracy Trade-Off (SAT) scores where possible.[18,19] Cognitive decline over time was calculated for each participant individually, by subtracting baseline scores from the follow-up scores. In this study we focused on global cognitive function (cognitive index) and psychomotor speed, as these test scores showed the strongest associations with network efficiency[8,9] and are a major deficit in SVD. For the overall cognitive index, we calculated the mean of the z-scores of all tests from the neuropsychological test battery. Psychomotor speed was calculated as the mean of the z-scores of the PPMST, the reading and color naming tasks of the Stroop Test, and the SDST.

MRI acquisition

MR images were acquired on a Siemens Magnetom Avanto 1.5-Tesla MRI. The protocol included a T1-weighted 3D MPRAGE sequence (repetition time (TR) = 2250 ms, echo time (TE) = 2.95 ms, inversion time (TI) = 850 ms, flip angle = 15°, voxel size = 1.0 mm isotropic), a FLAIR sequence (TR = 14240 ms, TE = 89 ms, TI = 2200 ms, voxel size = 1.2 × 1.0 × 2.5 mm, interslice gap = 0.5 mm) and a DTI sequence (TR = 10200 ms, TE = 95 ms, voxel size = 2.5 mm isotropic; 7 scans with b = 0 s/mm², 61 scans with b = 900 s/mm²). Full acquisition details have been described previously.[12,20] Patients were carefully instructed to remain still on the scanner.

T1 images processing

Raw T1 images were corrected for the bias field using the N4 algorithm,[21] then processed by Freesurfer 6.0 (surfer.nmr.mgh.harvard.edu/) after lesion filling. Freesurfer is a software suite that automatically carries out processing and analysis of MR images, including skull stripping, segmentation, and cortical parcellation.[22] After cross-sectional processing, T1 images were further processed using FreeSurfer's longitudinal stream. This involved the creation of an unbiased within-participant template image followed by re-processing of the cross-sectional T1w images using common information from the template. This approach increases the reliability of individual segmentations.[23]

Radiological markers of SVD

WMH volumes were segmented using a semiautomatic method[24] and visually inspected for misclassification by one trained rater, blinded for clinical data. These volumes were normalised to intracranial volume, which was calculated by summing the grey matter, white matter, and cerebrospinal fluid volumes obtained from the Freesurfer output. We have divided the group into mild-to-moderate WMH group and severe WMH group using a median split based on the WMH-load. Lacunar infarcts were manually rated on the T1 and FLAIR images and microbleeds on the T2-weighted MRI scans by two trained raters who were blind to the clinical data following consensus criteria.[20]

DTI analysis

Using a local principal component analysis filter,[25] the raw diffusion data were denoised using in-house developed and matlab-based software, which were then corrected for head movement, cardiac motion and eddy currents using the PATCH algorithm.[26] Susceptibility distortions were unwarped by normalising the images to the T1 images in the phase-encoding direction using SPM12. FSL was then used to extract brain tissue and calculate the diffusion tensor.[27] Next, the diffusion parameters (fractional anisotropy) were fed into TBSS pipeline.[28] After performing this pipeline, data

were then fed into the voxel-wise cross-subject statistics. Furthermore, we have calculated the mean fractional anisotropy and mean diffusivity within the skeleton for each participants.

Probabilistic tractography

Single-fibre white matter response functions were estimated on the skull stripped DWI images using a previously described algorithm[29] implemented in MRtrix3 (mrtrix.org/). These response functions were then used for single-shell single-tissue constrained spherical deconvolution[30], which estimated fibre orientation distributions (FOD) from the b = 900 s/mm^2 shell using lmax = 6. The resulting FOD image was used for anatomically constrained tractography,[31] which uses anatomical priors to improve tractography results. This required masks of the cortical and subcortical grey matter, white matter, and cerebrospinal fluid (CSF), as well as a lesion mask to delineate areas where no anatomical priors should be applied. This lesion mask was defined as WMH in our study. The first four masks were obtained from the longitudinal Freesurfer segmentations. WMH segmentations for each participant were brought to Freesurfer space by applying a rigid body transformation calculated by registering the FLAIR images to the corresponding T1w images. Areas of WMH were then subtracted from the Freesurfer-estimated white matter mask to produce a mask of normal-appearing white matter (NAWM). The resulting grey matter, NAWM, CSF, and WMH masks were registered to the b0 image using an inverse boundary-based registration transformation with nearest neighbour interpolation. Probabilistic streamlines were seeded at the white matter-grey matter boundary and computed using the iFOD2 algorithm in MRtrix3. Tracking was constrained to be as similar to the deterministic approach used in our paper, with step size = 0.5 mm, minimum length = 20 mm, turning angle = 40°, and FOD amplitude cutoff = 0.1. Backtracking was enabled to re-computed streamlines if poor structural terminations were encountered. Tractography continued until 10^6 valid streamlines were generated, standardizing the total number of streamlines for each participant.

White matter network construction

Network nodes were defined using the Desikan-Killiany atlas,[32] which divides the cortex into sulcal and gryal-based ROIs. This was automatically completed thorough the Freesurfer pipeline. The resulting cortical atlas ROIs were combined with Freesurfer's segmentation of subcortical structures, resulting in a total of 82 nodes (41 per hemisphere). A weighted edge (connection) was constructed, where weights were defined based on the product of number of streamlines and the mean of fractional anisotropy of each connection. Prior to analysis, we thresholded the matrices by the group threshold of 90% (e.g. a connection was retained when this connection was present in at least 90% of participants) to eliminate the false positive connections. This resulted in an undirected weighted 82x82 matrix for each participant.

Network analysis

The brain connectivity toolbox (http://www.brain-connectivity-toolbox.net/) was used to compute graph-theoretical measures. We calculated the following network measures: density, total network strength and global efficiency. Global efficiency is the average inverse shortest path length in the network and reflects the extent to which information communication is globally integrated in the network. In this study, we particularly focus on global efficiency, as this measure has been previously shown to correlate with cognitive performance, especially processing speed, and conversion to dementia.[9,33]

Statistical analyses

Group differences between the participants with mild-to-moderate WMH and those with severe WMH at follow-up assessment were calculated with an independent T-test for nominal variables with age and follow-up duration as covariates, Chi-square test for categorical variables, and the Mann-Whitney-U test for non-parametric values. To test whether severe WMH group had lower fractional anisotropy compared to mild-to-moderate WMH group, we performed voxel-wise analyses by applying permutation-based statistical interference tool for non-parametric approach, with number of permutation tests set to 5000.[34] Next, we applied threshold-free cluster enhancement with a p-value< 0.05, corrected for multiple comparisons.[35] To examine which connections are disconnecting over time and which are related to cognitive decline, we have performed regional network analyses using the Network Based Statistic toolbox (nitrc.org/projects/nbs). Edges were significant at $t \geq 3.1$, and component sizes determined using cluster extent. Multiple comparisons were controlled using the NBS, and data was permuted 5000 times. Multiple linear regression analyses were performed adjusted for potential confounders (age, sex, education and baseline cognitive performance) to assess the relation between changes in cognitive performance and global efficiency. Since adjusting for the time to follow-up did not alter the results, we did not include this variable in the model. Cumulative mortality was estimated using Kaplan-Meier analysis. Differences between the mild-to-moderate and severe WMH groups were investigated using log-rank test. To investigate whether MRI measures were associated with all-cause mortality, Cox proportional regression analyses were used to obtain hazard ratios (HR) for each MRI measure (baseline global efficiency, WMH and brain volume, lacunes and microbleeds), adjusted for age. Sex and education were excluded from the model because they were not significantly associated with all-cause mortality and baseline.

Data availability

The dataset used in this study are available from the corresponding author on request.

RESULTS

Baseline demographic and neuroimaging characteristics and change over time are presented in Table 1 and Table 2 for the 277 participants included at the first follow-up in 2011. Mean age at baseline was 68.0 years (SD 8.0) and 56% were men. 227 out of 277 participants completed MR and clinical assessments at second follow-up that were available for subsequent analyses. Mean follow-up duration was 3.4 years (SD 0.2) and follow-up duration was similar in both WMH severity groups. A decline in processing speed was observed across the whole group (average z-score difference between two time points -0.29 (SD 0.43)) and in both WMH severity group (Table 1). Severe WMH group had significantly lower FA in the corpus callosum, mainly genu of the corpus callosum and frontal lobe, compared to mild-to-moderate WMH group at baseline ($p<0.05$, FWE-corrected for multiple comparisons; Figure 2).

Progression of radiological markers of SVD

WMH volume significantly increased (median 1.4 ml, interquartile range 0.5 – 3.1 ml) over the 3.4 years, which was mainly explained by an increase in the severe WMH group (median 3.3 ml, interquartile range 1.7 – 6.6 ml). 23 incident lacunes and 25 microbleeds developed during the follow-up period, with significantly higher number of incident lacunes in the severe WMH group compared to the mild-to-moderate WMH group. Mean MD significantly increased over time across the whole group (mean increase 5.7×10^{-6}), mild-to-moderate WMH group (mean increase 3.2×10^{-6}) and severe WMH group (mean increase 8.6×10^{-6}). Brain volume decreased significantly across the whole group (mean decrease 17.4ml, SD 12 ml). The decrease in brain volume was however not statistically different between the WMH groups.

Longitudinal changes in structural networks

Global efficiency was not significantly decreased for the whole group ($p=0.21$) and mild-to-moderate WMH group ($p=0.79$; Table 1). A significant decrease of global efficiency was observed for the severe group (-1.6 (6.6); $p=0.01$). A significant decrease of network density were observed for the whole group, the severe WMH group and mild-to-moderate WMH group (all $p<0.001$). Although no significant decrease of global efficiency was found in the mild-to-moderate group, these changes of network measures did not significantly differ between the WMH severity groups. A significant change was found for network strength for the whole group ($p<0.001$), for the severe WMH group ($p<0.001$) and mild-to-moderate group ($p=0.04$). Decrease in global efficiency was associated with an increase in WMH volume ($r=-0.15$, $p=0.02$), whereas changes in global efficiency were not significantly related to

brain atrophy (r=0.05, p=0.47), incident lacunes (r=-0.01, p=0.83) and incident microbleeds (r=0.03, p=0.69). Regional analyses using NBS did not reveal significant clusters of connections that are disconnection over time.

Association between change in diffusion parameters and cognitive decline

Change of mean FA was significantly associated with change in psychomotor speed (β=0.15, p=0.001), and to the lesser extent to change in cognitive index (β=0.10, p=0.03), adjusted for potential confounders, while change of mean MD was significantly associated with change in cognitive index (β=-0.13, p=0.006) and to the lesser extent with change of psychomotor speed (β=-0.13, p=0.02).

Associations between global efficiency and cognition

Association between baseline global efficiency and cognitive decline

First, we investigated the association between global efficiency at baseline and decline in processing speed. Baseline global efficiency was not associated with a decline in processing speed or cognitive index (β=0.07, p=0.221; β=0.03, p=0.538, respectively).

Association between change in global efficiency and cognitive decline

Second, we tested whether a decline in global efficiency was associated with a decline in processing speed in the whole group. The decline in global efficiency was not significantly related to a decline in processing speed across the whole group (β=0.05, p=0.34). Given the differential association between the mild-to-moderate and severe WMH group, we investigated this relationship further by subgrouping our sample based on SVD severity, using a median split based on the WMH-load. The decline in global efficiency was not associated with a decline in psychomotor speed in the mild-to-moderate group (β=-0.04, p=0.62), but the decline in global efficiency was significantly associated with a decline in psychomotor speed in the group with severe WMH (β=0.18, p=0.03) (Figure 3). After adjustment for the conventional MRI markers for SVD, this association diminished and was not significant (β=0.12, p=0.13). We reran the analyses using robust regression to account for the outliers that showed similar results (data not shown).

Changes in global efficiency were not significantly associated with changes in cognitive index in the whole group and mild-to-moderate group. There was a trend in the association between changes in global efficiency and changes in cognitive index (β=0.14, p=0.068), which diminished after adjustment for imaging markers for SVD. Sensitivity analysis in participants without lacunes showed that changes in global efficiency were not significantly associated with cognitive index in the whole group or mild-to-moderate WMH group. In the severe WMH group, changes in global efficiency was significantly

associated with changes in cognitive index (β=0.18, p=0.045), which remained significant after adjusting for imaging marker for SVD.

As the actual decline may be larger due to learning effects, we have subdivided the group based on the median split of cognitive decline (based on psychomotor speed). These analyses revealed that changes in global efficiency were not significantly related to changes in cognitive decline in both groups.

Global efficiency as predictor for mortality

15 participants died during the follow-up period (5.4%). Figure 4 shows the cumulative mortality between low and high global efficiency, showing higher mortality in participants with low global efficiency (log-rank test p=0.02). In the Cox regression model adjusted for age, baseline global efficiency was significantly associated with all-cause mortality (HR per decrease of 1 SD 0.43, 95% CI 0.23–0.80, p = 0.008, C-statistic 0.76). Presence of lacunes (HR 3.51, 95% CI 1.24–9.88, p = 0.018, C-statistic 0.76) at baseline was also associated with all-cause mortality, while baseline WMH, presence of microbleeds and brain volumes did not predict all-cause mortality. In the multivariate model including baseline global efficiency and presence of lacunes, only baseline global efficiency remained significantly associated with all-cause mortality. Similarly, accounting for cardiovascular risk factors, such as hypertension, hypercholesterolemia, diabetes, smoking and BMI, did not alter the results; baseline global efficiency was associated with increased mortality. Furthermore, the results remained similar when generating sensitivity models restricted to participants without lacunes or microbleeds.

DISCUSSION

In this prospective study, we demonstrate that the global efficiency of the structural networks, which is related to the progression of SVD, decreases over time. Moreover, these longitudinal changes in network efficiency are associated with a decline in processing speed in the group with severe WMH and with decline in cognitive index in participants without lacunes. Change in global efficiency, a metric assessing the efficiency of network information transfer, was however not significantly associated with change in psychomotor speed, after adjusting for conventional MRI markers of SVD. Baseline global efficiency predict all-cause mortality over a 3.5-year observation period in SVD patients, independent of cardiovascular risk factors.

Emerging evidence suggests that SVD is characterised by white matter network disruption that may play a pivotal role in the development of cognitive impairment and dementia.[36] Previous studies have shown that the degree of disruption of network efficiency is associated with the conventional MRI

markers of SVD.[8,9] Moreover, the associations between conventional markers of SVD or total burden of SVD and cognition are partly mediated through global efficiency.[8,9,37] Here, we demonstrated significant deterioration of the structural networks in SVD, characterised by reduced global efficiency and network density over the 3.5-year interval. During this time-interval a concomitant increase of WMH volume was observed, suggesting that the progression of SVD is associated with disruption of white matter network efficiency. Using NBS, we were not able to find significant cluster of connections that are disconnecting over time. This might suggest that the disconnection at regional level are minimal, which can only be observed when performing whole-brain analysis. Importantly, the disruption of network efficiency is associated with decline in processing speed in the group with severe WMH, though this association is diminished after adjusting for the conventional MRI markers of SVD. This suggest that the network efficiency based on the tractography technique employed in our study does not solely explain the cognitive decline in the whole population.

The trajectories of cognitive (dys)function over time vary considerably across individual participants. Some show a stable performance on cognitive tests or even improvements over time, while others perform substantially worse at follow-up. One possible explanation for this observation is that the impact of SVD on the brain depends on the extent to which it causes large-scale disruption in the efficient wiring of the white matter network. In other words, if the cumulative burden of damage, or strategically located damage, caused by the conventional MRI markers of SVD does not reach a critical threshold that is enough to cause network disruption, it will not result in subsequent cognitive decline.[33,37] Structural networks are robust to random perturbation (e.g., removal of random nodes or edge), whereas removal of targeted centrally located nodes or edges have greater impact on the efficiency of the network.[38,39] This might explain why incident lacunes do not significantly alter the network efficiency, because they probably do not reach a critical threshold of damage. Additional analyses showed that global efficiency in the participants with lacunar infarcts was not significantly decreased over time. The different cognitive trajectories also explain why baseline network efficiency (or other neuroimaging markers[18]) is not associated with *change* in cognitive functions.

Here, we included patients with a wide spectrum of SVD burden, thus allowing us to investigate the differential associations of global efficiency on cognitive decline at different stages of SVD burden. At baseline, participants with severe WMH had lower microstructural integrity (indicated by lower fractional anisotropy) in corpus callosum and frontal white matter compared to participants with mild-to-moderate WMH. Importantly, cognitive decline was related to a decrease in global efficiency, which was more pronounced in the more severe SVD group, although both groups showed a significant decline in cognitive functioning. An explanation for this observation is that the underlying (vascular)

damage of the white matter in the severe SVD group contributes more to cognitive decline than in the mild-to-moderate SVD group. Any additional deleterious effects of SVD are likely to have a greater impact on structural network integrity and, consequently, on cognitive outcomes. In contrast, other factors than the underlying white matter damage might play a more significant role in cognitive decline in mild-to-moderate SVD, for instance effects related to normal ageing. This aligns with studies showing stronger associations between network disruption and cognitive functioning in the more severely affected SVD patients than in less affected participants with SVD.[9]

Previous studies have shown that conventional MRI markers of SVD, such as WMH, are associated with an increased risk of mortality.[3,10,40] Here we extend these findings by demonstrating that network efficiency is significantly associated with mortality, while conventional markers of SVD are not or only weakly associated with mortality. The latter is possibly due to the relatively low statistical power (with a limited number of deaths at the follow-up of our study). This suggests that the disruption of structural network efficiency is not only an important marker for cognitive decline[33,41] or dementia,[9,33] but also for increased risk of mortality. As the participants died before second MR scanning could be performed, we are unable to examine whether changes in global efficiency was association with an increased risk of mortality. The underlying mechanism is not fully understood, but a possible explanation could be that the disruption of network efficiency could serve as intermediate marker for the underlying vascular damage. Previous studies have shown that global efficiency was strongly related to the conventional MRI markers of SVD.[8,42] This suggests that global efficiency can be used as an useful marker for the underlying vascular damage in SVD.[37] Structural network measures may be able to capture the burden of SVD more accurately than conventional markers of SVD and are associated with vascular risk factors.[9] Another explanation may be that structural network disruption reflects, in part, ongoing neurodegeneration.[43] Neurodegenerative processes, such as Alzheimer pathology, are also characterized by alterations in organizational properties of structural networks.[44] Previous studies have shown that brain atrophy (as a marker for neurodegenerative process) also predicts mortality.[6]

Limitations

Due to a scanner upgrade between 2006 and 2011, we were only able to use the follow-up data from 2011 and 2015, resulting in a substantial reduction of the sample size. This is unfortunate, but hard to avoid in long-running prospective studies. The reduced sample size also limited the statistical power for the mortality analysis, as fewer patients were monitored who deceased during follow-up. This restricted our analysis to all-cause mortality and we were not able to perform subgroup-analysis based on the cause of mortality. Also, as same cognitive tests have been administered during follow-up,

learning effects may have occurred, especially cognitive tests with a memory-component. To account for possible learning effects, parallel versions have been used, where possible. If any, we think that the learning effects would have underestimated the degree of decline of cognitive function in our study. Another limitation is that control data on this scanner or cognitive data on cognition is not available. Furthermore, we have acquired single-shell DTI on 1.5 Tesla scanner with relatively low resolution images. Despite the sophisticated probabilistic tractography applied in this study, DTI tractography can be confounded by regions of crossing fibers, partial volume effects and insufficient signal/noise ratio, thus limiting the interpretation of the results. Future studies are therefore warranted to replicate our results by performing improved tractography techniques on newer diffusion protocols (higher angular and spatial resolution, modelling the effects of WMH on fibre orientation, multiple b-shells and/or higher field strength).[45] It is also important to note that network analysis is not yet available for the large studies as this type of analysis is relatively new, requires specific imaging sequences and analysis. Future large studies should be conducted that might be able to address this in time.

CONCLUSION

This study provides further evidence that SVD causes cognitive decline through a disruption of white matter network efficiency, especially in participants with severe SVD. However, the association between change in global efficiency and change in psychomotor speed was not significant after adjusting for conventional MRI markers for SVD, suggesting that the network efficiency based on the tractography technique used in our study does not solely explain cognitive decline in this population. Moreover, baseline global efficiency predicts the risk of mortality, which may reflect the vital health status of individuals. This emphasises the importance of structural network analysis in context of SVD research and the use of network measures as surrogate markers in research settings, though more research is needed.

Contributor Statement

Dr. Tuladhar: involved in data collection, analysis and interpretation of data, and drafting and revising the manuscript.

Drs. Tay: involved in analysis of data

Dr. van Leijsen: involved in data collection and in revising the manuscript.

Dr. Lawrence: involved in analysis and revising the manuscript.

Dr. van Uden: involved in data collection and revising the manuscript.

Drs. Bergkamp: involved in data collection and revising the manuscript.

Dr. van der Holst: involved in data collection, data analysis and in revising the manuscript.

Prof. Kessels: involved in data collection, data analysis and revising the manuscript.

Prof. Norris: involved in study concept and design and revising the manuscript.

Prof. Markus: involved in data analysis and revising the manuscript.

Prof. de Leeuw: involved in study concept and design, interpretation of data, revising the manuscript and obtaining funding.

	N = 277	N = 227 (complete follow-up)
Age, yr (SD)	68.0 (8.0)	67.0 (7.6)
Sex, men (%)	155 (56)	127 (56)
Education, median (range)	5 (5-6)	5 (5-6)
MMSE, median (range)	29 (28-30)	29 (28-30)
Vascular risk factors		
Hypertension, n (%)	219 (79)	172 (76)
Diabetes, n (%)	35 (13)	25 (11)
Smokers, n (%)	38 (14)	30 (13)
Hypercholesterolemia, n (%)	121 (44)	94 (41)
Body mass index, mg/kg^2, mean (SD)	27.8 (4.7)	27.7 (4.2)
Neuroimaging		
WMH, ml, median (range)	2.9 (1.3 – 8.1)	2.5 (1.1 – 6.4)
Lacunes, n (%)	65 (23.5)	46 (20.3)
Microbleeds, n (%)	53 (19.1)	32 (14.1)
Total brain volume, ml (SD)	1073 (104)	1086 (104)
Mean diffusivity, (SD)	8.2×10^{-4} (3.9×10^{-5})	8.2×10^{-4} (3.7×10^{-5})
Network density, (SD)	0.52 (0.02)	0.53 (0.01)
Network strength, (SD)	75.8 (6.2)	76.3 (5.8)
Global efficiency, (SD)	142.1 (16.2)	144.3 (14.5)

Table 1. Baseline and neuroimaging characteristics of the whole population and of the population with complete followup

Data represent mean ± SD, n of participants (%) or median (interquartile range).

Abbreviations: MMSE = Mini Mental State Examination. N = number. WMH = White matter hyperintensities.

		Follow-up	
	Whole group	**Mild-to-moderate WMH**	**Severe WMH**
	N = 227	N = 124	N = 103
Psychomotor speed, z-score (SD)	-0.29^ (0.4)	-0.32^ (0.4)	-0.25^ (0.5)
Cognitive index, z-score (SD)	-0.24^ (0.4)	-0.26^ (0.4)	-0.22^ (0.4)
Neuroimaging			
WMH, ml, median (range)	1.4^* (0.5 – 3.1)	0.7 (0.2 - 1.4)	3.3^ (1.7 – 6.6)
Lacunes, incident	23^{+}*	5^{+}	18^{+}
Microbleeds, incident	25^{+}	9^{+}	16^{+}
Total brain volume, ml (SD)	-17.4^ (12)	-17.3^ (11)	-17.5^ (14)
Mean diffusivity, (SD)	5.7^ x 10^{-6} (0.1 x 10^{-5})	3.2^ x 10^{-6} (9.8 x 10^{-6})	8.6^ x 10^{-6} (1.3 x 10^{-5})
Network density, (SD)	-4.4^ x 10^{-3} (1.2 x 10^{-2})	-4.1^ x 10^{-4} (1.3 x 10^{-2})	-4.7^ x 10^{-3} (1.2 x 10^{-2})
Network strength, (SD)	1.2^ (3.2)	1.5^ (3.2)	0.67^ (3.2)
Global efficiency, (SD)	-0.6 (7.5)	0.2 (8.1)	-1.6^ (6.6)

Table 2. Cognitive and neuroimaging changes over time

Data represent mean ± SD, n of incident lacunes or microbleeds at follow-up or median (IQR).

Abbreviations: WMH = White matter hyperintensities.

^Significant change from baseline based on independent T-test ($p<0.05$)

^Significant change from baseline based on Chi-square test ($p<0.05$)

*Significant difference between the groups ($p<0.05$)

Legends to the figures

Figure 1. Flowchart study-population at baseline and follow-up. Due to a scanner upgrade between 2006 and 2011, we were only able to include the study population from 2011 and 2015.

Figure 2. Voxel-wise analysis of the difference in fractional anisotropy between participants with mild-to-moderate and severe WMH, adjusted for age, sex, education and conventional markers of SVD, performed with a 2-sample t-test, thresholded at $p<0.05$ and corrected for multiple comparisons. The statistical maps are superimposed onto the spatially normalized (Montreal Neurological Institute stereotactic space) T1 map.

Figure 3. Longitudinal associations between global efficiency and psychomotor speed. No association was found between change in global efficiency and change in psychomotor speed in the mild-to-moderate WMH group (N = 124), whereas in the severe WMH group decline in global efficiency was significantly related to decline in psychomotor speed, adjusted for age, sex, education and baseline psychomotor speed. Negative values represent decline in scores over time.

Figure 4. Cumulative mortality was estimated using Kaplan-Meier analysis and the difference between the low and high global efficiency was estimated with the log-rank test. Higher mortality was observed in participants with low global efficiency compared to high global efficiency (log-rank test $p=0.02$)

REFERENCES

1 Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *The Lancet Neurology* 2013;12(8):822-38. doi: 10.1016/S1474-4422(13)70124-8

2 Pasi M, van Uden IW, Tuladhar AM, et al. White Matter Microstructural Damage on Diffusion Tensor Imaging in Cerebral Small Vessel Disease: Clinical Consequences. *Stroke* 2016;47(6):1679-84. doi: 10.1161/STROKEAHA.115.012065

3 Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *Bmj* 2010;341:c3666. doi: 10.1136/bmj.c3666

4 de Groot JC, de Leeuw FE, Oudkerk M, et al. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. *Annals of neurology* 2000;47(2):145-51.

5 Zeestraten EA, Lawrence AJ, Lambert C, et al. Change in multimodal MRI markers predicts dementia risk in cerebral small vessel disease. *Neurology* 2017;89(18):1869-76. doi: 10.1212/WNL.0000000000004594

6 van der Holst HM, van Uden IW, Tuladhar AM, et al. Factors Associated With 8-Year Mortality in Older Patients With Cerebral Small Vessel Disease: The Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Cohort (RUN DMC) Study. *JAMA neurology* 2016;73(4):402-9. doi: 10.1001/jamaneurol.2015.4560

7 Reijmer YD, Fotiadis P, Martinez-Ramirez S, et al. Structural network alterations and neurological dysfunction in cerebral amyloid angiopathy. *Brain : a journal of neurology* 2015;138(Pt 1):179-88. doi: 10.1093/brain/awu316

8 Lawrence AJ, Chung AW, Morris RG, et al. Structural network efficiency is associated with cognitive impairment in small-vessel disease. *Neurology* 2014;83(4):304-11. doi: 10.1212/WNL.0000000000000612

9 Tuladhar AM, van Uden IW, Rutten-Jacobs LC, et al. Structural network efficiency predicts conversion to dementia. *Neurology* 2016;86(12):1112-9. doi: 10.1212/WNL.0000000000002502

10 Debette S, Schilling S, Duperron MG, et al. Clinical Significance of Magnetic Resonance Imaging Markers of Vascular Brain Injury: A Systematic Review and Meta-analysis. *JAMA neurology* 2018 doi: 10.1001/jamaneurol.2018.3122

11 Rensma SP, van Sloten TT, Launer LJ, et al. Cerebral small vessel disease and risk of incident stroke, dementia and depression, and all-cause mortality: A systematic review and meta-analysis. *Neuroscience and biobehavioral reviews* 2018;90:164-73. doi: 10.1016/j.neubiorev.2018.04.003

12 van Norden AG, de Laat KF, Gons RA, et al. Causes and consequences of cerebral small vessel disease. The RUN DMC study: a prospective cohort study. Study rationale and protocol. *BMC neurology* 2011;11:29. doi: 10.1186/1471-2377-11-29

13 Van der Elst W, van Boxtel MP, van Breukelen GJ, et al. Rey's verbal learning test: normative data for 1855 healthy participants aged 24-81 years and the influence of age, sex, education, and mode of presentation. *J Int Neuropsychol Soc* 2005;11(3):290-302. doi: 10.1017/S1355617705050344

14 Caffarra P, Vezzadini G, Dieci F, et al. Rey-Osterrieth complex figure: normative values in an Italian population sample. *Neurol Sci* 2002;22(6):443-7. doi: 10.1007/s100720200003

15 Van Der Elst W, Van Boxtel MP, Van Breukelen GJ, et al. Assessment of information processing in working memory in applied settings: the paper and pencil memory scanning test. *Psychological medicine* 2007;37(9):1335-44. doi: 10.1017/s0033291707000360

16 van der Elst W, van Boxtel MP, van Breukelen GJ, et al. The Letter Digit Substitution Test: normative data for 1,858 healthy participants aged 24-81 from the Maastricht Aging Study (MAAS): influence of age, education, and sex. *J Clin Exp Neuropsychol* 2006;28(6):998-1009. doi: 10.1080/13803390591004428

17 Mahurin RK, Cooke N. Verbal series attention test: Clinical utility in the assessment of dementia. *Clin Neuropsychol* 1996;10(1):43-53. doi: Doi 10.1080/13854049608406662
18 van Uden IW, van der Holst HM, Schaapsmeerders P, et al. Baseline white matter microstructural integrity is not related to cognitive decline after 5 years: The RUN DMC study. *BBA clinical* 2015;4:108-14. doi: 10.1016/j.bbacli.2015.10.001
19 Heitz RP. The speed-accuracy tradeoff: history, physiology, methodology, and behavior. *Front Neurosci* 2014;8:150. doi: 10.3389/fnins.2014.00150
20 van Leijsen EMC, van Uden IWM, Ghafoorian M, et al. Nonlinear temporal dynamics of cerebral small vessel disease: The RUN DMC study. *Neurology* 2017;89(15):1569-77. doi: 10.1212/WNL.0000000000004490
21 Tustison NJ, Avants BB, Cook PA, et al. N4ITK: improved N3 bias correction. *IEEE transactions on medical imaging* 2010;29(6):1310-20. doi: 10.1109/TMI.2010.2046908
22 Fischl B. FreeSurfer. *NeuroImage* 2012;62(2):774-81. doi: 10.1016/j.neuroimage.2012.01.021
23 Reuter M, Schmansky NJ, Rosas HD, et al. Within-subject template estimation for unbiased longitudinal image analysis. *NeuroImage* 2012;61(4):1402-18. doi: 10.1016/j.neuroimage.2012.02.084
24 Ghafoorian M, Karssemeijer N, van Uden IW, et al. Automated detection of white matter hyperintensities of all sizes in cerebral small vessel disease. *Medical physics* 2016;43(12):6246. doi: 10.1118/1.4966029
25 Manjon JV, Coupe P, Concha L, et al. Diffusion weighted image denoising using overcomplete local PCA. *PloS one* 2013;8(9):e73021. doi: 10.1371/journal.pone.0073021
26 Zwiers MP. Patching cardiac and head motion artefacts in diffusion-weighted images. *NeuroImage* 2010;53(2):565-75. doi: 10.1016/j.neuroimage.2010.06.014
27 Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage* 2004;23 Suppl 1:S208-19. doi: 10.1016/j.neuroimage.2004.07.051
28 Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *NeuroImage* 2006;31(4):1487-505. doi: 10.1016/j.neuroimage.2006.02.024
29 Tournier JD, Calamante F, Connelly A. Determination of the appropriate b value and number of gradient directions for high-angular-resolution diffusion-weighted imaging. *NMR in biomedicine* 2013;26(12):1775-86. doi: 10.1002/nbm.3017
30 Tournier JD, Calamante F, Connelly A. Robust determination of the fibre orientation distribution in diffusion MRI: non-negativity constrained super-resolved spherical deconvolution. *NeuroImage* 2007;35(4):1459-72. doi: 10.1016/j.neuroimage.2007.02.016
31 Smith RE, Tournier JD, Calamante F, et al. Anatomically-constrained tractography: improved diffusion MRI streamlines tractography through effective use of anatomical information. *NeuroImage* 2012;62(3):1924-38. doi: 10.1016/j.neuroimage.2012.06.005
32 Desikan RS, Segonne F, Fischl B, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* 2006;31(3):968-80. doi: 10.1016/j.neuroimage.2006.01.021
33 Lawrence AJ, Zeestraten EA, Benjamin P, et al. Longitudinal decline in structural networks predicts dementia in cerebral small vessel disease. *Neurology* 2018;90(21):e1898-e910. doi: 10.1212/WNL.0000000000005551
34 Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Human brain mapping* 2002;15(1):1-25.
35 Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *NeuroImage* 2009;44(1):83-98. doi: 10.1016/j.neuroimage.2008.03.061
36 Ter Telgte A, van Leijsen EMC, Wiegertjes K, et al. Cerebral small vessel disease: from a focal to a global perspective. *Nature reviews Neurology* 2018;14(7):387-98. doi: 10.1038/s41582-018-0014-y

37 Heinen R, Vlegels N, de Bresser J, et al. The cumulative effect of small vessel disease lesions is reflected in structural brain networks of memory clinic patients. *NeuroImage Clinical* 2018;19:963-69. doi: 10.1016/j.nicl.2018.06.025

38 Alstott J, Breakspear M, Hagmann P, et al. Modeling the impact of lesions in the human brain. *PLoS Comput Biol* 2009;5(6):e1000408. doi: 10.1371/journal.pcbi.1000408

39 van den Heuvel MP, Sporns O. Rich-club organization of the human connectome. *J Neurosci* 2011;31(44):15775-86. doi: 10.1523/JNEUROSCI.3539-11.2011

40 Sabayan B, van der Grond J, Westendorp RG, et al. Accelerated progression of white matter hyperintensities and subsequent risk of mortality: a 12-year follow-up study. *Neurobiology of aging* 2015;36(6):2130-5. doi: 10.1016/j.neurobiolaging.2015.03.003

41 Reijmer YD, Fotiadis P, Riley GA, et al. Progression of Brain Network Alterations in Cerebral Amyloid Angiopathy. *Stroke* 2016;47(10):2470-5. doi: 10.1161/STROKEAHA.116.014337

42 Tuladhar AM, van Dijk E, Zwiers MP, et al. Structural network connectivity and cognition in cerebral small vessel disease. *Human brain mapping* 2016;37(1):300-10. doi: 10.1002/hbm.23032

43 de la Torre JC. Alzheimer disease as a vascular disorder: nosological evidence. *Stroke* 2002;33(4):1152-62.

44 Reid AT, Evans AC. Structural networks in Alzheimer's disease. *Eur Neuropsychopharmacol* 2013;23(1):63-77. doi: 10.1016/j.euroneuro.2012.11.010

45 Sun W, Amezcua L, Shi Y. FOD Restoration for Enhanced Mapping of White Matter Lesion Connectivity. *Med Image Comput Comput Assist Interv* 2017;10433:584-92. doi: 10.1007/978-3-319-66182-7_67