White matter integrity in small vessel disease is related to cognition

Anil M. Tuladhara, Anouk G.W. van Norden, Karlijn F. de Laat, Marcel P. Zwiers, Ewoud J. van Dijk, David G. Norris, Frank-Erik de Leeuw

Abstract

Cerebral small vessel disease, including white matter hyperintensities (WMH) and lacunes of presumed vascular origin, is common in elderly people and is related to cognitive impairment and dementia. One possible mechanism could be the disruption of white matter tracts (both within WMH and normal-appearing white matter) that connect distributed brain regions involved in cognitive functions. Here, we investigated the relation between microstructural integrity of the white matter and cognitive functions in patients with small vessel disease. The Radboud University Nijmegen Diffusion tensor and Magnetic resonance Cohort study is a prospective cohort study among 444 independently living, non-demented elderly with cerebral small vessel disease, aged between 5500 and 85 years. All subjects underwent magnetic resonance imaging and diffusion tensor imaging scanning and an extensive neuropsychological assessment. We showed that loss of microstructural integrity of the white matter at specific locations was related to specific cognitive disturbances, which was mainly located in the normal-appearing white matter (p < 0.05, FWE-corrected for multiple comparisons). The microstructural integrity in the genu and splenium showed the highest significant relation with global cognitive function and executive functions, in the cingulum bundle with verbal memory performance. Associations between diffusion tensor imaging parameters and most cognitive domains remained present after adjustment for WMH and lacunes. In conclusion, cognitive disturbances in subjects with cerebral small vessel disease are related to microstructural integrity of multiple white matter fibers (within WMH and normal-appearing white matter) connecting different cortical and subcortical regions.

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1. Introduction

Cerebral small vessel disease (SVD) manifests on conventional MR images (i.e. T1 and Fluid Attenuated Inversion Recovery (FLAIR)) as white matter hyperintensities (WMH) and lacunes of presumed vascular origin (Wardlaw et al., 2013). These SVD markers are commonly observed in the elderly population. SVD is related to cognitive impairment and may, in some, ultimately lead to dementia (de Groot et al., 2000; Vermeer et al., 2003). This is supposedly due to the disruption of important white matter (WM) tracts. Despite the high prevalence of SVD (de Leeuw et al., 2001; Vernooij et al., 2007), relatively few develop evident cognitive decline or dementia (Vermeer et al., 2003). Other factors, apart from WMH and lacunes, presumably play a role in the transition from relative intact cognitive performance to severe cognitive decline in these few individuals. One of these factors could be the loss of microstructural integrity of the largest part of the WM; the on FLAIR imaging normal-appearing white matter (NAWM) surrounding the SVD. Pathological studies have demonstrated loss of microstructural integrity in the NAWM (Grafton et al., 1991) that cannot be visualized with conventional imaging, but can be investigated with diffusion tensor imaging (DTI).

DTI provides information on the microstructural integrity of the WM. DTI measures the local water diffusion profiles by: fractional anisotropy (FA), which represents a normalized ratio of diffusion directionality; mean diffusivity (MD), which reflects the overall magnitude of water diffusion; axial diffusivity (AD), which reflects the diffusivity parallel to the WM tracts and radial diffusivity (RD), which is the diffusivity perpendicular to these tracts (Pierpaoli et al., 1996). Loss of...
region. The cognitive index is a compound score that was calculated as the mean of the z-scores of the 1-letter subtask of the Paper–Pencil Memory Scanning Task, the mean of the reading subtask of the Stroop test, the mean of the Symbol–Digit Substitution Task and the mean of the added score on the three learning trials of the Rey Auditory Verbal Learning Test and the delayed recall of this last test (Vermeer et al., 2003). Verbal memory is a compound score of the mean of two z-scores from the Rey Auditory Verbal Learning Test; one for the added scores of the three learning trials of this test, and one for the delayed recall of this test. Visuospatial memory is a compound score of the mean of the z-scores of the immediate recall trial and the delayed recall trial of the Rey’s Complex Figure Test. Psychomotor speed was calculated as the mean of the z-scores of the 1-letter subtask of the Paper–Pencil Memory Scanning Task, the reading subtask of the Stroop test and the Symbol–Digit Substitution Task. Fluency was calculated from the mean of the z-scores of both verbal fluency tasks. Concept shifting was calculated as the z-score of the third subtask of the Stroop. Attention is a compound score of the z-score of the total time of the Verbal Series Attention Test (de Groot et al., 2000).

2.3. Magnetic resonance imaging scanning protocol

MRI scans of all participants were acquired on a single 1.5-T MRI. The protocol included, among other sequences, the following whole brain scans: 3D T1 magnetization-prepared rapid gradient echo (MPRAGE) sequence (TR/TE/TI 2250/3.68/850 ms; flip angle 15°; voxel size 1.0 x 1.0 x 1.0 mm), a fluid-attenuated inversion recovery (FLAIR) sequence (TR/TE/TI 9000/84/2200 ms; voxel size 1.0 x 1.2 x 5.0 mm, interslice gap 1 mm) and DTI sequences (TR/TE 10.100/93 ms; voxel size 2.5 x 2.5 x 2.5 mm; 4 unweighted scans, 30 diffusion weighted scans with b-value 900 s/mm2).

2.4. Conventional magnetic resonance imaging analysis

WMH were manually segmented on FLAIR images and the number of lacunes was rated according to a standardized protocol (van Norden et al., 2011). In addition, the visual Fazekas scale was used on the FLAIR images to rate the severity of changes in the white matter (Fazekas et al., 1987). All imaging analyses were performed by two trained raters blinded to clinical information. In a random sample of 10%, interrater variability for total WMH volume yielded an intra-class correlation coefficient of 0.99. The probability map of the white matter hyperintensities were created using a method previously described by de Laat and colleagues (de Laat et al., 2011). In short, we registered the WMH maps to the T1 images using the transformation matrix from the registration parameters of skull-stripped FLAIR images to the T1-images that were obtained using Functional MRI of the Brain linear image registration tool (http://www.fmrib.ox.ac.uk/fsl/fnirt). Next, we normalized the WMH maps non-linearly to the group-specific template using the transformation parameters of T1 images to the group-specific template obtained from Functional MRI of the Brain linear registration tool (http://www.fmrib.ox.ac.uk/fsl/flirt). Finally, we averaged the normalized WMH maps to create a probability map of the WMH of the study population (Fig. 1).

We computed gray (GM) and WM tissue and cerebrospinal fluid (CSF) probability maps using SPM 5 unified segmentation routines on the T1 MPRAGE images (Ashburner and Friston, 2005). Total GM, WM and CSF volumes were calculated by summing all voxel volumes that had a p > 0.5 for belonging to that tissue class. Total brain volume (TBV) was taken as the sum of total GM and WM. Intracranial volume (ICV) was a summation of all tissue classes. To normalize for head size, TBV was expressed as percentage of total ICV.

2.5. DTI analysis

Tract-based spatial statistics (TBSS) is a relatively new method that mitigates the limitations of VBM analysis (Smith et al., 2006). This analysis is restricted to those WM voxels that constitute the skeleton (core) of the brains connectional architecture. This skeleton can be matched...
more accurately (compared to whole-brain normalization) across sub-
jects, enabling robust voxel-wise analysis of the microstructural WM in-
tegrity across subjects.

Diffusion data were first preprocessed to detect and correct head
and cardiac motion artifacts, using an in-house developed iteratively
re-weighted-least-squares algorithm named ‘PATCH’ (Zwiers, 2010).
FA, MD, AD and RD images were then calculated using DTIFit within
the Functional MRI of the Brain diffusion toolbox, which were fed into
the TBSS pipeline (Smith et al., 2006). The thinning procedure was
conducted on the mean FA image to create a common skeleton, which
represents the core-structure of the WM tract. This skeleton was
thresholded at the FA-value 0.3 to include the major WM tracts and to
account for the inter-subject variability. All normalized FA data were
then projected onto this skeleton. These skeleton projection factors
were then applied to the MD, AD and RD images. These data were
then fed into the voxel-wise cross-subject statistics. In addition, we ob-
tained FA, MD, AD and RD for three parts of the corpus callosum by
performing region-of-interest analyses. The corpus callosum provides
interhemispheric connections between cortical and subcortical regions
and might play an important role in cognitive function (Bloom and
Hynd, 2005). We created masks for genu, body and splenium of the cor-
pus callosum by applying the WM atlas (Johns Hopkins University WM
labels, provided by Functional MRI of the Brain Software Library (FSL))
on the mean FA skeleton. The masks were visually inspected and miscel-
naneous voxels that belonged to other regions, such as the cingulum
bundle, were excluded.

2.6. Other measurements

Age, sex and level of education, depressive symptoms and normal-
ized TBV were considered possible confounders. Depressive symptoms
were assessed using the Center of Epidemiologic Studies on Depression
Scale (CES-D) (Radloff, 1977). Functional independence was assessed
using the Barthel Index (range 0–20) (Mahoney and Barthel, 1965).
For assessment of vascular risk factors, structured questionnaires were
used together with measurements of blood pressure taken on separate
occasions. The risk factors included presence of hypertension (mean
blood pressure ≥140/90 mm Hg and/or use of anti-hypertensive medica-
tions) (Rosendoff, 2007), diabetes (treatment with antidiabetic
drugs), hypercholesterolemia (treatment with lipid-lowering drugs)
and smoking status.

2.7. Statistical analysis

Baseline characteristics were presented as mean ± standard devia-
tion (SD) and for the skewed parameters the median and interquartile
ranges were calculated.

For the TBSS analyses, we assessed voxel-wise correlations between
the skeletal DTI parameters (FA and MD) and cognitive performance on
several cognitive domains, while adjusting for age, sex, education, de-
pressive symptoms and normalized TBV. To test whether these associa-
tions were independent of WMH and lacunes, we adjusted for WMH
volume and number of lacunes in a second model. For the voxel-wise
statistical analyses, we applied permutation-based statistical interfer-
tance tool for non-parametric approach, with number of permutation
tests set to 5000 (Nichols and Holmes, 2002). Significant clusters
were identified using the threshold–free cluster enhancement with a
p-value < 0.05, corrected for multiple comparisons (Smith and Nichols,
2009).

For the ROI analyses, we computed regression coefficients of the
mean FA, MD, AD and RD of the three ROI in the corpus callosum with
cognitive performance, while adjusting for age, sex, education, depres-
sive symptoms, normalized TBV, white matter hyperintensities and
number of lacunes. Regression coefficients were presented as standard-
ized b-values. Bonferroni corrections were applied.

3. Results

Demographic and neuroimaging characteristics are shown in
Table 1. Mean age of the population (n = 444) was 65.3 years (SD
8.9) and 54.7% were male. Mean WM volume was 467.4 ml (SD 65.4).
The largest part of the WM consisted of NAWM, with a median per-
cent-hyperechogenicity of 98.6 (IQR 96.4–99.3). According to Fazekas visual rating scale, 10
participants (2.3%) had Fazekas score 0, 296 participants (66.7%) had
Fazekas-score 1, 88 participants (19.8%) had Fazekas score 2 and 50 par-
ticipants (11.3%) had Fazekas score 3. White matter hyperintensities

Table 1. Baseline characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n = 444</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.3 (8.9)</td>
</tr>
<tr>
<td>Male*</td>
<td>243 (54.7)</td>
</tr>
<tr>
<td>Only primary education*</td>
<td>44 (10)</td>
</tr>
<tr>
<td>CES-D scale</td>
<td>11.2 (9.5)</td>
</tr>
<tr>
<td>Mini mental state examination</td>
<td>28.1 (1.6)</td>
</tr>
<tr>
<td>Hypertension*</td>
<td>320 (72.1)</td>
</tr>
<tr>
<td>Diabetes*</td>
<td>61 (13.7)</td>
</tr>
<tr>
<td>Hypercholesterolemia*</td>
<td>194 (43.7)</td>
</tr>
<tr>
<td>Smokers, current*</td>
<td>69 (15.5)</td>
</tr>
<tr>
<td>Smokers, former*</td>
<td>239 (53.8)</td>
</tr>
<tr>
<td>Neuroimaging</td>
<td></td>
</tr>
<tr>
<td>TBV, ml</td>
<td>1098.0 (1205)</td>
</tr>
<tr>
<td>ICV, ml</td>
<td>1673.8 (1582)</td>
</tr>
<tr>
<td>WMH volume, ml†</td>
<td>6.4 (3.3; 16.8)</td>
</tr>
<tr>
<td>White matter volume, ml</td>
<td>467.4 (65.4)</td>
</tr>
<tr>
<td>Lacunes*</td>
<td>102 (23.0)</td>
</tr>
</tbody>
</table>

Data represent N of subjects* (%), mean (SD), or median (interquartile range).
TBV: total brain volume; ICV: intracranial volume; WMH: white matter hyperintensities.
were predominately located in the frontal periventricular regions (Fig. 1). 13 participants (2.9% of the study population) were diagnosed with mild cognitive impairment.

Fig. 2 shows the relation between FA and the cognitive domains tested \((p < 0.05, \text{FWE-corrected for multiple comparisons})\). FA in the frontal, parietal, occipital, temporal but also in the infratentorial voxels of the skeleton was positively related to the cognitive index, attention and verbal memory performance. A lower FA in almost all regions was associated with higher scores on psychomotor speed and concept shifting (indicating lower performance). We found a similar distribution for the inverse association with MD and the cognitive domains. With regard to MMSE, visuospatial memory and fluency, no significant associations were found for FA and MD. A significant \((p\text{-corrected} < 0.01)\) association was identified in almost all voxels of the skeleton in the relation between FA and cognitive index and concept shifting. The strongest significant \((p\text{-corrected} < 0.01)\) relation between MD and psychomotor speed and cognitive index was located in the frontal lobe and the corpus callosum. The relation between FA and MD and verbal memory was most outspoken along the whole course of the corpus callosum and the cingulum bundle \((p\text{-corrected} < 0.01)\).

Additional adjustment for WMH and lacunes (Fig. 2B) weakened the relations between the FA and cognitive index, psychomotor speed, verbal memory and concept shifting, but remained present \((p\text{-corrected} < 0.05)\). There were no associations between for FA and attention after additional adjustment for WMH and lacunes. There were no associations between MD and attention after additional adjustment for WMH and lacunes, while the associations between MD and psychomotor speed and concept shifting weakened but remained significant. Voxels with the highest significance for FA in relation to cognitive index, psychomotor speed, verbal memory and concept shifting were located in the corpus callosum \((p\text{-corrected} < 0.01)\). In addition, FA in the cingulum bundle and corpus callosum remained highly associated with verbal memory performance \((p\text{-corrected} < 0.01)\).

Fig. 2. Voxel-wise analysis of the relation between fractional anisotropy and the cognitive index and verbal memory performance and negatively associated with psychomotor speed, concept shifting and attention. A) Adjusted for age, sex, education, depressive symptoms and normalized TBV, depicted by red-yellow color thresholded at \(p < 0.05\) and corrected for multiple comparisons and B) additional adjustment for white matter hyperintensities and lacunes, depicted by blue color 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.
As post-hoc analysis we analyzed the corpus callosum in more anatomical detail by segmenting it in three regions (Table 2). We found that microstructural integrity of genu and splenium of the corpus callosum related to cognitive index, but also with executive domains, psychomotor speed and concept shifting (p < 0.05 after Bonferroni corrections).

The microstructural integrity of body was associated with verbal memory. We found no relations between microstructural integrity in corpus callosum and attention, visuospatial memory, MMSE and fluency. The associations with MD and cognitive performance were mainly driven by changes in RD, and not by changes in AD.

4. Discussion

In this study, we examined the relation between the microstructural integrity of the WM and cognitive performance in subjects with cerebral SVD. We demonstrated that low FA and high MD in multiple regions of the WM were associated with lower scores on cognitive performance. The corpus callosum showed the highest significant relation with cognitive function, especially in the genu and splenium. The microstructural integrity of the cingulum bundle showed the highest significant relation with verbal memory performance and the frontal WM with psychomotor speed.

A limitation is the cross-sectional nature of our study, which limits causal inference. The RUT DMC study has a longitudinal design and follow-up is currently being executed to evaluate the effect of progression of SVD on changes in cognitive performance (van Norden et al., 2011). In addition, we did not intentionally adjust for vascular risk factors, such as hypertension or diabetes, as they were considered a part of the causal chain between SVD and cognitive performance. Major strengths of the study included the large sample size, the single center design, the use of a single scanner and the high response rate. Furthermore, we manually segmented the WMH. Extensive neuropsychological assessment was performed by two investigators and all analyses were adjusted for potential confounders.

Structural abnormality of the white matter tracts has been found in Alzheimer’s disease and mild cognitive impairment and has been demonstrated in a population-based cohort and a cohort of healthy adults (Acosta-Cabronero et al., 2010; Barrick et al., 2010; Damoiseaux et al., 2009; Vernooij et al., 2008; Zhuang et al., 2010). In patients with SVD, clinical manifestations of cognitive impairment are usually of a characteristic and fairly homogeneous 'subcortical' pattern and include psychomotor slowing due to impaired executive function, deficits of attention, planning and set-shifting and forgetfulness (Roman et al., 2002). In this study, we demonstrated that in patients with SVD the white matter’s microstructural integrity was associated with these measures of cognition.

We showed that WM integrity at specific locations was related to specific cognitive performance. The strongest significant relations between microstructural integrity and verbal memory performance were located in the cingulum bundle. This bundle, which connects the medial temporal lobe and the posterior cingulate cortex, is an important structure in verbal memory performance (Sepulcre et al., 2008; van der Holt et al., 2013). We also found verbal memory to be associated with the anterior parts of the corpus callosum. Episodic memory is partially dependent on interhemispheric interaction (Christman and Propper, 2001). Loss of microstructural integrity of the corpus callosum can lead to impaired interhemispheric interaction, resulting in impaired (verbal) memory performance. With regard to psychomotor speed, we found that the strongest significant relation with microstructural integrity was located in the corpus callosum and frontal lobe. The prefrontal–subcortical circuits are known to be involved in executive function, which affects the psychomotor speed (Roman et al., 2002). Corpus callosum has been related to global cognitive status and, based on a topographical organization, genu has been related to frontal-lobe mediated executive function and attention (Jokinen et al., 2007), whereas splenium is associated with visuospatial construction (Fryer et al., 2008), which corresponds with its connection with the parietal, temporal and occipital regions (Huang et al., 2005). We found no relations between WM integrity and fluency and MMSE. This might be explained by the fact that MMSE is a too crude measure not designed for subcortical damaged related function loss and hence not sensitive to detect subtle cognitive changes that correlate with macro- and microstructural SVD changes (Pasi et al., 2015).

The whole spectrum of cognitive disturbances in patients with SVD cannot solely be explained by the degree of WMH and lacunes. Our results show that the majority of the relations between microstructural integrity and cognitive performance were located in the NAWM. This suggests that the microstructural integrity of the NAWM has an important role in the cognitive disturbances in our subjects with SVD and might explain a part of the cognitive impairment beyond the SVD-visible on conventional MRI. Findings from functional and pathological studies in combination with our results suggest a disruption of cortical–cortical and cortical–subcortical connections, and a subsequent ‘disconnection-syndrome’ accounting for cognitive disturbances in patients with SVD (O’Sullivan et al., 2001a). In concordance with the findings from previous studies (van Norden et al., 2012; Vernooij et al., 2009), this suggests that microstructural integrity of the NAWM should also be taken into account when investigating the relation between SVD and cognitive function and that DTI should be considered part of the imaging protocol in future studies on cognitive performance.

The abnormalities in microstructural integrity of the NAWM are partially SVD-related, as our results showed that the presence of WMH and lacunes weakened the relation between the WM integrity and cognitive performance. Several explanations could be proposed by which SVD might influence this relation. First, it may be that these associations were, at least in part, explained by the presence of the coexisting WMH and not so much by the loss of integrity of the NAWM. Second, the structural changes of the NAWM could be caused by the same risk factors that are involved in the development of SVD, such as hypertension (Gons et al., 2012). This hypothesis is supported by the finding of increased blood–brain barrier permeability in the NAWM in subjects with SVD (Topkian et al., 2010). Finally, the observed association between the microstructural integrity of the NAWM and cognitive performance could reflect indirect damage to the NAWM by distant effects of WMH by means of antero-(Wallarian) or retrograde degeneration (Dziedzic et al., 2010; Thomalla et al., 2004).

In conclusion, our study showed that cognitive disturbances in elderly with SVD are related to the microstructural integrity of the

Table 2

Association between the microstructural integrity of the corpus callosum and cognitive performance.

<table>
<thead>
<tr>
<th></th>
<th>Cognitive index</th>
<th>Verbal memory</th>
<th>Psychomotor speed</th>
<th>Concept shifting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genu</td>
<td>.19*</td>
<td>.12</td>
<td>.16*</td>
<td>.16*</td>
</tr>
<tr>
<td>MD</td>
<td>−.19</td>
<td>−.13</td>
<td>−.14*</td>
<td>−.11</td>
</tr>
<tr>
<td>AD</td>
<td>−.12</td>
<td>−.10</td>
<td>−.06</td>
<td>−.01</td>
</tr>
<tr>
<td>RD</td>
<td>−.19</td>
<td>−.12*</td>
<td>−.16*</td>
<td>−.14*</td>
</tr>
<tr>
<td>Body</td>
<td>.14</td>
<td>.13*</td>
<td>.09</td>
<td>.15*</td>
</tr>
<tr>
<td>MD</td>
<td>−.16</td>
<td>−.14*</td>
<td>−.10</td>
<td>−.09</td>
</tr>
<tr>
<td>AD</td>
<td>−.08</td>
<td>−.05</td>
<td>−.05</td>
<td>−.06</td>
</tr>
<tr>
<td>RD</td>
<td>−.16</td>
<td>−.14*</td>
<td>−.10</td>
<td>−.13*</td>
</tr>
<tr>
<td>Splenium</td>
<td>.19*</td>
<td>.09</td>
<td>.18*</td>
<td>.19*</td>
</tr>
<tr>
<td>MD</td>
<td>−.15*</td>
<td>−.09</td>
<td>−.13</td>
<td>−.13*</td>
</tr>
<tr>
<td>AD</td>
<td>−.02</td>
<td>−.04</td>
<td>−.01</td>
<td>−.01</td>
</tr>
<tr>
<td>RD</td>
<td>−.18</td>
<td>−.09</td>
<td>−.17*</td>
<td>−.18*</td>
</tr>
</tbody>
</table>

Standardized β-values adjusted for age, sex, education, depressive symptoms, normalized TIV, white matter hyperintensities and number of lacunes. FA: fractional anisotropy. MD: mean diffusivity. AD: axial diffusivity. RD: radial diffusivity.

* p < .05 (Bonferroni corrected).
cerebral WM areas with WMH and lacunes, but also in NAWM, the largest part of WM. Our results suggest that loss of microstructural integrity in the NAWM is part of the SVD spectrum, which is not visible on conventional MRI. DTI could therefore serve as an additional tool to conventional MRI in order to investigate the cognitive consequences of cerebral SVD. If the predictive value is proven, DTI could possibly serve as a surrogate marker for development of cognitive decline and dementia and could be a starting point for therapeutic trials aiming to prevent disease progression.

Conflicts of interest

The authors have no conflicts of interest.

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