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Diffusion tensor imaging and cognition in cerebral small vessel disease The RUN DMC study ☆

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ABSTRACT

Background: Cerebral small vessel disease (SVD) is very common in elderly and related to cognition, although this relation is weak. This might be because the underlying pathology of white matter lesions (WML) is diverse and cannot be properly appreciated with conventional FLAIR MRI. In addition, conventional MRI is not sensitive to early loss of microstructural integrity of the normal appearing white matter (NAWM), which might be an important factor. Diffusion tensor imaging (DTI) provides alternative information on microstructural white matter integrity and we have used this to investigate the relation between white matter integrity, in both WML and NAWM, and cognition among elderly with cerebral SVD.
Methods: The RUN DMC study is a prospective cohort study among 503 independently living, non-demented elderly with cerebral SVD aged between 50 and 85 years. All subjects underwent MRI and DTI scanning. WML were segmented manually. We measured mean diffusivity (MD) and fractional anisotropy (FA), as assessed by DTI in both WML and NAWM.
Results: Inverse relations were found between MD in the WML and NAWM and global cognitive function ($\beta = -.11$, $p<0.05$; $\beta = -.18$, $p<0.001$), psychomotor speed ($\beta = -.15$, $p<0.01$; $\beta = -.18$, $p<0.001$), concept shifting ($\beta = -.11$, $p<0.05$; $\beta = -.10$, $p<0.05$) and attention ($\beta = -.12$, $p<0.05$; $\beta = -.15$, $p<0.001$). The relation between DTI parameters in both WML and NAWM and cognitive performance was most pronounced in subjects with severe WML.
Conclusion: DTI parameters in both WML and NAWM correlate with cognitive performance, independent of SVD. DTI may be a promising tool in exploring the mechanisms of cognitive decline and could function as a surrogate marker for disease progression in therapeutic trials.
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1. Introduction

Cerebral small vessel disease (SVD) includes white matter lesions (WML) and lacunar infarcts and is very common in the elderly. Both patient and population based studies have shown that cerebral SVD is an important cause of cognitive impairment, and may ultimately lead to dementia in some [1,2]. Despite the high prevalence of WML and lacunar infarcts in the population over 50 years of age (90% and 20% respectively) [3,4], surprisingly few individuals develop cognitive decline or dementia [2]. Apparently there are other factors apart from the SVD visible on conventional MRI that determine the transition from intact cognitive performance to cognitive decline in some, while leaving the cognition unaffected in most.

As identical appearing WML on conventional MRI are actually histopathologically heterogeneous [5], it could be that only WML with the highest loss of structural integrity are related to cognitive

Abbreviations: CES-D, Center of Epidemiologic Studies on Depression Scale; DTI, diffusion tensor imaging; FA, fractional anisotropy; FLAIR, fluid attenuated recovery; MD, mean diffusivity; MMSE, Mini Mental State Examination; NAWM, normal appearing white matter; OR, odds ratio; RAVLT, Rey Auditory Verbal Learning Test; RCFT, Rey's Complex Figure Test; RUN DMC, Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort; SD, standard deviation; SVD, small vessel disease; TBV, total brain volume; VSAT, verbal series attention test; WML, white matter lesions.

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impairment. It is also important to realize that only a small proportion of the white matter (usually less than a few percent) is affected by SVD, even among individuals with severe SVD. As conventional MRI is not sensitive to early loss of microstructural integrity in the normal appearing white matter (NAWM), possible changes in this largest part of the white matter cannot be assessed. The integrity of the NAWM might be an important factor for a better understanding of the relation between white matter integrity and cognitive performance and decline.

These limitations of conventional MRI can potentially be overcome with the use of Diffusion Tensor Imaging (DTI) which allows the assessment of the microstructural integrity of the whole white matter [6,7]. DTI provides two scalar parameters: mean diffusivity (MD), a measure of the magnitude of diffusion of water averaged in all spatial directions, and fractional anisotropy (FA), a measure which provides information about the directionality of water diffusion. A reduction in FA and increase in MD are believed to represent reduced microstructural integrity in SVD [8]. We therefore hypothesized a relation between the degree of structural integrity of WML and NAWM and cognitive performance.

Studies on CADASIL and multiple sclerosis showed a relation between DTI parameters in WML and NAWM and cognitive function [9,10], whereas only a few studies investigated this relation in individuals with SVD [11–14]. Most of these studies had small sample sizes ($n<105$) and did not adjust for possible confounders. In addition, most studies addressed only few cognitive domains of the spectrum observed in WML related cognitive decline. Recently a population based cohort study demonstrated a relation between microstructural integrity of both the WML and NAWM and cognitive function [15].

In this study we report on the relation between various domains of cognitive function and the structural integrity of both the WML and NAWM, as assessed by DTI, in 503 independently living, non-demented elderly with cerebral SVD. We hypothesized that a higher MD and lower FA in the WML as well as in the NAWM are related to cognitive function. The second aim was to determine whether these associations in the NAWM are independent of WML and lacunar infarcts.

2. Methods

2.1. Study population

The Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study, is a prospective cohort study that investigates risk factors and clinical consequences of functional and structural brain changes among elderly with cerebral SVD.

Cerebral SVD is characterized on neuroimaging by either WML or lacunar infarcts. Symptoms of SVD include acute symptoms, such as TIAs or lacunar syndromes, or subacute manifestations, such as cognitive, motor (gait) disturbances and/or depressive symptoms [15]. As the onset of cerebral SVD is often insidious, clinically heterogeneous, and typically with mild symptoms, it has been suggested that the selection of subjects with cerebral SVD in clinical studies should be based on the more consistent brain imaging features [16]. Accordingly, in 2006, consecutive patients referred to the Department of Neurology between October 2002 and November 2006, were selected for participation. Inclusion criteria were: (a) age between 50 and 85 years; and (b) cerebral SVD on neuroimaging (WML and/or lacunar infarcts). Subsequently, the above mentioned acute or subacute clinical symptoms of SVD were assessed by standardized structured assessments. Patients who were eligible because of a lacunar syndrome were included only >6 months after the event to avoid acute effects on the outcomes.

Exclusion criteria were: (a) dementia [17] (b) parkinson(−ism) [18] (c) life expectancy of less than six months; (d) intracranial space occupying lesion; (e) (psychiatric) disease interfering with cognitive testing or follow-up; (f) recent/current use of acetylcholine-esterase inhibitors, neuroleptic agents, L-dopa or dopa-a(nta)gonists; (g) WML or SVD mimics (e.g. multiple sclerosis and irradiation induced gliosis); (h) prominent visual or hearing impairment; (i) language barrier; and (j) MRI contraindications or known claustrophobia.

From 1004 invited individuals by letter, 727 were eligible after contact by phone of whom 525 agreed to participate. In 22 individuals exclusion criteria were found during their visit to our research center, yielding a response of 71.3% (503/705).

All participants signed an informed consent form. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study.

2.2. Conventional MRI scanning protocol

All participants underwent a 1.5-Tesla MRI scanning on the same Magnetom scanner. (Siemens, Erlangen, Germany). The protocol included the following whole brain scans: 3D T1 MPRAGE imaging (TR/TE/TI 2250/3.68/850 ms; flip angle15º; voxel size $1.0\times1.0\times1.0$ mm); FLAIR pulse sequences (TR/TE/TI 9000/84/2200 ms; voxel size $1.0\times1.2\times5.0$ mm, interslice gap 1 mm); DTI (TR/TE 10100/93 ms; voxel size $2.5\times2.5\times2.5$ mm; 4 unweighted scans, 30 diffusion weighted scans with b-value 900 s/mm²). The complete protocol took 31 min.

2.3. Conventional MRI analysis

White matter signal hyperintensities in both supra and infratentorial regions on FLAIR scans, which were not, or only faintly, hypo-intense on T1 weighted images, were considered WML, except for gliosis surrounding infarcts. WML were manually segmented on FLAIR images by two trained raters. Total WML volume was calculated by summing the segmented areas multiplied by slice thickness. Lacunar infarcts were defined as hypo-intense areas with a diameter >2 mm and <15 mm with low signal intensity on T1 and FLAIR, ruling out enlarged perivascular spaces and infraputaminal pseudolacunes [19]. All imaging analyses were performed by raters blinded to clinical information. In a random sample of 10%, inter-rater variability for total WML volume yielded an intra-class correlation coefficient of 0.99; intra- and inter-rater reliability for the lacunar infarcts yielded a weighted kappa of 0.80 and 0.88.

Normalization parameters to the ICBM152 linear template (as provided with SPM5; Wellcome Department of Cognitive Neurology, University College London, UK) and gray (GM) and white matter (WM) tissue and cerebrospinal fluid (CSF) probability maps were computed by using SPM5 unified segmentation routines on the T1 MPRAGE images [20]. Total GM, WM and CSF volumes were calculated by summing all voxel volumes that had a $p>0.5$ for belonging to the 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, i.e. total GM, total WM and CSF volume. To normalize for head size, TBV was expressed as percentage of total ICV.

Co-registration parameters of the FLAIR image to the T1 image were computed (SPM5 mutual information co-registration) and used to bring both the FLAIR and WML segmentation images into the subject's (anatomical) reference frame. Transformed images were visually checked for co-registration errors. Subsequently, the WML segmentations were resampled to and combined with the white matter maps to yield to a WML map (the intersection of WML and white matter) and NAWM map (the complement of WML in white matter) in the T1 reference space.

2.4. DTI analysis

Affine distortion in our DW images from residual eddy-currents was minimized during MR acquisition and did not require further

correction using post-processing methodology [21]. The diffusion weighted images of each subject were realigned on the unweighted image using mutual information based co registration routines from SPM5. Then, the diffusion tensor [6] and its eigenvalues were computed using an SPM5 add-on (http://sourceforge.net/projects/spmtools). Unphysical spurious negative eigenvalues of the diffusion tensor were set to zero, after which the tensor derivatives the MD and FA were calculated [22]. The mean unweighted image was used to compute the co-registration parameters to the anatomical reference T1 image (SPM5 mutual information co-registration), which were then applied to all diffusion weighted images and derivates. All images were visually checked for motion artifacts and co registration errors, which resulted in a final sample of 499 subjects because of the exclusion four due to technical artifacts. The mean MD and FA were then calculated in both the WML and NAWM.

2.5. Measurement of cognitive function

Cognitive function was measured with a neuropsychological test battery that proved to be sensitive and suitable for this purpose in other, large epidemiological studies [1]. The tests included the Mini Mental State Examination (MMSE) [23], and the Rey Auditory Verbal Learning Test (RAVLT) [24]. To evaluate speed of mental processes we used the Stroop test [25], the Paper-Pencil Memory Scanning Task [26], the Symbol-Digit Substitution Task [27] and a verbal fluency task in which as many animals followed by as many professions as possible had to be named within 60 s. Attention was measured by the verbal series attention test (VSAT) [28]. To evaluate visuospatial memory we used the Rey's Complex Figure Test (RCFT) [29]. Performance across tests was made comparable by transforming the raw test scores into z-scores as described elsewhere [1].

Subsequently, we calculated compound scores for seven cognitive domains. Global cognitive function was evaluated by the MMSE and the Cognitive Index. 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 RAVLT and the delayed recall of this last test [1]. Verbal memory is a compound score of the mean of two z-scores from the RAVLT; 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 RCFT. 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 [1]. 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 VSAT [1].

2.6. Other measurements

Age, sex, level of education [30], depressive symptoms, lacunar infarcts and TBV were considered possible confounders. Depressive symptoms were present if a subject had a score ≥16 on the Center of Epidemiologic Studies on Depression Scale (CES-D) and/or the present use of anti-depressive medication [31].

2.7. Statistical analysis

Baseline characteristics were presented as mean ± standard deviation (SD) and for the skewed distributed parameters the median and interquartile range were calculated.

The relation between DTI parameters in WML and NAWM, WML volume and lacunar infarcts with cognitive performance was assessed by means of linear regression analysis. Regression coefficients are presented as standardized beta's.

To investigate if the burden of WML was an intermediate in this relation we analyzed this in strata (tertiles) of WML volume.

All analyses were adjusted for age, sex, education, depressive symptoms, TBV normalized for head size, lacunar infarcts and WML whenever appropriate.

All data were analyzed using SPSS statistical software, version 16.0.

Table 1
Baseline characteristics of the RUN DMC study population.

Demographic and clinical characteristics	
Number of participants	499
Age at admission (yrs)	64.2 ± 8.8
Age at enrolment (yrs)	65.6 ± 8.8
50-60 years (number, %)	161 (32)
60-70 years (number, %)	161 (32)
70-85 years (number, %)	177 (36)
Disease duration (years)[a]	1.4 ± 1.1
Sex (% male)	56.5
Only primary education (number, %)	49 (10)
Depressive symptoms (number, %)[b]	168 (34)
Neuro-imaging characteristics	
Total brain volume (ml)	1093.1 (121.1)
Total white matter volume (ml)	464.4 (66.5)
Total white matter lesion volume (ml)	7.1 (3.4;18.1)
Total normal appearing white matter volume (ml)	450.3 (70.3)
Presence lacunar infarcts (number, %)	171 (34)

Numbers represent means (SD), percentages or medians (inter-quartile range).
[a] Defined as age at enrolment-age at admission.
[b] Depressive symptoms defined as CES-D (Center of Epidemiologic Studies Depression Scale) ≥ 16 and/or present use of anti depressants.

3. Results

Demographics and neuro-imaging characteristics of 499 subjects are shown in Table 1. Mean age of the population was 65.6 years (SD 8.8) and 56.5% were male. In Table 2 the test scores of the cognitive test battery are shown. The mean MMSE of the study population was 28.1 (SD 1.6).

Table 2
Cognitive test scores.

Mini mental state examination (range 0–30)	28.1 (1.6)
15 word verbal learning task (no. of words recalled)	
Immediate recall trial 1	5.1 (1.7)
Immediate recall trial 2	7.3 (2.2)
Immediate recall trial 3	8.7 (2.5)
Total trial 1-3	21.1 (5.8)
Delayed recall	6.0 (3.1)
Delayed recognition	27.0 (3.2)
Stroop test (time in s.)	
trial 1 (words)	25.8 (6.3)
trial 2 (colors)	33.2 (7.7)
trial 3 (concept-shifting)	63.8 (22.1)
Paper and pencil memory scanning task (time in s)	
1 character	45.0 (13.4)
2 characters	62.3 (19.7)
3 characters	77.4 (26.5)
Symbol digit substitution task (no. in 60 s)	27.1 (9.7)
Fluency (no. in 60 s)	
Animals	22.0 (6.5)
Jobs	16.5 (5.3)
Verbal series attention test	
Total time (s)	90.6 (32.9)
Total faults (no.)	2.1 (2.7)
Rey complex figure test (range 0–36)	
Copy trial	33.5 (3.4)
Immediate recall trial	18.2 (6.8)
Delayed recall trial	18.1 (6.6)

Numbers represent means (SD).

Table 3
The relation between DTI parameters in both the white matter lesions and the normal appearing white matter and cognitive performance.

	White matter lesions		Normal appearing white matter	
	Mean diffusivity	Fractional anisotropy	Mean diffusivity	Fractional anisotropy
Global cognitive function				
MMSE	−.12 (−.21 to −.01)*	.06 (−.02 to .15)	−.17 (−.29 to −.47)*	.06 (−.21 to .16)
Cognitive index	−.11 (−.16 to −.02)*	.03 (−.03 to .09)	−.18 (−.22 to .06)**	.11 (.04 to .17)**
Memory				
Verbal memory	−.08 (−.17 to .02)	.04 (−.04 to .12)	−.18 (−.28 to −.06)**	.11 (.01 to .19)*
Visuospatial memory	−.09 (−.16 to .01)	.03 (−.05 to .10)	−.13 (−.21 to .02)*	.10 (−.01 to .19)
Executive function and attention				
Psychomotor speed	−.15 (−.21 to −.06)**	.02 (−.04 to .09)	−.18 (−.24 to −.06)**	.12 (.04 to .18)**
Fluency	−.09 (−.18 to .01)	.03 (−.05 to .11)	−.11 (−.21 to −.02)*	.07 (−.03 to .15)
Concept shifting	−.11 (−.20 to −.02)*	.09 (.01 to .17)*	−.10 (−.16 to −.06)*	.10 (.01 to .19)*
Attention	−.12 (−.22 to −.02)*	.11 (.02 to .20)*	−.15 (−.27 to −.03)**	.16 (.06 to .25)**

Numbers represent standardized β's and are adjusted for age, sex, education, depressive symptoms, total brain volume normalized for head size, lacunar infarcts and in the NAWM also for white matter lesions.
* p<0.05.
** p<0.01.

In the WML, MD was related to global cognitive function (MMSE:β = −.12,p = 0.02; cognitive index:β = −.11,p = 0.01) and executive function (psychomotor speed:β = −.15,p<0.001; concept shifting:β = −.11,p = 0.02; attention:β = −.12,p = 0.02). (Table 3) MD in the WML did not significantly correlate with either of the memory domains and fluency. FA in the WML was only related with concept shifting and attention (β = .09,p = 0.03; β = .11,p = 0.01). In the NAWM, MD was related to all cognitive domains independent of the WML load (Table 3). The FA in the NAWM was related with the cognitive index (β = .11,p = 0.002), verbal memory (β = .11, p = 0.01), psychomotor speed (β = .12,p<0.001), concept shifting (β = .10,p = 0.03) and attention (β = 0.16,p = 0.001), again independent of WML volume.

Stratification on tertiles of WML severity revealed a relation between the MD in the WML with all cognitive domains, except for verbal memory performance and fluency, in subjects with severe WML, not in those with mild or moderate WML. (Table 4) The FA in the WML was related to the MMSE (β = .17,p<0.05), the cognitive index (β = .12,p<0.05), visuospatial memory (β = .16,p<0.05), psychomotor speed (β = .16,p<0.01), concept shifting (β = .18, p<0.05), and attention (β = .27,p<0.001) in subjects with severe WML, whereas no significant relations were found in subjects with mild or moderate WML. Identical correlations were found in the NAWM between MD and FA and cognitive performance in subjects with severe WML, except for concept shifting and the cognitive index.

Table 4
The relation between DTI parameters in white matter lesions and the normal appearing white matter and cognitive performance in subjects with mild, moderate and severe white matter lesions (defined as tertiles of the distribution).

	White matter lesions					
	Mean diffusivity			Fractional anisotropy		
	Mild WML	Moderate WML	Severe WML	Mild WML	Moderate WML	Severe WML
Global cognitive function						
MMSE	.04 (−.17 to .25)	−.04 (−.16 to .25)	−.18 (−.39 to −.01)*	.01 (−.12 to .15)	.00 (−.17 to .17)	.17 (.01 to .35)*
Cognitive index	.03 (−.11 to .17)	−.08 (−.22 to .07)	−.19 (−.29 to −.06)**	.02 (−.07 to .11)	-.03 (−.15 to .09)	.12 (.01 to .23)*
Memory						
Verbal memory	.11 (−.10 to .32)	−.11 (−.30 to .09)	−.13 (−.30 to .04)	.03 (−.11 to .16)	.01 (−.15 to .17)	.10 (−.05 to .26)
Visuospatial memory	.03 (−.15 to .20)	−.05 (−.23 to .13)	−.23 (−.41 to −.06)**	−.03 (−.14 to .08)	−.02 (−.18 to .13)	.16 (.01 to .32)*
Executive function and attention						
Psychomotor speed	−.07 (−.23 to .08)	−.07 (−.23 to .10)	−.23 (−.35 to −.08)**	−.01 (−.11 to .09)	−.06 (−.21 to .08)	.16 (.04 to .27)**
Fluency	−.10 (−.32 to .10)	.01 (−.19 to .21)	−.07 (−.23 to .09)	.08 (−.06 to .22)	-.06 (−.23 to .11)	.02 (−.12 to .16)
Concept shifting	−.03 (−.19 to .13)	.03 (−.16 to .23)	−.24 (−.45 to −.07)**	.06 (−.05 to .16)	.06 (−.10 to .23)	.18 (.02 to .35)*
Attention	.04 (−.16 to .26)	−.05 (−.26 to .16)	−.28 (−.50 to −.12)**	.11 (−.03 to .25)	−.02 (−.19 to .15)	.32 (.16 to .49)**
	Normal appearing white matter					
	Mean diffusivity			Fractional anisotropy		
	Mild WML	Moderate WML	Severe WML	Mild WML	Moderate WML	Severe WML
Global cognitive function						
MMSE	−.03 (−.28 to .22)	−.05 (−.17 to .26)	−.24 (−.44 to −.04)*	.03 (−.16 to .23)	−.02 (−.18 to .15)	.13 (−.03 to .29)
Cognitive index	−.19 (−.35 to −.01)*	−.10 (−.25 to .07)	−.17 (−.27 to −.02)**	.11 (−.02 to .24)	.11 (−.02 to .22)	.11 (.00 to .20)
Memory						
Verbal memory	−.08 (−.34 to .16)	−.15 (−.38 to .01)	−.16 (−.32 to −.02)*	.12 (−.07 to .32)	.15 (−.01 to .30)	.07 (−.07 to .21)
Visuospatial memory	−.08 (−.29 to .13)	−.09 (−.16 to .10)	−.22 (−.39 to −.05)*	.13 (−.02 to .29)	.05 (−.19 to .10)	.16 (.01 to .29)*
Executive function and attention						
Psychomotor speed	−.19 (−.37 to −.02)*	−.07 (−.18 to .24)	−.23 (−.35 to −.08)**	.08 (−.06 to .22)	.08 (−.05 to .22)	.17 (.05 to .27)**
Fluency	−.07 (−.33 to .17)	−.03 (−.18 to .22)	−.16 (−.32 to −.01)*	.22 (.04 to .43)*	−.05 (−.21 to .11)	.15 (.02 to .30)*
Concept shifting	−.07 (−.26 to .13)	−.03 (−.17 to .22)	−.12 (−.32 to .07)	.11 (−.04 to .26)	.09 (−.07 to .24)	.15 (−.01 to .31)
Attention	.12 (−.10 to .40)	.08 (−.11 to .32)	−.36 (−.56 to -.19)**	.03 (−.16 to .23)	.09 (−.08 to .25)	.27 (.12 to .42)**

Numbers represent standardized β's and are adjusted for age, sex, education, depressive symptoms, total brain volume normalized for head size and lacunar infarcts.
* p<0.05.
** p<0.01.

WML volume was related to MMSE ($\beta = -.11, p = 0.02$), visuospatial memory ($\beta = -.14, p = 0.004$), psychomotor speed ($\beta = -.09$, $p = 0.05$), concept shifting($\beta = -.09, p = 0.04$) and attention ($\beta = -.13, p = 0.003$). We did not find a relation between cognitive performance and presence or number of lacunar infarcts.

4. Discussion

In this large cohort of patients with cerebral SVD, we found that the microstructural integrity, of both WML and NAWM, as assessed by DTI, is related to global cognitive function, memory and executive function. In the NAWM this relation persisted even after controlling for presence of SVD on conventional MRI.

In contrast to previous DTI studies we measured MD and FA using a whole brain voxel-based analysis differentiating between NAWM and WML, whereas others investigating the relation between cognitive decline and WML used whole brain histogram approach, not accounting for the absence or presence of WML, or region of interest (ROI) analyses [9,30]. An ROI provides a local estimate of possible changes in the microstructure within the brain, not necessarily reflecting the structural integrity of the remainder of the white matter. However, this whole-brain analysis does not allow dissociating between specific brain areas in relation to cognitive performance. As cognitive function is the resultant of the integrated action of many parts of the brain we wanted to investigate the structural integrity of the global white matter with respect to cognitive performance, rather than an ROI approach. In addition an overall assessment is less prone to partial volume artifacts.

The co-registration of different image types and their co-registration to standard space could be a methodological limitation of our study. Two co-registration steps should be discerned, i.e. from the FLAIR to T1 and from the mean-b0 to T1. Furthermore, two sources of error should also be discerned, i.e. contrast differences between the source and target image and geometric distortion differences between source and target. The contrast source of error in our study should be minimal, as normalized mutual information was used a cost function to compute the optimal co-registration parameters. The distortions in the FLAIR and T1 images are both minimal and of no concern in general. There can be significant distortion differences between the DW images and the T1 and these will increase the variability of our analyses. Ideally one would therefore like to first correct the distortions in the DW images before co-registering them to the T1, which would require one to have information on these distortions, e.g. from measured fieldmaps. We did not acquire such fieldmaps, but we did visually inspect all co-registrations for optimality and found no apparent inaccuracies or errors.

Major strengths of this study included its large sample size and use of novel imaging techniques, such as DTI. Furthermore, our study is a single center study, with a high response rate and all subjects examined by only two investigators. Another strength is the manual segmentation of the WML without prior knowledge of the clinical data.

The relation between DTI parameters and cognitive function was investigated with extensive adjustments for possible confounders as education, depressive symptoms, TBV, and SVD on FLAIR.

Adjustment for SVD visible on conventional MRI is important in order to determine whether DTI, on top of conventional MRI measures, explains more of the variance in the relation between DTI measures and cognitive performance or whether it represents the same degree of white matter damage. There are only a few studies adjusting for WML volume; two adjusted for WML as measured by a visual scale [12,32], two other studies adjusted for WML volume but had small sample sizes ($n = 36$ and $n = 42$) and did not adjust for depressive symptoms, another well known confounder in the relation between white matter pathology and cognitive performance [11,13,32].

A limitation is the cross-sectional nature of our study, which prevents us from proving causality. The RUN DMC study had a longitudinal design and follow-up is already planned to evaluate the effect of progression of SVD on (changes in) cognition [33].

Our findings are in agreement with other studies relating DTI parameters to cognitive function in individuals with symptomatic SVD, however these studies had a small sample size and depressive symptoms were not taken into account [10,11,14]. Recently a population based cohort study demonstrated a relation between microstructural integrity of both the WML and NAWM and cognitive function [14]. They did not adjust for depressive symptoms. A longitudinal study of normal aging demonstrated a correlation between MD and working memory and showed that DTI is sensitive to detect microstructural change over a 2-year period [34].

The relation between DTI parameters of the WML and NAWM and cognitive performance has also been investigated in individuals with WML other than sporadic vascular WML, such as multiple sclerosis, CADASIL and auto immune mediated WML [9,10,35,36]. Our results are in accordance with their results, however, they had small sample sizes ($n < 105$) and did not, or rather selective adjust for possible confounders.

The relation between DTI parameters and cognitive performance has also been studied in subjects with mild cognitive impairment (MCI) and Alzheimer's Disease (AD). In most of these studies, subjects with SVD were excluded [37,38]. Although the assessment of cognitive performance was limited and the sample sizes were relatively small, they all demonstrated a significant increase in MD and decrease in FA in cognitively impaired subjects. Two studies did not exclude subjects with WML [39,40]. One study found a clear inverse relation between MD in the posterior cingulate gyrus and the MMSE. However, they did not report whether MD was measured in WML or NAWM and did not adjust for WML volume [39].

The relation between DTI parameters and cognitive performance has also been investigated in healthy elderly without severe WML [41,42]. They demonstrated a relation between lower FA in the pericallosal frontal region and in the genu of the corpus callosum and the relationship between perceptual speed and episodic retrieval reaction time and FA in the frontoparietal white matter and reaction time [40,41].

In our study, the relation between cognitive performance was less pervasive for FA than for MD. This could be due to the fact that the value of MD is relatively independent of the location measured in the brain whereas FA values very much depend on the degree of white matter organization and thus on the location in which it is measured [35]. In addition, because of the voxel size in which the DTI parameters are measured multiple fibers are present within a single voxel, that may all have different destinations. Due to this intervoxel incoherence the measured FA within a voxel may be low and does not necessarily reflect an underlying lower structural integrity [7]. These factors are likely to be important in the strength of the relation between FA and cognitive performance. In addition, correlations in the WML may be harder to demonstrate as WML constitute a very small proportion ($\pm 1\%$) of the whole white matter.

Our findings suggest that the structural integrity of the NAWM should also be taken into account when investigating the relation between SVD and cognitive function, as microstructural pathology extends in the NAWM beyond the detection limit of conventional FLAIR imaging. In addition our findings show that especially in those with severe WML, damage to the microtsructural integrity of the NAWM is related to impairment of cognitive performance. This suggests a positive relation between WML load and severity of damage to the NAWM, which is demonstrated before [43]. This is in line with our understanding of the underlying neuropathological changes that occur in the "NAWM", corresponding with mild tissue changes, with lower myelin density and a looser but largely preserved axonal network and glial cell density [44]. Microstructural changes in the NAWM are postulated to reflect remote effects in tracts damaged by the WML themselves and/or white matter changes that have not

yet become visible on conventional MRI. This is supported by the finding of an increased blood–brain barrier permeability in the NAWM in subjects with SVD [45]. This suggests that the microstructural changes of the white matter detected by DTI have important clinical consequences.

Another issue is that other studies have shown that particularly lacunar infarcts drive the association between WML and cognitive performance and incident dementia [2]. We tried to overcome this issue to the best of our ability by rating all lacunar infarcts and adjusting for them in the statistical analysis. We found a relation between the structural integrity of both NAWM and WML and cognitive performance, independent from SVD visible on FLAIR MRI. DTI could therefore serve as an additional tool to conventional MRI in order to investigate cognitive dysfunction. The relation of DTI parameters within the WML with cognitive performance and impairment may explain why subjects with identical WML on FLAIR differ in cognitive performance. Future studies should prospectively investigate the predictive value of DTI parameters for incident cognitive decline or dementia. When proven, DTI could possibly be a surrogate marker for disease progression, and can as such be used in therapeutic trials.

5. Disclosure statement

A.G.W. van Norden no conflicts of interest.
K.F. de Laat no conflicts of interest.
E.J. van Dijk no conflicts of interest.
L.J.B. van Oudheusden no conflicts of interest.
I.W.M. van Uden no conflicts of interest.
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D.G. Norris no conflicts of interest.
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