Changes in Normal-Appearing White Matter Precede Development of White Matter Lesions

Marius de Groot, MSc*; Benjamin F.J. Verhaaren, MD*; Renske de Boer, PhD; Stefan Klein, PhD; Albert Hofman, MD, PhD; Aad van der Lugt, MD, PhD; M. Arfan Ikram, MD, PhD; Wiro J. Niessen, PhD; Meike W. Vernooij, MD, PhD

**Background and Purpose**—It is unknown whether white matter lesions (WML) develop abruptly in previously normal brain areas, or whether tissue changes are already present before WML become apparent on MRI. We therefore investigated whether development of WML is preceded by quantifiable changes in normal-appearing white matter (NAWM).

**Methods**—In 689 participants from the general population (mean age 67 years), we performed 2 MRI scans (including diffusion tensor imaging and Fluid Attenuation Inversion Recovery [FLAIR] sequences) 3.5 years apart using the same 1.5-T scanner. Using automated tissue segmentation, we identified NAWM at baseline. We assessed which NAWM regions converted into WML during follow-up and differentiated new WML into regions of WML growth and de novo WML. Fractional anisotropy, mean diffusivity, and FLAIR intensity of regions converting to WML and regions of persistent NAWM were compared using 3 approaches: a whole-brain analysis, a regionally matched approach, and a voxel-wise approach.

**Results**—All 3 approaches showed that low fractional anisotropy, high mean diffusivity, and relatively high FLAIR intensity at baseline were associated with WML development during follow-up. Compared with persistent NAWM regions, NAWM regions converting to WML had significantly lower fractional anisotropy (0.337 vs 0.387; P<0.001), higher mean diffusivity (0.910×10–3 mm2/s vs 0.729×10–3 mm2/s; P<0.001), and relatively higher normalized FLAIR intensity (1.233 vs −0.340; P<0.001). This applied to both NAWM developing into growing and de novo WML.

**Conclusions**—White matter changes in NAWM are present and can be quantified on diffusion tensor imaging and FLAIR before WML develop. This suggests that WML develop gradually, and that visually appreciable WML are only the tip of the iceberg of white matter pathology. *(Stroke. 2013;44:1037-1042.)*

**Key Words:** aging • diffusion tensor imaging • MRI • white matter disease • white matter hyperintensities • white matter lesions

See related article, p 951.

Cerebral white matter lesions (WML) in the elderly are frequently seen on MRI. They are considered to reflect subclinical vascular brain disease and are associated with an increased risk of dementia and stroke. Preventing or slowing down WML development may thus have the potential to decrease disease burden. To date, several potentially modifiable risk factors, such as smoking and high blood pressure, have been associated with WML development. Yet, the pathogenesis of WML is still poorly understood. Most important, it is unknown whether WML develop abruptly in previously normal brain regions or whether development of WML on MRI is a gradual process, in which tissue changes are already present before they become apparent on MRI as WML. This is especially important to identify in which persons and at what moment preventive measures should be installed.

On MRI, WML are best visualized by the Fluid Attenuation Inversion Recovery (FLAIR) sequence, on which WML appear as hyperintense regions in the white matter. WML can be quantified using visual rating scales or automated measurements. Both methods measure visually appreciable WML, that is, the macrostructural changes of the white matter that are clearly distinguished on a FLAIR scan. However, pathology studies suggest that these WML are only the tip of the iceberg of white matter pathology. If WML development is indeed a gradual process, early stages of its development might be accompanied by subtly increased FLAIR intensities.

Diffusion tensor imaging (DTI) is a relatively recent MR imaging technique that allows in vivo study of tissue microstructure, and is often applied to study cerebral white matter. DTI provides multiple imaging metrics, such as fractional anisotropy (FA) and mean diffusivity (MD). These metrics have been shown to detect changes in white matter development.
microstructure that are not distinguished on conventional MRL. Performing DTI in longitudinal MR imaging studies enables investigation of normal-appearing white matter (NAWM) microstructure before WML develop.

New WML form either as lesion growth (ie, adhering to already present WML) or as de novo WML. It is important to take this distinction into account. First, this allows investigation of potentially different pathogeneicities. Second, around the border of existing WML, NAWM voxels can contain a fraction of WML tissue that affects measurements in that voxel (so-called partial volume effect). This introduces a potential bias, which is not present for de novo WML.

It is also important to take into account that DTI measurements vary considerably across brain regions because of neuronal tract-width and tract-geometry, and that WML preferentially occur in specific brain regions. These observations demand that longitudinal analyses of NAWM features and WML development take spatial location into account. Finally, to investigate the generic pathophysiology of WML development, this should preferably be studied in the general population.

Therefore, in 689 participants from the population-based Rotterdam Scan Study, we investigated whether DTI measures and the FLAIR intensity of NAWM at baseline are associated with growing WML and de novo WML over a period of 3.5 years. To take into account the lesion location of measurements in the white matter, we used regional matching and a voxel-based approach.

Methods

Study Population

This study is based on participants from a large, prospective, population-based cohort study in the Netherlands that investigates determinants of various chronic diseases in elderly people. The original study population consisted of 7983 people in the general population, aged ≥55 years and all residents of the Ommoord suburb of Rotterdam. In 2000 to 2001, the cohort was expanded with 3011 people aged ≥55 years of age. The institutional review board approved the study, and written informed consent was obtained from all participants.

In 2005, 1073 from these 3011 people were randomly selected for MRI scanning. After exclusion of demented people (N=4) and people who had MRI contraindications (N=94), 975 were eligible, of whom 907 (93%) participated. Physical inabilities precluded image acquisition in 12 individuals. Imaging was incomplete for 3 subjects, leaving 892 people with complete MRI examinations. In 2008, these people were invited for a follow-up MRI scan. After exclusion of people who had died (N=21) or had new MRI contraindications (N=7), 864 people were eligible. From these, 770 (89%) were willing to participate, of whom 754 had complete MRI examinations. After exclusion of people with cortical infarcts at either baseline or follow-up (N=32), 722 people were included in this study.

MRI Protocol

The MRI protocol performed at both time points was identical and was performed on the same 1.5T GE Signa Excite MR scanner in a standardized way. Details of this protocol have been described previously. In short, structural imaging included a T1-weighted 3D Fast RF Spoiled Gradient Recalled Acquisition in Steady State with an inversion recovery prepulse sequence, a proton density weighted sequence, and a T2-weighted FLAIR sequence. For DTI, we performed a single shot, diffusion-weighted spin echo echo-planar sequence, and a T2-weighted FLAIR sequence. Maximum b-value was 1000 s/mm² in 25 noncollinear directions; 1 volume was acquired without diffusion weighting (b-value=0 s/mm²).

Tissue Segmentation

Brain tissue was classified into NAWM, WML, grey matter, and cerebrospinal fluid. For classification of all tissues except WML, a multispectral tissue classification strategy with 6 manually labeled atlases for learning subject-specific tissue intensities. The FLAIR intensity was used to identify WML in an automated postprocessing step. Tissue segmentations were visually inspected. Subjects with artifacts in the segmentation of either scan (eg, because of motion) were excluded (33), leaving 689 subjects for analysis.

Spatial and Intensity Normalization

Nonrigid image was used to align the T1w structural images of both time points. Registration was performed using FMRIB’s Linear and Non-linear Image Registration Tools (FLIRT and FNIRT) [1] part of the FMRIB Software Library (FSL). To prevent biasing toward a particular time point, both scans were transformed to the (subject-specific) intermediate space by inverting half the deformation fields of the transformations between both scans. For each subject, the mean intermediate T1w images were registered to the 1-mm MNI_152 template, supplied with FSL using FNIRT. A schematic overview of the spatial normalization process is given in Figure 1. WML were masked to minimize their influence on the registration.

FLAIR intensities were normalized across subjects by matching grey matter intensity histograms for each subject (matching peak and full width at half maximum using linear transformations). This normalization was driven by grey matter intensities to avoid potential influence of subclinical white matter pathology. Nonuniformity correction (before normalization) and coregistration to the T1w image were performed as described by de Boer et al.10

Diffusion Data Processing

Diffusion data were corrected for motion and eddy currents by affine coregistration of the diffusion-weighted volumes to the b=0 volume. Registrations were performed with Elastix. The rotation component of each transformation was used to realign each gradient vector to compensate for motion during the acquisition. Transformed diffusion-weighted images were resampled at an isotropic resolution of 1.0 mm. The Brain Extraction Tool from FSL was used to mask out nonbrain tissue. Tensor fits were performed with a Levenberg-Marquard nonlinear least squares optimization algorithm, available in ExploreDTI. Data quality was examined by visual inspection of axial FA slices, every 4 mm, combined with 2 coronal and 2 sagittal slices around the center of the brain. Resampling of diffusion data in standard space was performed in 1 pass by concatenating an affine coregistration of the FA to the baseline T1w image, the nonlinear transformations of T1w space to mean structural space, and the transformation from that space to standard space. All registrations were checked by visually inspecting the warped structural and FA images in standard space. No unacceptable misregistrations were found.

Definition of New WMLs, Growing and De Novo WMLs

WMLs were defined as each group of voxels, classified as WMLs in the tissue segmentation, connected in a 3-dimensional 18-voxel-neighborhood (spherical kernel with a diameter of 3 voxels). In standard space, brain tissue segmentations for both time points were combined per subject to obtain voxelwise persistent NAWM, persistent WML, and new WML (ie, converting from NAWM to WML) tissue classes (Figure 1). Every WML in the follow-up image was then checked for overlap with WML in the baseline image. The new WML voxels were subdivided into WML growth and de novo WML tissue, according to this overlap. New WML voxels were identified as WML growth, if they were part of a WML that overlapped with a WML at baseline. Accordingly, if not overlapping with a baseline WML, new WML voxels were classified as de novo WML.
Regional Matching

A schematic overview of the approach is given in the online-only Data Supplement (Figure 1). To take into account that diffusion metrics and WML formation depend on anatomic location, we performed an analysis in which regions developing into WML were compared with anatomically corresponding regions of persistent NAWM. Regional matching was completed by confining measurements to the overlap between new WML in the driving subject and persistent NAWM in the matched subject to account for registration errors and potential (new) WML in the matched subject in those locations. In those regions in standard space, we averaged FLAIR and DTI metrics in the baseline scans. This process was repeated 4 times with different age- and sex-matched subjects for each driving subject for additional robustness.

Statistical Analyses

We investigated baseline tissue properties of NAWM developing into WMLs during follow-up using 3 approaches. First, we performed a whole-brain analysis without regional matching. We averaged FLAIR intensity and diffusion metrics in all persistent NAWM voxels and compared these with measures inside NAWM converting into WML, further partitioned into WML growth and de novo WMLs. Hereto, we used paired-samples t tests (2-sided; α-value=0.05) using SPSS statistical software (version 20).

Second, we used regional matching to compare baseline measurements in NAWM developing into WML with those in regionally matched persistent NAWM of age- and sex-matched controls. Persistent NAWM and WML measurements were averaged across the 4 repetitions to generate the measurement pairs to be used in paired-samples t tests. To test the added information of DTI metrics over FLAIR intensity and vice versa on a voxelwise level, by adding measures from both modalities as voxelwise-independent variable in the same model.

Third, to investigate regional dependence of the associations, we tested for voxelwise differences in diffusion and FLAIR measurements between new WML and persistent NAWM. For each voxel, a regression was performed using the metric of interest as dependent variable, and age and lesion status as (voxelwise) independent variables provided that each tissue class was represented by at least 10 subjects. This constraint effectively limited the analysis to the periventricular watershed area. Analyses were performed using t tests in Randomise19 available in FSL, using 5000 permutations to correct for multiple comparisons (α-value=0.05). Threshold-free cluster enhancement20 was used to cluster significant results. We repeated the test for added information of diffusion metrics over FLAIR intensity and vice versa on a voxelwise level, by adding measures from both modalities as voxelwise-independent variable in the same model.

Results

Characteristics of the study population are presented in Table 1. The median WML volume at baseline was 3.4 mL. After an average of 3.5 years of follow-up, we observed a net increase in WML volume in 81% of the participants and a net decrease in WML volume in the remaining 19% of the participants (median increase 1.4 mL; loss 0.8 mL). Table 2 represents the DTI and FLAIR parameters of persisting NAWM versus NAWM converting to WML for the whole-brain analysis. Compared with persistent NAWM regions, NAWM regions converting to WML had significantly lower FA (0.337 [standard deviation: 0.030] vs 0.387 [standard deviation: 0.017]; P<0.001), higher MD (0.910×10–3 [0.054×10–3] mm2/s vs 0.729×10–3 [0.027×10–3] mm2/s; P<0.001) and relatively higher normalized FLAIR intensity (1.233 [0.150] vs –0.340 [0.190]; P<0.001). This applied to both NAWM regions of growing and de novo WML.

In Table 3, the difference in DTI and FLAIR parameters is shown between converting NAWM versus persisting NAWM with respect to the regionally matched analyses, using 698 sets of matched subjects. Again, compared with persistent NAWM regions, NAWM regions converting to WML had lower FA (difference [95% confidence interval]:
Table 1. Population Characteristics

<table>
<thead>
<tr>
<th></th>
<th>N=689</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>66.9 (5.0)</td>
</tr>
<tr>
<td>Female</td>
<td>52% (355)</td>
</tr>
<tr>
<td>Baseline WML volume, mL*</td>
<td>3.4 (2.1-6.5)</td>
</tr>
<tr>
<td>New WML volume, mL*</td>
<td>1.4 (0.8-2.8)</td>
</tr>
<tr>
<td>De novo WML volume, mL*</td>
<td>0.2 (0.1-0.3)</td>
</tr>
<tr>
<td>Growing WML volume, mL*</td>
<td>1.1 (0.6-2.4)</td>
</tr>
<tr>
<td>Lost WML volume, mL*</td>
<td>0.8 (0.5-1.3)</td>
</tr>
<tr>
<td>Baseline NAWM volume, mL</td>
<td>397 (53)</td>
</tr>
<tr>
<td>NAWM FA</td>
<td>0.387 (0.017)</td>
</tr>
<tr>
<td>NAWM MD, 10⁻³ mm²/s</td>
<td>0.730 (0.028)</td>
</tr>
<tr>
<td>NAWM FLAIR</td>
<td>-0.332 (0.194)</td>
</tr>
<tr>
<td>Follow-up time, y</td>
<td>3.5 (0.2)</td>
</tr>
</tbody>
</table>

FA indicates fractional anisotropy; FLAIR, Fluid Attenuated Inversion-Recovery (normalized signal intensity); MD, mean diffusivity; NAWM, normal-appearing white matter; and WML, white matter lesion.

Values are means (standard deviation) or percentages (numbers).

*Median (interquartile range).

Table 2. DTI and FLAIR Parameters of Persisting NAWM Versus NAWM Converting to WMLs; Whole-Brain Analysis

<table>
<thead>
<tr>
<th></th>
<th>Persisting NAWM</th>
<th>NAWM Converting to WMLs</th>
<th>Total</th>
<th>Growing</th>
<th>De Novo</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>0.387 (0.017)</td>
<td>(Ref)</td>
<td>0.337 (0.030)</td>
<td>P&lt;0.001</td>
<td>0.335 (0.033)</td>
</tr>
<tr>
<td>MD, 10⁻³ mm²/s</td>
<td>0.729 (0.027)</td>
<td>(Ref)</td>
<td>0.910 (0.054)</td>
<td>P&lt;0.001</td>
<td>0.919 (0.055)</td>
</tr>
<tr>
<td>FLAIR</td>
<td>-0.340 (0.190)</td>
<td>(Ref)</td>
<td>1.233 (0.150)</td>
<td>P&lt;0.001</td>
<td>1.295 (0.151)</td>
</tr>
</tbody>
</table>

DTI indicates diffusion tensor imaging; FA, fraction anisotropy; FLAIR, Fluid Attenuated Inversion Recovery (normalized signal intensity); MD, mean diffusivity; NAWM, normal-appearing white matter; and WMLs, white matter lesions.

Values are means (SD). P values are based on the paired-samples t test results of the comparisons of mean FA, MD, and FLAIR values of persisting NAWM versus the values of NAWM converting to WMLs.
analysis. Therefore, if anything, this misclassification will either not have influenced our results or may have led to a slight underestimation.

Only one other study reported on the relationship between changes of the NAWM at baseline and the development of WML in a longitudinal MR study, but it did not distinguish between growing and de novo WML. In line with our findings, it found FA and FLAIR intensity to be independently associated with the development of WML in a heterogeneous population of 119 people with Alzheimer disease, mild cognitive impairment, and normal cognitive function. Together with our findings, this further corroborates that WML are the result of a gradual process because we now assess that this also applies to the general population, and that it holds for both growing as well as de novo WML. In addition, using the voxelwise analysis, we found no evidence that this process is spatially varying along the ventricles.

WML in the elderly are considered to be mainly vascular in origin. This is based on numerous epidemiological studies that found vascular risk factors, such as high blood pressure, to be associated with WML, and pathology studies that found damage of the cerebral small vessels, signs of blood–brain barrier dysfunction, and ischemic pathology in WML. Previous longitudinal studies have shown that baseline WML load is strongly associated with WML progression, and cross-sectional studies found abnormalities in the NAWM to be related to WML burden. Yet, it was unknown whether the disease process develops gradually or abruptly. This information is essential because if WML would develop abruptly, other causes would be more likely (eg, acute ischemia) than when WML develop over a longer period (eg, chronic ischemia). In addition, it was unknown whether WML growth and de novo WML development have a similar pathophysiology. Our findings suggest that both growing and de novo WML develop

| Table 3. Difference in DTI and FLAIR Parameters Between Persisting NAWM and NAWM Converting to WML; Regionally Matched Analysis |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
| Persisting NAWM                | NAWM converting to WMLs |
| FA (Ref)                       | Total            | Growing        | De Novo        |
| 0.0646 (0.0625, 0.0668)        | 0.0678 (0.0656, 0.0700) | 0.0435 (0.0413, 0.0456) | 0.0233 (0.0234, 0.0232) |
| MD, 10⁻³ mm²/s (Ref)           | 0.0646 (0.0625, 0.0668) | 0.0678 (0.0656, 0.0700) | 0.0435 (0.0413, 0.0456) | 0.0233 (0.0234, 0.0232) |
| FLAIR                          | 0.895 (0.884, 0.906) | 0.942 (0.931, 0.953) | 0.682 (0.671, 0.693) | 0.0233 (0.0234, 0.0232) |

DTI indicates diffusion tensor imaging; FA, fraction anisotropy; FLAIR, Fluid Attenuated Inversion Recovery (normalized signal intensity); MD, mean diffusivity; NAWM, normal-appearing white matter; and WMLs, white matter lesions.

Values are mean differences (95% CI) of FA, MD, or normalized FLAIR signal intensity of NAWM converting regions and NAWM persisting regions, matched on age, sex, and anatomic region. P values are based on paired-samples t tests.

Figure 2. Voxelwise comparison of baseline diffusion tensor imaging (DTI) measures and Fluid Attenuated Inversion Recovery (FLAIR) intensity in normal-appearing white matter (NAWM) converting to white matter lesions (WML) vs persisting NAWM. Only voxels in which there were at least 10 subjects in both categories were analyzed (shown in red and blue). This effectively constrained the analysis to the periventricular watershed area. Shown in blue are voxels in which DTI and FLAIR measures at baseline were significantly related to WML development during follow-up. For the fraction anisotropy (FA), the left panel shows regions of significantly lower FA, corrected for age. The right panel shows the same analysis, additionally corrected for voxelwise FLAIR intensity. Left panels in mean diffusivity (MD) and FLAIR show regions of significantly higher MD or relatively higher normalized FLAIR intensity related to WML development adjusted for age. The right panel for the MD is additionally adjusted for FLAIR intensity. The right panel for the FLAIR is additionally adjusted for FA values. FLAIR adjusted for MD showed similar results (not shown).
gradually. Therefore, a shared pathophysiological process is likely. Our results also suggest that DTI measures and FLAIR signal intensity provide some information independently from each other, implying that both measures partially capture different elements of tissue pathology. Furthermore, our findings confirm that WML are the extremes, or the tip of the iceberg, of white matter pathology.1,2,26,28

Our findings may have clinical implications. A clinician should take into account that the true white matter pathology may be more extensive than what is visually appreciable on structural MRI. This could lead to an improved estimate of a patients’ risk of stroke, dementia, and death.2,29 However, further research is needed to confirm these hypotheses.

In summary, in this longitudinal MRI study, we found NAWM changes to be present before WML develop. This suggests that the pathophysiology of WML is a gradual process. Furthermore, our results suggest that WML are only the tip of the iceberg of white matter pathology.

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Disclosure
Dr de Boer has ownership interest in Quantib B.V. In Quantib B.V., has Institution/Employer, has stocks in Quantib B.V., and has served as part-time scientific director at Quantib B.V.

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Supplemental Material

Changes in normal-appearing white matter precede development of white matter lesions

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### Supplemental Table 1. Odds Ratios of WML development per SD increase in baseline DTI and FLAIR parameters of NAWM

<table>
<thead>
<tr>
<th></th>
<th>total</th>
<th>growth</th>
<th>de-novo</th>
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</thead>
<tbody>
<tr>
<td><strong>MODEL I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>0.31 (0.29; 0.34)</td>
<td>p≤0.001</td>
<td>0.33 (0.30; 0.36)</td>
</tr>
<tr>
<td>MD</td>
<td>3.99 (3.66; 4.35)</td>
<td>p≤0.001</td>
<td>3.85 (3.54; 4.19)</td>
</tr>
<tr>
<td>FLAIR</td>
<td>25.96 (19.37; 34.79)</td>
<td>p≤0.001</td>
<td>25.45 (19.05; 34.01)</td>
</tr>
<tr>
<td><strong>MODEL II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>0.84 (0.74; 0.95)</td>
<td>p=0.008</td>
<td>0.78 (0.69; 0.88)</td>
</tr>
<tr>
<td>MD</td>
<td>1.19 (1.03; 1.39)</td>
<td>p=0.02</td>
<td>1.42 (1.22; 1.65)</td>
</tr>
<tr>
<td>FLAIR*</td>
<td>23.99 (17.66; 32.58)</td>
<td>p≤0.001</td>
<td>23.97 (17.48; 32.87)</td>
</tr>
</tbody>
</table>

Values are Odds Ratios (95%-CI) per SD increase in FA, MD or normalized FLAIR signal intensity.

Model I: conditional logistic regression with age, sex and region matched converting and persisting NAWM regions, adjusted for time between scans.

Model II: as Model I with additional adjustment for normalized FLAIR signal intensity. *adjusted for FA and MD.

Abbreviations: NAWM = normal-appearing white matter, FA = fraction anisotropy, MD = mean diffusivity,

FLAIR = Fluid Attenuated Inversion Recovery (normalized signal intensity).
driving subject in standard space defines objective ROI

matched subject 1
e.g. full tissue match

matched subject 2
e.g. ventricle mis-registration

matched subject 3
e.g. full tissue match

matched subject 4
e.g. overlapping WML

De-novo WML
WML growth
Persistent WML

Objective ROI

Tissue matching
- WML
- NAWM
- Ventricle
- Driving subject tissue borders

Measurements
- Matched ROI

measurements in case & control

measurements in case & control

measurements in case & control

measurements in case & control
Supplemental Figure 1. Schematic overview of the regional matching procedure, illustrated with enlarged axial cutouts for 5 subjects. The left panel shows how the new WML in standard space for the driving subject are treated as objective region of interest (ROI). This objective ROI is distinguished into WML growth and de-novo WML (not shown). For age and gender matched subjects in the same population, the overlap between the matched subject NAWM and the objective ROI is determined. The matching is performed four times to increase robustness, e.g. to be robust against a situation where new lesions in the driving subject overlap with WML in the matched subject. The repetition also means that every subject is driving-subject once, and matched subject exactly four times in the analysis. In the graph, matched subjects 1 and 3 show a complete overlap between the objective ROI and the persistent NAWM. For subject 2, the enlarged ventricle is not perfectly registered, leading to part of the ventricle overlapping with the objective ROI. Subject 4 shows WML overlapping with the objective ROI. The unmatched regions are excluded from the objective ROI in calculating the average baseline DTI and FLAIR metrics. The four resulting measurement pairs for each metric are averaged across the pairs. Abbreviations: WML = white matter lesion, NAWM = normal-appearing white matter, ROI = region of interest, DTI = diffusion tensor imaging, FLAIR = fluid attenuated inversion recovery.