Diffusion Tensor Imaging Surpasses Cerebrospinal Fluid as Predictor of Cognitive Decline and Medial Temporal Lobe Atrophy in Subjective Cognitive Impairment and Mild Cognitive Impairment

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Abstract. Neuropathological correlates of Alzheimer’s disease (AD) emerge years before dementia. Biomarkers preceding cognitive decline and reflecting the causative processes can potentially aid early intervention and diagnosis. Diffusion tensor imaging (DTI) indirectly reflects tissue microstructure. To answer whether DTI is an early biomarker for AD and to explore the relationship between DTI and the established biomarkers of medial temporal lobe atrophy and cerebrospinal fluid (CSF) Aβ42, T-tau, and P-tau, we longitudinally studied normal controls and patients with subjective (SCI) or mild (MCI) cognitive impairment. 21 controls and 64 SCI or MCI cases recruited from a university-hospital based memory clinic were re-examined after two to three years. FreeSurfer was used for longitudinal processing of morphometric data, and DTI derived fractional anisotropy, radial diffusivity, and mean diffusivity were analyzed in Tract-Based Spatial Statistics. Using regression models, we explored and compared the predictive powers of DTI and CSF biomarkers in regard to cognitive change and atrophy of the...
medial temporal lobe. Both DTI and CSF biomarkers significantly predicted cognitive decline and atrophy in the medial temporal lobe. In this population, however, DTI was a better predictor of dementia and AD-specific medial temporal lobe atrophy than the CSF biomarkers. The case for DTI as an early biomarker for AD is strengthened, but further studies are needed to confirm these results.

Keywords: Alzheimer’s disease, biomarker, cerebrospinal fluid, diffusion tensor imaging, longitudinal study, magnetic resonance brain imaging, mild cognitive impairment, preclinical Alzheimer’s disease, subjective cognitive impairment

INTRODUCTION

Increasing evidence suggests that neuropathological correlates of Alzheimer’s disease (AD) emerge decades before clinical dementia [1–3] but the preclinical stages are hard to define. The recently published recommendations from the National Institute on Aging-Alzheimer’s Association [4] provide a conceptual framework for future research. The recommendations adapt the hypothetical dynamic model of biomarkers for AD first published by Jack et al. [3]. This model proposes a sequence of events in AD induction that may be measured by different biomarkers (e.g., amyloid markers becoming abnormal before markers of neuronal injury) reflecting temporally related specific pathologic changes, but awaits further testing in clinical studies. We have recently documented increasing loss of axonal integrity, as measured by white matter (WM) magnetic resonance diffusion tensor imaging (DTI), in pre-dementia stages of increasing severity. The DTI changes are seen independently of, and earlier than, corresponding cortical atrophy [5]. We also found that DTI changes were associated with cognitive impairment in pre-dementia cases [6]. Change in DTI derived anisotropy in AD is well documented [7, 8], and our findings thus suggest that loss of axonal integrity is of earlier occurrence in AD than cortical atrophy.

DTI measures a diffusion tensor that (for each voxel) is represented by three eigenvectors with corresponding eigenvalues defining an ellipsoid. Water diffusion indirectly reflects the microstructure of the underlying tissue; the diffusion of water molecules will be more pronounced parallel (or axial) to, rather than perpendicular (or radial) to the long myelinated isocortical and cortico-subcortical (often reciprocal) projections that constitute WM. DTI can thus be used to measure properties believed to reflect neural fiber integrity (and orientation). Fractional anisotropy (FA), axial diffusivity (DA), radial diffusivity (DR), and mean diffusivity (MD) are DTI indices of axonal bundle microstructure, but their relations to pathophysiology are not obvious (reviewed in [9] and expanded in [10]). FA is frequently used and reflects degree of directionality of water diffusion within a given voxel [11], decreasing in neurodegeneration [12]. DR is a measure of the degree of restricted radial diffusion due to myelin and membranes, and MD is a similar measure, both reported to increase in neurodegeneration [12], but the pattern of diffusivity changes may depend both on degree and time interval since initial damage [13].

Mild cognitive impairment (MCI) and subjective cognitive impairment (SCI) are heterogeneous clinical conditions associated with an increased risk of dementia [14–17]. Incipient AD, other neurodegenerative diseases, and potentially reversible somatic conditions are possible etiologies. Cerebrovascular small vessel disease in itself may constitute the etiological basis of some SCI and MCI cases, but is also an AD risk factor [18] and the two often co-exist.

The majority of MCI patients already have extensive neuroimaging changes and amyloid plaques, consistent with a diagnosis of AD, and frequently also show medial temporal lobe atrophy, reflecting neuronal loss [19, 20]. In a research setting, AD can be diagnosed at the MCI stage on the basis of memory testing and biomarkers [21]. SCI patients score according to norms on cognitive screening tests (i.e., do not fulfill AD research criteria [4, 16, 21]), but the extent of AD-related changes (or other pathology) is unknown. It further remains to be confirmed whether the neuropathological process in SCI patients destined to develop AD is amenable to intervention or even reversible. DTI changes, however, may be reversible [22] and with the added benefit of being non-invasive and repeatable, DTI may prove useful in AD intervention trials.

The levels of cerebrospinal fluid (CSF) amyloid-β42 (Aβ42), total tau (T-tau), and phosphorylated tau (P-tau) are well established surrogate markers in AD pathogenesis, and have been shown to provide good diagnostic accuracy and predict conversion from MCI to AD [23]. Aβ42 is a major constituent of amyloid plaques, and reduced CSF Aβ42 levels are associated with the amount of plaques. Tau is an intracellular microtubule associated and stabilizing protein, and...
increased CSF levels of T-tau reflect neuronal damage and tau release. Tau phosphorylation diminishes microtubule stabilization and leads to aggregation of phosphorylated tau (P-tau) in neurofibrillary tangles. Elevated CSF P-tau levels are markers of tangle formation and increased tau phosphorylation [23]. Building on the dynamic model of biomarkers for AD, we herein propose DTI as an additional early stage biomarker further elucidating the pathologic continuum from healthy to AD. In the present study, we examine the relationship between DTI measures and future clinical change and cortical atrophy, and we contrast DTI with the established AD-related CSF biomarkers Aβ(42), T-tau, and P-tau. To our knowledge, this is the first longitudinal study to combine DTI and CSF biomarkers in SCI and MCI patients.

In short, we ask the following five research questions:

1. Does baseline WM DTI predict cognitive decline?
2. Do CSF biomarkers (Aβ(42), T-tau, and P-tau) predict cognitive decline?
3. Does baseline WM DTI predict atrophy of the medial temporal lobe?
4. Do CSF biomarkers (Aβ(42), T-tau, and P-tau) predict atrophy of the medial temporal lobe?
5. What is the relative performance and interdependence of CSF and DTI biomarkers in prediction of cognitive decline and atrophy of the medial temporal lobe?

MATERIALS AND METHODS

Eligibility criteria, measures of cognitive impairment, and ethical conduct

15 patients with SCI, 51 with MCI, and 28 normal controls (NC) were recruited consecutively from referrals to a university-hospital based memory clinic between 2006 and 2009, and re-assessed 2–3 years later (21 NC, 43 MCI, and 11 SCI; drop out is accounted for in Fig. 1). Inclusion criteria for all groups were age 50–79 and established normality (NC) or impaired cognition (SCI or MCI) for at least 6 months. SCI and MCI are largely congruent with and herein defined as the second (SCI) and third (MCI) stages of the global deterioration scale (GDS) [24, 25]. GDS stage (at baseline and follow-up) was determined from a clinical interview and the following screening tests: Mini-Mental Status Examination (MMSE) ([26], Stepwise comparative status analysis (STEP) parameters 13–20 (12); L-Flex (fluency, interference and numeral-letter items) [16, 27, 28]; and Cognistat [29] (memory, including cued recall, and executive functions). Further, the Clinical Dementia Rating (CDR) [30] was administered. To be classified as GDS 2 (SCI), patients had to score above published cutoff on all screening tests (28 for MMSE); patients scoring below were classified as GDS 3 (MCI) [16]. Patients scoring GDS 3, CDR >0.5 or ≥1 (in sum) of STEP variables 13–20 were considered demented. This method of determining GDS stage is in agreement with a previously used method [31]. As such, all subjects classified as GDS 3/MCI will fulfill general criteria for MCI as revised in [32] by Petersen and colleagues. Further, subjects classified herein as GDS 2/SCI will fulfill the SCI/pre-MCI criteria as outlined in [16]. Spouses of participating patients were potentially eligible as controls provided they had a GDS score of 1, i.e., clinically established normality with regard to memory, emotionality, and tempo. The GDS scores for controls were determined by a clinical interview. Exclusion criteria were impaired activities of daily living (i.e., dementia), established psychiatric disorder, cancer, drug abuse, solvent exposure, and anoxic brain damage.

After two to three years, patients and controls underwent a follow up clinical examination, cognitive evaluation, and MRI scanning. Figure 1 outlines inclusions and exclusions in the cohort, and the sample is summarized in Table 1. A diagnosis of Alzheimer’s Disease required the subject to be clinically demented (GDS score >3) and fulfill research criteria according to (Dubois et al. [21]) for Alzheimer’s Disease. Patients developing other types of dementia were excluded. One patient developed mixed AD and vascular dementia and was not excluded. The study protocol was approved by the regional ethical committee for medical research, and informed consent was obtained from all subjects before any study-specific procedures were performed.

MRI/DTI acquisition

MRI scans were obtained from two sites. At site 1 we used a Siemens Symphony 1.5 T system with a conventional quadrature head coil. For structural imaging, we used a T1-weighted volumetric (3D) magnetization prepared rapid gradient echo (MPRAGE) sequence. Two MPRAGE were obtained in succession (TR/TE/ TIP = 2730 ms/3.19 ms/1100 ms/15°; matrix = 256 × 192), 128 sagittal slices, thickness = 1.33 mm,
in-plane resolution of 1.0 × 1.33 mm. At site 2, using a Siemens Espree 1.5 T system, one MPRAGE sequence was acquired (TR/TE/TI/FA = 2400/3.65/1000°, matrix = 240 × 192), 160 sagittal slices, thickness = 1.2 mm, in-plane resolution of 1 × 1.2 mm. The protocol also included 2D axial fluid-attenuated inversion recovery (FLAIR) images with the following parameters: site 1: TR/TE/TI = 9000/105/2500, 19 slices, spaced at 7.9 and 5 mm thick; site 2: TR/TE/TI = 13420/121/2500, 36 slices, spaced at 3.0 and 3.9 mm thick.

The pulse sequences for DTI at the two sites were: Site 1: b = 700, 12 directions repeated twice; one b0-value per slice, TR = 4300 ms, TE = 131 ms.
number of axial slices: 19, slice thickness = 5 mm (gap 1.5 mm), in-plane resolution: 1.8 × 1.8 mm², bandwidth: 955 Hz/pixel and Site 2: b = 750; 12 directions repeated 5 times; 5 b-values per slice, TR = 6000 ms, TE = 117 ms, number of slices: 30, slice thickness: 3 mm (gap 1.9 mm), in-plane resolution: 1.2 × 1.2 mm², bandwidth: 840Hz/ pixel. All follow up scans were performed at site 2.

As previously described [33], six of the included controls were scanned on both scanners. Volumes of the hippocampi and thickness of the entorhinal and parahippocampal cortices were estimated and correlated across scanners. The Pearson coefficients were 0.99, 0.96, and 0.92, respectively for these tests. Mean differences in cortical thickness were generally within ±0.1 mm across the brain surface. This indicates that change of scanner did not introduce a large degree of bias in the structural data. Mean FA, DR, and MD was also estimated in the subgroup scanned on both scanners. The Pearson coefficients were around 0.8 between scanners, and possible scanner bias in the DTI measures was therefore corrected for in statistical analyses.

MRI segmentations and analyses

Cortical reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite version 4.5.0 (http://surfer.nmr.mgh.harvard.edu/). This includes segmentation of the subcortical WM and deep gray matter volumetric structures [34] and parcellation of the cortical surface [35] according to a previously published parcellation scheme [36]. This labels cortical sulci and gyri, and thickness values are calculated in the regions of interest (ROIs).

The Oxford Centre for Functional MRI of the Brain (FMRIB) Software Library (FSL) version 4.1 [37, 38] was used for DTI analyses and calculations. Initially, FMRIB’s Linear Image Registration Tool [39] was used for affine registrations of each DTI volume to the low-b (b=0) image. Motion between scans and residual eddy-currents were corrected for, before creation of FA and eigenvalue maps. DR was defined as the mean of eigenvalue 2 and 3, and MD as the mean of all three eigenvalues. Tract-Based Spatial Statistics (TBSS) [11] (part of FSL) was used for voxel-wise statistical analysis of the DTI variables (FA, DR, and MD). FMRIB’s Diffusion Toolbox was used to create DTI images by fitting a tensor model to the raw diffusion data, and FSL’s Brain Extraction Tool was used for subsequent brain extraction. All subjects’ FA data were then aligned into a common space using the nonlinear registration tool FMRIB’s Non-linear Image Registration Tool, which uses a b-spline representation of the registration warp field [40]. Next, the mean FA image was created and thinned to create a mean FA skeleton that represents the centers of all tracts common to the group. Each subject’s aligned FA data were then projected onto this skeleton and the resulting data fed into voxel-wise cross-subject statistics. DR and MD data were then extracted from each subject according to the skeletonized FA map.

Based on the FLAIR images, white matter lesions were qualitatively assessed according to the method published by Fazekas et al. [41].

CSF biomarkers

CSF samples were collected from all patients through lumbar puncture. The lumbar puncture was performed consecutively after inclusion at a standardized time of day. CSF Aβ42, T-tau, and P-tau were routinely examined. CSF T-tau level was considered abnormal if T-tau ≥300ng/L for patients under 50 years >450ng/L for patients from 50 to 69 years, and ≥500ng/L for patients from 70 years and above [42]. CSF P-tau was considered pathological if ≥80ng/mL, and CSF Aβ42 was considered pathological if ≤550ng/L.

Statistics

Whole-brain voxel-wise statistics were initially performed using Randomise from the FSL suite to examine baseline differences between the subjects (NC, SCI, and MCI) who underwent cognitive decline and those who did not. Threshold-free cluster enhancement (TFCE) [43] was employed to correct for multiple comparisons. The threshold was set at p<0.05. Initially, (separate) analyses were performed for DR, MD, and FA as associated with cognitive decline at follow-up. Scanner, age, and gender were treated as nuisance variables. This produces whole-brain statistical maps of voxels (in the mean FA skeleton) whose DTI index is associated with future cognitive decline. Further, the mean values of the different DTI indices in the voxels according to the skeletonized FA map were extracted and used for further analysis in SPSS. The WM underlying the entorhinal, parahippocampal, retrosplenial, posterior cingulate, precuneous, inferior parietal, supramarginal, and middle temporal cortices were chosen as regions of interest (ROIs) to further spatially examine these relationships. (The choice of ROIs was motivated
by our previous findings of compromised WM integrity in these ROIs in SCI and MCI [5]. These WM ROIs (based on the FreeSurfer WM parcellations [36]) were extracted for FA, DR, and MD. The FSL FMRIB58 FA template (to which every subjects FA volume initially was registered) was coregistered to the standard space T1 volume MNI152, which subsequently went through the FreeSurfer processing stream to create a volume with WM parcellations. The registration between the FA template and the MNI152 volume was applied to the volume with the WM parcellations, and the resulting volume was used to extract the skeletonized FA, DR, and MD data from each WM ROI. ROIs were averaged across hemispheres to reduce the number of statistical operations. These DTI variables were corrected for the effects of age, gender, and scanner by regression, yielding standardized residuals for further use.

1. Does baseline WM DTI predict cognitive decline?
   Analyses of variance (ANOVA) were used to assess the differences in baseline indices of DTI between subjects with different clinical outcome (cognitive improvement, decline, or no change). Planned contrasts were employed to examine differences between the subjects undergoing cognitive decline and the remaining subjects, and between those who improved cognitively and the remaining subjects. Logistic regression with cognitive status at follow-up (cognitive decline or not) as the dependent variable and the different (standardized) DTI indices as independent variables were used to study the discriminatory abilities of the DTI indices.

2. Do CSF biomarkers (Aβ42, T-tau, and P-tau) predict cognitive decline?
   As in 1, ANOVA (with planned contrasts) were used to assess the relationship between cognitive change (improvement, no change, or deterioration) and the baseline CSF biomarkers. The relationship between cognitive decline and baseline CSF biomarkers (Aβ42, T-tau, and P-tau) were modeled using binary logistic regression with cognitive decline (or not) at follow-up as the dependent variable and the CSF biomarkers as independent variables. Separate analyses were performed with the CSF biomarkers as continuous variables, and a binary composite variable representing one or more pathological CSF biomarkers (in accord with the research criteria for AD [Dubois et al. [21]]).

3. Does baseline WM DTI predict atrophy of the medial temporal lobe?
   The most prominent atrophy in the majority of AD subjects is seen in the medial temporal lobe, and we therefore chose to examine how baseline DTI indices were associated with ensuing atrophy of the hippocampus as well as the entorhinal and parahippocampal cortices. To reduce the number of statistical operations, the left and right hemispheres were averaged. Quantitative measures of the rate of atrophy (from baseline to follow-up) were determined by dividing thickness (or volume for the hippocampus) at follow-up by the same measure at baseline (producing δ thickness or volume quotients). T-tests were performed to determine whether rates of atrophy were different between the subjects who underwent cognitive decline and those who did not. The different baseline DTI indices’ associations with ensuing morphometric change were modeled using linear regression with the DTI indices as independent variables and the relative change in thickness (cortical grey matter) or volume (hippocampus) as dependent variables. We have previously described that differences in DTI indices are widespread and not confined to particular areas [5]. We therefore chose to use the DTI variables (as described above) and not DTI measurements from the WM in the medial temporal lobe. As stated in Fig. 1, follow up MRI could not be performed in some subjects. These subjects were excluded from this analysis. Also, we used logistic regression to determine whether DTI predicts future atrophy in the medial temporal lobe. The different DTI indices were used as independent variables and a dichotomous variable representing medial temporal lobe atrophy was entered as the dependent variable. Subjects with more relative atrophy than two standard deviations (as estimated from δ quotients among the healthy controls) were said to have undergone medial temporal lobe atrophy from baseline to follow-up.

4. Do CSF biomarkers (Aβ42, T-tau, and P-tau) predict atrophy of the medial temporal lobe?
   Whether the CSF biomarkers could predict morphometric change was modeled using linear regression with the different CSF biomarkers as continuous variables and the composite variable representing one or more pathological biomarkers (in separate analyses) as the independent variable, and the rate of atrophy (as described in 2) as the dependent variables. Also, in the same manner as above with DTI, we used logistic regression to determine whether CSF biomarkers predict future atrophy in the medial temporal lobe.
5. What is the relative performance and interdependence of CSF and DTI biomarkers in prediction of cognitive decline and atrophy of the medial temporal lobe?

Pearson correlations between the CSF biomarkers and DTI indices were determined. To see whether there was interdependence between the effects of the CSF biomarkers and DTI variables, we repeated the regression analyses as multiple logistic (for cognitive decline) and linear regression (for atrophy). For clinical change as the dependent variable, MD was entered with each of the CSF biomarkers (continuous) as independent variables in separate multiple linear regression analyses. For atrophy as the dependent variable, MD was entered with each of the CSF biomarkers as independent variables in separate multiple linear regression analyses.

RESULTS

ANOVA did not show any significant differences between groups for age or Fazekas scores, but there was a significant difference in MMSE scores. Chi square tests did not show any significant differences between SCI and MCI (Table 1).

Eleven patients (and zero controls) declined cognitively (eight of these to dementia), and nine patients had improved by follow-up. Table 2A summarizes the change in clinical status from baseline to follow-up.
Fig. 2. The statistical map (shown in red for radial diffusivity and yellow for mean diffusivity) represents voxels in the fractional anisotrophy skeleton (shown in blue) for which radial diffusivity and mean diffusivity were significantly associated with future cognitive decline. No voxels were significant for fractional anisotrophy (but several voxels were trend level significant). Multiple comparisons were corrected for by threshold-free cluster enhancement with the threshold set at $p<0.05$, and the significant voxels are inflated for ease of viewing. The statistical maps are shown as overlays on the Montreal Neurological Institute template (annotated with the corresponding y-coordinates).
Analyses of variance. Differences in baseline cerebrospinal fluid and diffusion tensor imaging biomarkers according to clinical outcome at follow-up. When analyzed as continuous variables, T-tau were not associated with cognitive decline (data not shown). When analyzed as continuous variables, T-tau and P-tau (but not Aβ142) were associated with future dementia, and T-tau was also associated with future cognitive decline (Table 4).

2. Do CSF biomarkers (Aβ142, T-tau, and P-tau) predict cognitive decline?

ANOVA demonstrated that T-tau (but not Aβ142 and P-tau) were different between subjects according to clinical outcome. Planned contrasts showed significant baseline differences in CSF T-tau between the patients who underwent decline and those who did not, whereas there was no significant difference between those who improved and those who did not (Table 3). In linear regression, when analyzed as one composite variable (representing pathological levels of one or more of Aβ142, T-tau, and P-tau), CSF biomarkers were not associated with cognitive decline (data not shown). When analyzed as continuous variables, T-tau and P-tau (but not Aβ142) were associated with future cognitive decline.

3. Does baseline WM DTI predict atrophy of the medial temporal lobe?

T-tests demonstrated that atrophy (δ thickness or volume quotient) in the medial temporal lobe (the hippocampal, the entorhinal, and parahippocampal cortices) were significantly different between the subjects who had undergone cognitive decline by follow-up and those who had not (hippocampal volume, p < 0.01; δ entorhinal thickness, p = 0.49; δ parahippocampal thickness, p = 0.23). DR, MD, and FA at baseline were all significantly associated with atrophy of the medial temporal lobe (δ quotient in all three examined regions of interest) (Table 4). When assessing atrophy dichotomously, eight subjects (four of them were among the demented) had undergone atrophy of the medial temporal lobe from baseline to follow-up. For logistic regression, DR, MD, and FA (but not FA) predicted atrophy.
Cerebrospinal fluid (CSF) and diffusion tensor imaging (DTI) biomarkers in relation to future cognitive decline and atrophy of the medial temporal lobe

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Independent variables, effect measure and significance levels (p)</th>
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<tbody>
<tr>
<td>1. Prediction of cognitive decline by DTI</td>
<td>Logistic regression Radial Diffusivity Mean Diffusivity Fractional anisotrophy</td>
</tr>
<tr>
<td>Cognitive decline</td>
<td>Exp(B)/R²/Clas P</td>
</tr>
<tr>
<td>Dementia at follow-up</td>
<td>Exp(B)/R²/Clas P</td>
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2. Association between atrophy and DTI

<table>
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<tr>
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<td>−0.37/0.13</td>
<td>Beta/R²</td>
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<tr>
<td>Entorhinal δ</td>
<td>−0.35/0.20</td>
</tr>
<tr>
<td>Parahippocampal δ</td>
<td>−0.37/0.12</td>
</tr>
</tbody>
</table>

4. Do CSF biomarkers (Aβ42, T-tau, and P-tau) predict atrophy of the medial temporal lobe?

When analyzed as one composite variable (representing pathological levels of one or more of Aβ42, T-tau, and P-tau), CSF biomarkers predicted atrophy of the hippocampi (but not the entorhinal and parahippocampal cortices) (data not shown). When analyzed as continuous variables, all CSF biomarkers were associated with future hippocampal atrophy. T-tau was associated with future entorhinal atrophy, whereas T-tau and P-tau were associated with future parahippocampal atrophy (Table 4).

5. What is the relative performance and interdependence of CSF and DTI biomarkers in prediction of cognitive decline and atrophy of the medial temporal lobe?

None of the Pearson correlations between the CSF biomarkers and MD in the WM parcellations were significant. For the multiple regression analyses with cognitive decline as the dependent variable, MD was still significant for all analyses, but none of the CSF biomarkers were significant. For the multiple regression analyses with MRI atrophy as the dependent variable, MD was significant for all analyses. Aβ42, T-tau, and P-tau were also significant in several of these analyses, but the significance levels/effect measures were in most cases weaker than for MD (Table 5).

DISCUSSION

We have previously shown that the pre-dementia stages SCI and MCI are characterized by loss of axonal integrity as measured by changes in DTI derived DR and MD and (for MCI) also FA [5]. The changes were detectable in SCI and even more extensively in MCI. We have further shown that these indices are associated with memory and executive impairment, commonly seen in early stages of AD [6, 44]. Herein, in one of the first longitudinal studies exploring the predictive

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*β² = Nagelkerke R Square. Clas = Percentage correctly classified in logistic regression; β² = Adjusted R Square.
properties of WM DTI for cognitive decline and medial temporal lobe atrophy, we have shown that the DTI indices FA, DR, and MD also predict cognitive decline and medial temporal lobe atrophy. However, the association with clinical decline was found to be stronger than with clinical improvement. We also reproduce earlier findings showing that CSF biomarkers predict cognitive decline and medial temporal lobe atrophy in a population of pre-dementia patients over 2-3 years follow-up. Together with the lack of correlations between the DTI measures and the CSF biomarkers, this suggests that these biomarkers reflect independent aspects of the disease process, and that DTI is an independent predictor of decline early in the hypothetical model of biomarkers of AD; predicting patterns of brain atrophy known to be associated with AD dementia. This strengthens the case for DTI as a biomarker for development of AD. The widespread extent of the DTI effects further suggests a distributed disease process at this stage. The finding that DTI indices predict not only cognitive decline but also atrophy of the medial temporal lobe suggests that WM changes as measured by DTI is not an unspecific finding but may be more directly related to downstream AD neuropathology. Thus, these findings support the hypothesis that biomarkers may follow an ordered temporal pattern [3]; changes in diffusivity appearing early in emerging AD, with the ensuing grey matter atrophy probably appearing later but more closely related to dementia. These findings suggest that DTI should have a place among the dynamic biomarkers for AD, although independent confirmation of our findings is necessary. The number of studies of DTI in MCI is growing, but there are still few reports of DTI measurements in SCI. However, we found only select longitudinal reports of WM DTI in MCI [47, 48] (and some concerning grey matter DTI, e.g., [49]), none of which employ the repertoire of biomarkers used here, which is essential as one third of clinically normal older subjects may harbor amyloid pathology [50]. As healthy aging also may influence diffusivity [51], more studies are clearly needed to evaluate DTI as an early biomarker for AD. However, the question of whether the diffusivity changes are directly related to AD specific processes or represents a more diffuse aging-associated neuronal injury (perhaps also induced by other neurodegenerative processes) is not settled. We have previously shown that cerebral small vessel disease co-existing with AD related to cerebral small vessel disease is closely related to levels of amyloid- β protein precursor metabolites in CSF [52], and we cannot exclude that diffusivity changes could also be related to cerebral small vessel disease co-existing with or interacting with the AD process (though Fazekas scores in this cohort was similar between patients and controls).

Sharp demarcation between cognitive impairment of differing severity is not possible; differences in cognitive reserve and test-specific limitations concerning premorbid cognitive abilities may blur the demarcation between SCI and MCI, and MCI and dementia. Our decision to primarily stage the subjects on basis of clinical screening tests is a limitation in the present material in some respects, but these tests directly reflect everyday function and similar tests are commonly used in clinical practice. The present study was performed on a 1.5 Tesla systems and use of higher field strength systems could potentially have resulted in better signal-noise ratio and improved statistical power [53]. The increasing availability of 3 Tesla systems combined with constant

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### Table 5

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Cognitive decline</th>
<th>Hippocampal atrophy</th>
<th>Entorhinal atrophy</th>
<th>Parahippocampal atrophy</th>
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<tr>
<td>P-tau</td>
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<td>1.01 &lt;0.04 &lt;0.01</td>
<td>1.01 &lt;0.04 &lt;0.01</td>
<td>1.01 &lt;0.04 &lt;0.01</td>
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*Associations with cognitive decline were determined by means of multiple logistic regression. Associations with atrophy were determined by means of multiple linear regression. In logistic regression R² refers to Nagelkerke R Square, whereas in linear regression it refers to Adjusted R Square.
improvements in analytical methods should make for more precise estimates of DTI-derived metrics, and with proper future standardization, DTI may even be used clinically in this patient group.

It is previously well documented that CSF biomarkers are predictors of future AD dementia in MCI cases [45], but performance has not been compared relative to DTI. Several of the subjects with pathological Aβ plaque formation may be present well in advance of cognitive impairment and is the first biomarker to become abnormal according to the already discussed hypothetical model of dynamic biomarkers. Further, a significant number of cognitively healthy elderly have evidence of significant Aβ plaque deposition [3]. Thus, clinical deterioration is expected to occur in this patient group, but may not have been observed due to the relatively short time to follow-up or the early disease stage. Short follow-up is a limitation of this (and many other) studies dealing with AD, the follow-up period is short as compared to the decade long disease evolution process. A different interpretation may be that this is an indication that the subjects who showed clinical decline did not do so because of AD. However, the patients underwent thorough clinical evaluation and were diagnosed according to guidelines. The subjects that were diagnosed with other conditions than AD after thorough baseline and follow-up examinations were excluded (though we cannot exclude contribution from non-stroke vascular components).

In addition, the small sample size of the SCI group is a limitation of the study and prevented us from performing the analyses separately in the SCI and MCI groups. Also, a higher number of subjects undergoing cognitive decline during the follow-up period would have made for more robust results. For whole-brain voxel-wise statistics (performed in FSL Randomise), the number of subjects in the smaller group is arguably low, and these results should be interpreted in light of results from the SPSS based analyses. The possibility that the use of two different scanners could bias the DTI data has been a concern. Scanner was included and corrected for in all relevant analyses. Pearson correlations showed the DTI data to be fairly consistent (but not identical) across scanners. This underlines the need to correct for scanner, but also shows that this is a valid approach. Further, the number of patients and controls on each scanner at baseline was almost equal, and previous studies on partially overlapping samples have shown that data collected from the scanners are comparable, not biasing the results [54, 55]. Among the study’s strengths are the multimodal subject characterization and the longitudinal design.

In conclusion, DTI predicts dementia and atrophy of the medial temporal lobe, and DTI is a better predictor of cognitive decline and medial temporal atrophy than CSF biomarkers in this cohort of pre-dementia patients.

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