The pathogenesis of lesions and normal-appearing white matter changes in multiple sclerosis
A serial diffusion MRI study

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Summary
The idea that the initiating event in the formation of all new multiple sclerosis lesions is a focal blood–brain barrier (BBB) leakage associated with perivascular inflammation has been challenged recently by the observation of subtle abnormalities in some quantitative magnetic resonance (MR) parameters (including the magnetization transfer ratio) prior to lesion enhancement. MR diffusion imaging can non-invasively quantify the average apparent diffusion coefficient (ADCav), a measure of water molecule random motion that is sensitive to pathological change in multiple sclerosis lesions and to abnormalities in the normal-appearing white matter (NAWM). We therefore used MR diffusion imaging to investigate the dynamic evolution of water diffusion measurements in new enhancing multiple sclerosis lesions, in the NAWM from which they arise, and in anatomically matched contralateral NAWM regions from which no visible lesions develop. Gadolinium diethylenetriaminepentaacetic acid (Gd)-enhanced MRI and MR diffusion studies were performed monthly for 1 year in five multiple sclerosis patients with clinically and radiologically active disease. The ADCav was calculated at each time point of the study (before, during and after lesion appearance on Gd-enhanced scans) for each new enhancing lesion, and for regions matched for size and position in the contralateral NAWM. A steady and moderate increase in ADCav in prelesion NAWM was observed, which was followed by a rapid and marked increase at the time of Gd enhancement and a slower decay after the cessation of enhancement. In matched contralateral NAWM regions there was a significant but milder increase in ADCav at the time of the first noted lesion enhancement. These findings indicate that new focal lesions associated with frank BBB leakage are preceded by subtle, progressive alterations in tissue integrity beyond the resolution of conventional MRI. The increases in ADCav in anatomically matched contralateral regions after lesions have appeared supports the concept that structural damage in lesions causes damage or dysfunction in connected areas of NAWM.

Keywords: multiple sclerosis; diffusion; magnetic resonance imaging; pathophysiology

Abbreviations: ADCav = average apparent diffusion coefficient; BBB = blood–brain barrier; Gd-DTPA = gadolinium-diethylenetriaminepentaacetic acid; EDSS = expanded disability status scale; NAWM = normal-appearing white matter; MR = magnetic resonance; MTR = magnetization transfer ratio; NAA = N-acetyl aspartate; PD = proton density

Introduction
The pathological features of the characteristic white matter plaques found in multiple sclerosis, namely demyelination, inflammation, gliosis and axonal loss, are well described (Lassmann, 1998). However, as tissue studies are available at only one time point, they are of limited value in elucidating lesion pathogenesis. Serial MRI studies have the potential to monitor lesion evolution in vivo. For example, conventional T2-weighted MRI demonstrates the evolution of multiple sclerosis lesions with excellent sensitivity, although T2 signal changes do not reflect a specific pathological process (Clanet and Berry, 1998). The contrast agent gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA) improves pathological specificity: areas of enhancement with Gd-DTPA reflect blood–brain barrier (BBB) disruption and inflammation.
The great majority of new lesions show a similar pattern of evolution, in which new T2-weighted signal abnormalities are accompanied or preceded by focal Gd-DTPA enhancement (Miller et al., 1988; Thompson et al., 1992), suggesting that inflammation, associated with BBB leakage, is a primary event in lesion evolution.

However, newer MRI techniques are challenging the notion that all lesions evolve in this stereotyped pattern, in which focal BBB leakage and perivascular inflammation is the initiating event. There is evidence from some studies using magnetization transfer imaging, which provides information on protons bound to macromolecules, thus giving a measure of tissue integrity, that subtle structural abnormalities in areas of NAWM may precede any detectable changes on Gd-DTPA-enhanced or T2-weighted scans (Filippi et al., 1998; Goodkin et al., 1998; Pike et al., 1998), although not all investigators have confirmed these findings (Dousset et al., 1998; Silver et al., 1998).

The sequence of events in lesion evolution is thus a key question that has important implications for understanding lesion pathogenesis and for targeting (or monitoring) therapeutic interventions. It is important to establish whether BBB leakage is the initiating event in new lesion formation or a consequence of earlier subtle pathological changes in NAWM that are not demonstrated on standard MRI. In order to investigate this question, a technique is required which is sensitive to subtle pathology. Diffusion magnetic resonance (MR) imaging is promising in this regard: it is sensitive to the random translational motion (diffusion) of water molecules in tissue. Even subtle pathological damage should disrupt the tissue architecture, increasing the mobility of water molecules and giving diffusion imaging the potential to detect structural changes inaccessible to other MRI methods.

Quantitation of diffusion is possible by applying magnetic field gradients of different degrees of diffusion sensitization, allowing the calculation of the apparent diffusion coefficient (ADC) in tissue (Le Bihan et al., 1992). The ADC is elevated in areas of NAWM in multiple sclerosis (Christiansen et al., 1993; Horsfield et al., 1996; Droogan et al., 1999; Werring et al., 1999), suggesting that MR diffusion imaging can indeed detect subtle pathological changes not apparent on conventional MRI. We therefore performed a serial diffusion MRI study in five multiple sclerosis patients with clinical and MRI evidence of disease activity over a period of 1 year. The primary aim was to monitor the dynamic evolution of diffusion properties, particularly in the NAWM regions from which lesions subsequently arose. Contralateral NAWM regions, matched as far as possible to lesion location, were examined in order to investigate whether measurable diffusion changes occur in anatomically connected regions before or after lesion appearance. Areas of NAWM that were not obviously connected to areas in which lesions appeared during the course of the study were also examined.

Methods
All patients gave written informed consent to participation in the study, which had been approved by the Joint Ethics Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery, London.

Patients
Five patients with clinically definite multiple sclerosis (Poser et al., 1993) were recruited from the population of patients attending the out-patient department of the National Hospital for Neurology and Neurosurgery. Patients were required to have radiological evidence of active disease (at least one Gd-DTPA-enhancing lesion) prior to inclusion in the study; this criterion was designed to increase the yield of enhancing lesions for longitudinal analysis. A trained observer (D.J.W.) examined all patients and an expanded disability status scale (EDSS) (Kurtzke, 1983) assessment was made at each visit.

MRI protocol
All studies were performed on a 1.5 T Signa echospeed MRI system (General Electric, Milwaukee, Wis., USA) with a quadrature head coil. A scan was carried out approximately monthly for a total duration of 12–13 months. For all studies, the patients were repositioned carefully in the scanner with reference to sagittal anatomical localizer images from previous imaging studies, as described elsewhere (Gallagher et al., 1997). High-resolution T2-weighted and proton density (PD)-weighted spin echo images were acquired at 26 contiguous axial slices, each 5 mm thick, covering the whole brain (TR 2000 ms, TE 14/100 ms, matrix 256 × 256, where TR = repetition time and TE = echo time). Diffusion imaging was then performed to include the brain tissue above and below the lateral ventricles (see below), and was followed by T1-weighted contrast-enhanced scans [TR 550 ms, TE 15 ms, matrix 256 × 256, 5 mm contiguous slices, 5 min after the administration of a standard dose of Gd-DTPA (0.1 mmol/kg)].

Diffusion imaging
A navigated spin-echo technique with cardiac gating was used. The field of view was 24 cm2, TR 2 × cardiac R–R interval, TE 75 ms (navigator echo TE = 102 ms), 5 mm thick slices, matrix 128 × 256, optimal b value = 738 s/mm–2, gradient strength up to 22 mTm–1, applied along each of the three principal axes. The total scan time required to obtain six to eight contiguous axial slices through the periventricular region and centrum semiovale was ~25 min, depending on heart rate. Standard foam-pad immobilization was used. The navigator echo strategy, in which a second echo without phase encoding is acquired, allows correction for artefacts due to head translation (Ordidge et al., 1994) and rotation (when diffusion sensitization is along the phase
direction) (Anderson and Gore, 1994). Semiquantitative evaluation of the residual motion artefact was applied as described elsewhere (Droogan et al., 1999). Data graded as severely affected on one or more of the diffusion-weighted images were discarded.

The ADC maps were constructed by calculating the ADC on a pixel-by-pixel basis from the MR signal intensity decay according to the formula:

$$\frac{S}{S_0} = \exp(-b \cdot ADC)$$

where $S$ and $S_0$ are the MR signal intensities in the presence and absence of the diffusion-sensitizing gradients, respectively, and $b$ is the gradient $b$ factor, a measure of the degree of diffusion sensitization. Both diffusion and imaging gradient contributions to the $b$ factor were accounted for.

$ADC_{av}$, a directionally averaged measure of diffusion, was calculated from the ADC maps according to the formula:

$$ADC_{av} = \frac{ADC_x + ADC_y + ADC_z}{3}$$

where $ADC_x$, $ADC_y$, and $ADC_z$ are the ADCs measured along each of the principle gradient axes.

To allow serial ADC measurement in a particular constant brain region over the course of the study, repositioning at each scanning session was used in conjunction with a registration program based on the Woods algorithm (Woods et al., 1992), which has been shown to provide robust alignment of serial MRI data (Silver et al., 1998). The $ADC_{av}$ map and the $T_2$- and PD-weighted images were all co-registered with the PD images of the first month for each patient. The Woods algorithm used trilinear interpolation to reslice the diffusion parameter maps, providing a small degree of blurring, both in-plane and through-plane. This registration method minimizes the variance caused by patient positioning in serial multislice scanning. Regions for which full data were not available for all three sets of images were excluded.

### Image analysis

Images were displayed on a Sun workstation (Sun Microsystems, Mountain View, Calif., USA) using DisImage software (D. L. Plummer, University College London, 1997). Lesions for study were defined on post-contrast $T_1$-weighted scans at the onset of Gd enhancement, with reference to the co-registered $T_2$- and PD-weighted scans. For each lesion, a region of interest was defined as the maximum area of signal change seen on the PD-weighted images during the study (Silver et al., 1998). Lesions of area $<20$ mm$^2$ or in close proximity to the CSF were excluded to eliminate small misregistration or partial volume artefacts. For each lesion, a matched contralateral region of interest in the NAWM was defined whenever possible; the contralateral regions were examined carefully to ensure that there was no contamination by MRI-visible lesions or partial volume of CSF at any time during the study. An attempt was also made to define contralateral NAWM regions not anatomically matched to evolving lesions in either the contralateral or the ipsilateral hemisphere.

The $ADC_{av}$ was calculated for lesion regions of interest at each time point of the study (before and after the appearance of lesions on Gd-DTPA-enhanced scans). It was therefore possible to define regions of NAWM which subsequently evolved into an enhancing lesion (prelesion regions). The scan at which Gd-DTPA enhancement was first noted defined the reference time point (time = 0) of the study, to which all lesion and homologous NAWM $ADC_{av}$ measurement profiles were temporally shifted.

### Quality assurance

During and subsequent to the period of the study, a water phantom was scanned using the same spin-echo diffusion sequence every 15 days. The mean values, normalized to 20°C (with standard deviations), were as follows: $ADC_x$ mean = 2.06 (0.056); $ADC_y$ mean = 2.06 (0.049); $ADC_z$ mean = 2.04 (0.040). There was no evidence of a significant upward drift over time.

### Statistical analysis

An analysis of the time-shifted $ADC_{av}$ profiles for lesions (before and after appearance on Gd-enhanced $T_1$-weighted images) and in contralateral NAWM regions was performed using mixed-model regression analysis (Hand and Crowder, 1996). The calculations were performed using SAS v6.12 PROC MIXED (SAS Institute, Cary, NC, USA). A random coefficients piecewise regression model was adopted, incorporating a transition at the time of lesion appearance (time = 0) and differing time dependencies during the pre- and post-enhancing periods. Two calculations were performed. The first used a model that allowed simultaneous analysis of the lesion and contralateral NAWM ADC profiles. Due to the larger number of lesion regions than contralateral NAWM regions, the analysis of homologous contralateral NAWM profiles was performed using the NAWM $ADC_{av}$ profiles in isolation. (In the combined lesion and contralateral NAWM data analysis, some parameter estimates for random effects are dominated by the lesion data.) Probability values were obtained after conversion of the REML (restricted maximum likelihood) Wald statistics to $F$ ratios. The non-homologous contralateral NAWM $ADC_{av}$ profiles were not time-shifted to the time of lesion appearance, but were tested for any linear trend during the study period. For each tissue region (prelesion NAWM, lesions, homologous NAWM and non-homologous NAWM), the rate of change of the $ADC_{av}$ per month was estimated and expressed as a percentage of the $y$ intercept $ADC_{av}$ value.
Table 1 Characteristics of patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Multiple sclerosis type</th>
<th>Age (years)</th>
<th>Sex</th>
<th>EDSS at entry</th>
<th>Number of relapses</th>
<th>EDSS at exit</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RR</td>
<td>27</td>
<td>F</td>
<td>2.5</td>
<td>2</td>
<td>2.5</td>
<td>First relapse (month 8) treated with IVMP, second (month 11) resolved spontaneously</td>
</tr>
<tr>
<td>2</td>
<td>RR</td>
<td>30</td>
<td>F</td>
<td>5.5</td>
<td>1</td>
<td>6.5</td>
<td>Relapse treated with IVMP and subsequently started interferon β. Incomplete recovery</td>
</tr>
<tr>
<td>3</td>
<td>RR</td>
<td>34</td>
<td>F</td>
<td>3.0</td>
<td>None</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>RR</td>
<td>38</td>
<td>F</td>
<td>3.0</td>
<td>None</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>SP</td>
<td>37</td>
<td>M</td>
<td>7.0</td>
<td>None</td>
<td>7.0</td>
<td></td>
</tr>
</tbody>
</table>

RR = relapsing–remitting; SP = secondary progressive; IVMP = i.v. methylprednisolone.

Results

Clinical data

The clinical characteristics of the patients studied are shown in Table 1. Five patients with clinically definite multiple sclerosis were studied [four female, one male; mean age 33.6 years (range 27–38 years), median EDSS at study entry 3.5 (range 3.0–7.0)], mean disease duration 6.6 years (range 1.0–13.0 years). Four had relapsing–remitting multiple sclerosis and one had secondary progressive multiple sclerosis, defined according to standard criteria (Lublin and Reingold, 1996).

Regions available for analysis

One hundred and forty-seven new enhancing lesions were seen on the Gd-enhanced, T₁-weighted images corresponding to slices for which diffusion data were available. Five lesions were excluded because of size <20 mm², five because of proximity to the CSF and 41 because of technical problems in diffusion acquisition, including motion artefact. Forty-five lesions had to be excluded because of the missing volume effect, which occurred when variations in the prescription of the slices between scans meant that a lesion did not exist on some registered images at one or more time points. A total of 51 lesions were therefore available for the study, with 25 matched contralateral NAWM regions; it was not possible to obtain a matched contralateral region for all lesions because of the presence of other lesions or CSF. Nineteen areas of contralateral NAWM unmatched to lesions appearing during the study were identified, but it was not possible to exclude completely the possible influences from lesions in the ipsilateral hemisphere. The monthly mean ADCav measurements from the various regions are provided in Tables 2 and 3.

ADCav measurements in new enhancing lesions

All ADCav maps surviving the motion artefact criteria provided images with a high signal-to-noise ratio and in-plane resolution. A sharp increase in the ADCav was observed at the time of initial enhancement (time = 0) in lesions. This was visible on inspecting the plotted ADCav data (Fig. 1),

Table 2 Mean ADCav (×10⁻³ mm² s⁻¹) at each time point for each type of brain region

<table>
<thead>
<tr>
<th>Month</th>
<th>Lesions</th>
<th>Homologous NAWM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>-8</td>
<td>0.866</td>
<td>0.017</td>
</tr>
<tr>
<td>-7</td>
<td>0.880</td>
<td>0.027</td>
</tr>
<tr>
<td>-6</td>
<td>0.865</td>
<td>0.109</td>
</tr>
<tr>
<td>-5</td>
<td>0.944</td>
<td>0.126</td>
</tr>
<tr>
<td>-4</td>
<td>0.929</td>
<td>0.136</td>
</tr>
<tr>
<td>-3</td>
<td>0.962</td>
<td>0.096</td>
</tr>
<tr>
<td>-2</td>
<td>0.970</td>
<td>0.134</td>
</tr>
<tr>
<td>-1</td>
<td>0.981</td>
<td>0.155</td>
</tr>
<tr>
<td>0</td>
<td>1.177</td>
<td>0.098</td>
</tr>
<tr>
<td>1</td>
<td>1.117</td>
<td>0.157</td>
</tr>
<tr>
<td>2</td>
<td>1.039</td>
<td>0.120</td>
</tr>
<tr>
<td>3</td>
<td>1.089</td>
<td>0.158</td>
</tr>
<tr>
<td>4</td>
<td>1.099</td>
<td>0.133</td>
</tr>
<tr>
<td>5</td>
<td>1.061</td>
<td>0.163</td>
</tr>
<tr>
<td>6</td>
<td>1.101</td>
<td>0.202</td>
</tr>
<tr>
<td>7</td>
<td>1.056</td>
<td>0.125</td>
</tr>
<tr>
<td>8</td>
<td>1.108</td>
<td>0.245</td>
</tr>
<tr>
<td>9</td>
<td>1.151</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Table 3 Mean ADCav (×10⁻³ mm² s⁻¹) at each time point (not time-shifted to lesion appearance) for unmatched contralateral regions of NAWM not obviously connected to new evolving lesions

<table>
<thead>
<tr>
<th>Month</th>
<th>Non–homologous NAWM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>1</td>
<td>0.783</td>
</tr>
<tr>
<td>2</td>
<td>0.844</td>
</tr>
<tr>
<td>3</td>
<td>0.868</td>
</tr>
<tr>
<td>4</td>
<td>0.881</td>
</tr>
<tr>
<td>5</td>
<td>0.879</td>
</tr>
<tr>
<td>6</td>
<td>0.914</td>
</tr>
<tr>
<td>7</td>
<td>0.871</td>
</tr>
<tr>
<td>8</td>
<td>0.896</td>
</tr>
<tr>
<td>9</td>
<td>0.908</td>
</tr>
<tr>
<td>10</td>
<td>0.933</td>
</tr>
<tr>
<td>11</td>
<td>0.891</td>
</tr>
</tbody>
</table>
The ADCav in lesions did not reduce to prelesion NAWM values during the study period, however (Fig. 2).

**ADCav measurements in prelesion NAWM regions**

An increase in ADCav for at least 6 months prior to lesion appearance was apparent on inspecting the plotted data (Fig. 1); the rate of change of the ADCav was estimated as +2.86% per month. The increase in the ADCav was statistically significant in comparison with the null hypothesis of no change over time, and in comparison with the ADCav observed in matched, contralateral NAWM regions up to the time of Gd enhancement ($P = 0.0001$) (Fig. 2).

**ADCav measurements in homologous contralateral NAWM**

The plotted data from homologous contralateral NAWM regions are shown in Fig. 1. Prior to lesion appearance, there was no significant change in ADC over time in comparison with the null hypothesis ($P = 0.678$) (Fig. 2). There was a small but significant increase (by 3.30% of the prelesion ADCav value) in the contralateral NAWM ADCav at the time of lesion appearance ($time = 0$), also visible on the plotted data (Fig. 1) ($P = 0.042$). After lesion appearance, the ADCav in matched contralateral NAWM continued to increase slightly; this upward slope was significant in comparison with the null hypothesis ($P = 0.004$). The estimated rate of change during this period was +0.76% per month; these data are shown in Table 3.

**ADCav measurements in non-homologous contralateral NAWM**

In these regions, which were not time-shifted to the time of lesion appearance, there was evidence for a small but significant linear upward trend throughout the study ($P < 0.001$), with an estimated rate of change of +1.40% per month; these data are shown in Table 3.

**Discussion**

This study provides a serial in vivo exploration of the pathology of multiple sclerosis using MR diffusion imaging. Our finding of a raised ADCav in multiple sclerosis lesions compared with NAWM is in agreement with previous cross-sectional studies using different diffusion imaging techniques, including single-axis diffusion (Larsson et al., 1992; Christiansen et al., 1993), three-axis navigator-corrected spin-echo diffusion (Droogan et al., 1999) and echoplanar diffusion tensor imaging (Werring et al., 1999). The ADCav values we have measured in lesions are comparable to those obtained in a previous cross-sectional study using a similar methodology.
**ADC<sub>av</sub> changes in prelesion NAWM**

The increase in ADC<sub>av</sub> before the onset of Gd-DTPA enhancement indicates that structural changes in prelesion NAWM occur prior to inflammation and BBB leakage, as detected by standard-dose Gd-DTPA-enhanced imaging. Inspection of the data (Fig. 1) indicates that the rise in ADC<sub>av</sub> is not obvious until 6 months prior to lesion appearance, but analysis using the mixed model regression approach suggests that structural abnormalities may evolve for the entire study period, up to 8 months before lesion appearance (Fig. 2), although the amount of data reduces at early time points. The statistical model describes the temporal behaviour of data during the period of the study, but cannot be extrapolated to time points outside this period. However, the trend for a steady increase in prelesion ADC<sub>av</sub> was present from the onset of the study, suggesting that the underlying process is slowly progressive, at least over a period of months. We are unaware of any comparable diffusion data in humans, but an experimental study in a model of inflammatory demyelination has reported changes on DWI (diffusion-weighted imaging) in the internal capsule prior to the appearance of signal abnormality on T<sub>2</sub>-weighted MRI (Heide et al., 1993).

Our data should be interpreted in the light of data obtained with other MRI techniques. Filippi and colleagues have reported a reduction in the magnetization transfer ratio (MTR), a marker of macromolecular integrity, up to 3 months prior to lesion appearance on enhanced scans in 10 patients studied monthly for 3 months (Filippi et al., 1998). A similar study using MT imaging for up to 12 monthly scans obtained comparable results, with NAWM changes preceding lesion appearance by at least several months (Goodkin et al., 1998). Another group has reported a gradual reduction in MTR preceding lesion appearance by months to years (Pike et al., 1998). These changes have not been observed in other studies, however; Silver and colleagues used more frequent (weekly) studies for 3 months in three multiple sclerosis patients and found no evidence of MTR reduction up to 7 weeks prior to lesion appearance on Gd-enhanced scans (Silver et al., 1997). In serial studies over 9–12 months, Dousset and colleagues also detected no MTR changes in prelesion NAWM (Dousset et al., 1998). The reason for the discrepancy between these MTR studies is unclear, but one possibility is that there are heterogeneous mechanisms of evolution such that, in some lesions and some patients, there is a pathological process occurring prior to detectable BBB breakdown, while in others, opening of the BBB (with Gd enhancement) is indeed the earliest event. An alternative possibility is that the difference between the methods and the MRI protocols is important, highlighting the need for a standardized MTR imaging protocol (Berry et al., 1999). It is therefore particularly important to seek corroborative data using other MRI techniques. Another diffusion MRI study has reported that, in six patients studied over 4 months at shorter scan intervals (of the order of days) than in the present work, there was a reduction in the diffusion coefficient in prelesion NAWM (Gass et al., 1999). The discrepancy between these data and those reported here is most likely to be due to differences in temporal sampling resolution, although it is possible that methodological differences also contribute: Gass and colleagues examined 11 new active lesions using an echoplanar diffusion-sensitized method in contrast to the navigated spin-echo sequence used in our study.

The pathological changes described in multiple sclerosis NAWM include astrocyte hyperplasia, activation of microglia, small areas of perivascular inflammation and myelin breakdown products (Allen and McKeown, 1979), and an increase in tissue water (Tourtellotte and Parker, 1968). Reduced axonal density (Evangelou et al., 2000) and alterations in neurofilament phosphorylation (Ferguson et al., 1997; Trapp et al., 1998) have also been reported, as has evidence for an oligodendropathy (Lucchinetti et al., 1996), which might subsequently lead to demyelination. A number of these processes would lead to a disruption of tissue structure and an increase in the extracellular space, thus increasing ADC<sub>av</sub>. Although the lack of visible Gd-DTPA enhancement or accompanying abnormality on T<sub>2</sub>-weighted images indicates that marked BBB leakage and associated oedema are not present, a subtle BBB leak is not excluded, since previous studies have shown that enhancement may occur only with higher doses of contrast (Filippi et al., 1996; Silver et al., 1997; van Waesberghe et al., 1997), and that quantitative signal changes suggesting a mild BBB leak may
occur either focally or diffusely in the NAWM prior to visible enhancement (Goodkin et al., 1998). On the other hand, a primary oligodendropathy with secondary demyelination could also account for the observed ADCav increase in the absence of visible enhancement or change on T2-weighted images, and a previous report of elevated lipid and choline peaks in prelesional NAWM using MR spectroscopy would be consistent with such a process (Narayana et al., 1998). The present study cannot distinguish between these possibilities, but further studies which employ higher doses of contrast, quantitation of BBB leakage and MR spectroscopy will help to do so.

**ADCav during lesion evolution**
A striking elevation of ADCav was seen at the time of appearance of lesions and visible evidence of BBB breakdown on Gd-DTPA-enhanced images. This is consistent with acute vasogenic oedema causing an increase in the number of water molecules in the more freely diffusing extracellular compartment. The predicted increase in ADCav associated with oedema has been confirmed in experimental models of inflammatory demyelination (Verhoey et al., 1996). Active demyelination, a classic finding in acute multiple sclerosis plaques, and possibly axonal destruction, could also contribute to the observed ADCav increase; both processes would be expected to reduce the barriers to water diffusion, and there is now increasing evidence that axon damage occurs even in acute lesions (Ferguson et al., 1997; Trapp et al., 1998). The steady increase in ADCav prior to lesion formation, followed by a rapid increase at the time of visible enhancement, is consistent with the concept that a low-grade pathological process is evolving in prelesion NAWM, which reaches a threshold of pathological change beyond which there is the acute development of a focal lesion with gross breakdown of the BBB, accompanied by frank inflammation and demyelination. It is noteworthy that the mean ADCav of the white matter immediately prior to lesion appearance (1.0 × 10^−3 mm^2 s^−1) was higher than the mean ADCav measured at any time from the contralateral NAWM regions.

After the appearance of a lesion on enhanced scans, the ADCav reduced. The mixed regression model indicates that this reduction initially occurs rapidly but subsequently slows (probability of curvature in the post-enhancing period P = 0.018). A rapid initial reduction in ADCav is consistent with the resolution of inflammation and oedema, in keeping with serial conventional MRI observations of a ‘disappearing element’ on T2-weighted images (Willoughby et al., 1989; McDonald et al., 1992). The sustained progressive reduction in ADCav (albeit occurring at a less rapid rate) could reflect a number of processes, including repair of the BBB, remyelination and gliosis. However, the ADCav in lesions did not decrease to the level of prelesional or contralateral NAWM during the follow-up period of this study, consistent with persistence of structural damage, including demyelination and axonal loss. This observation is also in agreement with diffusion studies of multiple sclerosis lesions demonstrating a raised ADCav in chronic lesions; the loss of structural integrity (indicated by the extent of ADCav elevation) is particularly severe in T1-hypointense lesions (Droogan et al., 1999; Werring et al., 1999).

**ADCav changes in the contralateral NAWM**
The contralateral homologous NAWM initially showed stable ADCav measurements (Fig. 1), with no significant difference in comparison with the null hypothesis of no change over time (P = 0.678). However, at the time of the first noted lesion enhancement there was an increase in ADCav (P = 0.042), albeit of smaller magnitude than that observed within the lesion itself. In subsequent months the ADCav continued to increase in contralateral NAWM steadily but slowly (P = 0.004); at the final time point it appeared to begin to reduce (Fig. 1). Although we are not aware of previous reports of abnormal ADC measurements in the hemisphere contralateral to an acute lesion, a reversible reduction in the relative N-acetyl aspartate (NAA) concentration has been observed in regions contralateral to three large, solitary demyelinating lesions (De Stefano et al., 1997; Trapp et al., 1998). The data indicate that the ADCav, subsequently continues to increase in contralateral NAWM for at least 6 months; this is consistent with the time course of developing Wallerian degeneration, which in the CNS has been shown to appear by 4 weeks after
injury, with subsequent evolution over several months on T2-weighted MRI (Kuhn et al., 1989).

Considerable care was taken to ensure that the contralateral homologous NAWM regions were not contaminated by visible tissue abnormality during this study. The lack of remaining NAWM in the brain volume we studied, and the large number of new lesions, made it difficult to examine an adequate number of uncontralateral NAWM regions unmatched to evolving lesions. In the regions identified, a small but significant linear upward trend provided an adequate model of the data. It is not meaningful to time-shift selected non-homologous NAWM regions to their contralateral lesions in the same way as for homologous NAWM, since their location is arbitrary (i.e. there may be a number of possible unmatched regions from which the choice of a single area is essentially random). It is therefore not possible to conclude from the available data whether there is a difference in the NAWM behaviour between anatomically homologous and non-homologous regions. The available results suggest that, in these active patients, there may be a small but steady increase in the ADC of NAWM remote from visible lesions. Several factors may contribute. First, it is possible that non-homologous NAWM regions could be influenced by lesions in connected regions in the ipsilateral hemisphere. Unfortunately the effects of all potentially connected lesions could not be avoided because of the large number of evolving lesions and the limited brain volume available for analysis in this study. Secondly, it is conceivable that the subtle NAWM ADC changes remote from lesions, in brain areas not obviously connected by traversing fibres, could result from the effects of diffusible factors associated with inflammation (Moreau et al., 1996). Inflammation would be expected to accompany the clinical and radiological evidence of disease activity seen in these patients, three of whom had either clinical relapse or disease progression during the period of study. Thirdly, it is possible that microscopic pathology develops independently of lesions in at least some regions of NAWM, especially in a clinically active subgroup such as this. At present it is not possible to determine definitively whether anatomical symmetry with a lesion confers increased susceptibility to damage or dysfunction in NAWM. Further studies in patients with fewer active lesions may help to clarify this question.

The present study was also limited by the small patient cohort and the limited region of brain coverage possible using the spin-echo diffusion sequence, although the latter limitation is partly offset by the good spatial resolution. The number of lesions per patient varied between three and 39, a large proportion of the lesions (77%) coming from a single patient, so it was not possible to perform statistical analyses on each individual. All patients did, however, show visually similar time courses, justifying the decision to analyse them together and indicating that the result was not confined to the patient with the high lesion activity. A key question for future studies in larger cohorts will be to define the prevalence, regional extent and duration of NAWM diffusion change in regions contralateral to evolving lesions, and its relationship to the present and future clinical course.

**Stability of ADC measurements**

The stability of serial ADC measurements obtained with the same MR diffusion sequence in a water phantom during the study period indicates that the changes in ADC seen in prelesional NAWM and in contralateral NAWM are unlikely to have been due to artefactual upward drift in diffusion measurements. Moreover, the ADC pattern observed in evolving lesions was seen not only in the group data but also in each of the five patients considered individually, indicating that the jump in ADC cannot be explained by an instrumental drift in ADC measurements.

**Conclusions**

This longitudinal study provides new information about the pathogenesis of multiple sclerosis lesions and NAWM changes. It suggests that focal inflammatory BBB leakage, indicated by contrast enhancement, is not necessarily the initiating event in multiple sclerosis plaque formation but may be preceded, and perhaps triggered, by subtle progressive alterations in tissue integrity beyond the resolution of conventional MRI. The changes in ADC in the contralateral NAWM imply that distant pathological damage or dysfunction in the white matter can result from damage or dysfunction of fibres traversing lesions, although other factors not directly related to focal lesions may also contribute.

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**References**


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