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Phil. Trans. R. Soc. Lond. B 1999 **354**, 1675-1686
doi: 10.1098/rstb.1999.0511

THE ROYAL SOCIETY

Characterization of tissue damage in multiple sclerosis by nuclear magnetic resonance

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Nuclear magnetic resonance (NMR) imaging is an established diagnostic medium to diagnose multiple sclerosis (MS). In clinically stable MS patients, NMR detects silent disease activity, which is the reason why it is being used to monitor treatment trials, in which it serves as a secondary outcome parameter. The absence of a clear correlation with clinical disability, the so-called 'clinico-radiological' paradox, and the poor predictive value of NMR prohibit the use of NMR as a primary outcome parameter in clinical trials. This is—among others—a result of the limited histopathological specificity of conventional, or 'T2-weighted' imaging, the most commonly used NMR technique. In this paper we review additional NMR techniques with higher tissue specificity, most of which show marked heterogeneity within NMR-visible lesions, reflecting histopathological heterogeneity.

Gadolinium enhancement identifies the early inflammatory phase of lesion development, with active phagocytosis by macrophages. Persistently hypointense lesions on T1-weighted images ('black holes') relate to axonal loss and matrix destruction, and show a better correlation with clinical disability. Marked prolongation of T1 relaxation time correlates with enlargement of the extracellular space, which occurs as a result of axonal loss or oedema. Axonal viability can also be measured using the concentration of *N*-acetyl aspartate (NAA) using NMR spectroscopy; this technique is also capable of showing lactate and mobile lipids in lesions with active macrophages. The multi-exponential behaviour of T2 relaxation time in brain white matter provides a tool to monitor the myelin water component in MS lesions (short T2 component) as well as the expansion of the extracellular space (long T2 component). Chemical exchange with macromolecules (e.g. myelin) can be measured using magnetization transfer imaging, and correlates with demyelination, axonal loss and matrix destruction. Increased water diffusion has been found in MS lesions (relating to oedema and an expanded extracellular space) and a loss of anisotropy may indicate a loss of fibre orientation (compatible with demyelination).

Apart from the histopathological heterogeneity within focal MS lesions, the normal-appearing white matter shows definite abnormalities with all quantifiable NMR techniques. A decrease in the concentration of NAA, decreased magnetization transfer values and prolonged T1 relaxation time values are probably all related to microscopic abnormalities, including axonal damage. This 'invisible' lesion load may constitute a significant proportion of the total lesion load but is not visible on conventional NMR. Similarly, mechanisms for clinical recovery exist, which are not distinguished using MR imaging. Therefore, it is highly unlikely that the clinico-radiological paradox will ever be solved completely. However, NMR provides an opportunity to sequentially measure tissue changes *in vivo*. Using MR parameters with (presumed) histopathological specificity, the development of (irreversible) tissue damage can be monitored, which perhaps allows the identification of factors that determine lesional outcome in MS. Since the absence of severe tissue destruction is prognostically favourable, NMR monitoring of the extent to which such changes can be prevented by treatment will ultimately benefit the selection of future treatment strategies.

Keywords: multiple sclerosis; magnetic resonance; histopathology; relaxation time; blood–brain barrier

1. BACKGROUND: THE CLINICO-RADIOLOGICAL PARADOX

Nuclear magnetic resonance (NMR) imaging has become the major confirmatory test for multiple sclerosis (MS), by sensitively showing disseminated lesions in the central nervous system (CNS). NMR imaging has not only improved the diagnostic certainty in MS, but has also greatly influenced our thinking about the evolution of the disease process itself. Clinically silent lesions are often found, not only in patients presenting with their first symptoms (Morrissey *et al.* 1993), but also in between relapses in patients with clinically definite MS (Barkhof *et al.* 1992*b*; Thompson *et al.* 1991; Miller *et al.* 1993). These 'silent' lesions are found throughout the brain, even in eloquent areas such as the optic nerve and spinal cord. New symptoms, on the other hand, are usually accompanied by new lesions on NMR (Smith *et al.* 1993); the reason why NMR

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Table 1. *NMR parameters and their presumed histopathological correlate*

NMR parameter	physical alteration	interpretation
gadolinium enhancement	uptake in CNS	blood–brain barrier disruption
T1 relaxation rate	mild prolongation	intracellular oedema, gliosis
	strong prolongation	extracellular widening and axonal loss
T2 relaxation rate	reduced short component	loss of myelin-associated water
	increased long component	increased free (extracellular) water
magnetization transfer	reduced chemical exchange	demyelination, axonal loss
diffusion weighting	reduced Brownian motion	tissue swelling
	increased Brownian motion	diminished compartmentalization
	reduced anisotropy	loss of tissue fibre orientation
spectroscopy	reduced NAA	loss of neuronal integrity
	increased myoinositol	gliosis
	increased Cho	increased membrane turnover
	mobile lipid peaks	myelin breakdown products
	lactate	macrophage activation

is now also frequently used to monitor treatment (Miller *et al.* 1996; Miller & Thompson, this issue).

Following great enthusiasm about the possibilities of such a sensitive disease measure, scepticism has arisen about what we have, optimistically, called the 'clinico-radiological paradox' (Barkhof & Filippi 1995). Disease progression on NMR imaging occurs without, at the moment, measurable clinical consequences to the patient. Sceptics wonder what the nature and significance of such lesions might be, since they have not been shown to predict clinical worsening very well (Kappos *et al.* 1999). It should be noted that the relevance of surrogate markers is not unique for radiology nor for MS. Pathologists too have noticed a discrepancy between the amount of lesions found at autopsy and the clinical status of the patient (Lumsden 1970). The clinico-radiological paradox is also present in other diseases; for example, in rheumatoid arthritis discordancies are observed between (absence of) clinical disease progression and subclinical parameters, such as bone erosions (Mulherin *et al.* 1996). On a more philosophical level, one might even regard the clinical situation of the patient as a surrogate marker of the disease, and the biological marker (when known) as the true disease process.

At a time that NMR imaging has become a widespread tool, not only diagnostically, but also to monitor therapies, it is becoming a relevant issue to understand (and perhaps resolve) the clinico-radiological paradox in MS. Apart from clinical factors, such as uncertainty about the moment of onset of the disease and limitations of clinical scales (Noseworthy *et al.* 1990), methodological factors may play a role, such as a cross-sectional type of analysis or an insufficiently long duration of follow-up. One of the most important factors, however, is the limited histopathological specificity of conventional, so-called 'T2-weighted' NMR. In fact, almost any alteration in brain tissue composition (e.g. oedema, inflammation, demyelination, gliosis and axonal loss) will increase the signal on such images. While this explains the high sensitivity of NMR imaging on a diagnostic level, it also results in a low specificity of NMR imaging for MS, and a lack of predictive value for future disability. In this review, other NMR parameters will be discussed that are histopathologically more specific, and could shed light on the clinico-radiological paradox. Following a short introduction on conventional NMR imaging, the various NMR parameters and their presumed histopathological correlates (table 1) will be discussed, together with some emerging concepts regarding the pathophysiological processes at work in MS.

2. CONVENTIONAL NMR IMAGING: A MIXTURE OF PHYSICAL PARAMETERS

In standard NMR imaging, contrast is dominated by three main physical properties, i.e. proton density, and the longitudinal and the transverse relaxation rates. The last two show exponential curves, characterized by the time constants T1 and T2, respectively. In biological tissues, typical T1 values range from about 50 ms to a few seconds. In general, the T2 relaxation time is shorter than the T1 relaxation time and ranges in biological tissues from a few microseconds in solids to a few seconds in liquids. The ability of MR imaging to create high contrast images of the brain depends on the differences in T1 and T2 relaxation times of brain structures. Based on these inherent tissue parameters, NMR image contrast can be manipulated by user-selectable pulse interval delays. Image contrast can be made T1-dominated ('T1-weighted') or T2-weighted (accentuating differences in T2 relaxation time). So-called 'proton-density weighted' images are obtained by minimizing T1 and T2 contrast effects.

Using conventional pulse sequences, several compromises have to be made in a clinical setting to achieve acceptable image acquisition times and an adequate signal-to-noise ratio. As a result, T2-weighted and proton-density-weighted images often display considerable T1-weighting, while T1-weighted images are usually not very heavily T1-weighted. In multislice imaging, the use of slice-selective gradient magnetic fields further complicates matters by introducing magnetic transfer and diffusion effects (see §§ 6 and 7). In the end, the contrast in conventional NMR imaging is a mixture of contrast mechanisms and often difficult to unravel. Several dedicated pulse sequences have been designed to disentangle the complex contrast mechanisms, in order to highlight one specific physical property. In doing so, one can obtain information about the physical properties

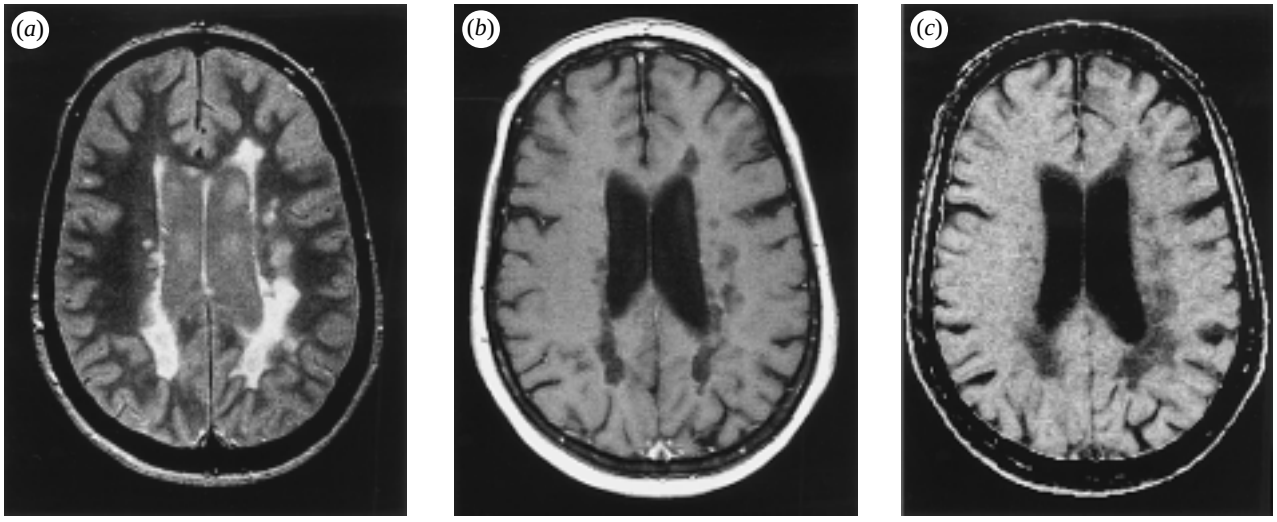

Figure 1. T2-weighted (*a*) and enhanced T1-weighted (*b*) MR images versus MT ratio (MTR) image (*c*) of an MS patient. Part of the abnormalities which are visible on the T2-weighted MR image (*a*) as increased signal intensity areas have low signal intensity (hypointense lesions or 'black holes') on the corresponding T1-weighted MR image (*b*). Note that the hypointense lesion load resembles the 'MTR lesion load', although more abnormalities are visible on the MTR image (*c*) than on the T1-weighted image, possibly due to a diffuse involvement of the brain matter surrounding the hypointense lesions.

listed in table 1, and study the tissue at hand with greater accuracy.

Conventional T2-weighted images are routinely acquired in the diagnostic setting. Their sensitivity is undisputed, even when comparing post-mortem scans with histopathology (Newcombe *et al.* 1991; Stewart *et al.* 1984; Van Waesberghe *et al.* 1999; Barkhof *et al.* 1993). The typical sclerotic lesions that are found macroscopically at post-mortem are displayed as clearly demarcated lesions with a very high signal. In addition, smaller and less bright abnormalities are observed, which can be difficult to see macroscopically. On a microscopical level such lesions range from partly demyelinated areas (or perhaps remyelinated lesions) to early reactive lesions with only minimal inflammatory changes and oedema (Van Waesberghe *et al.* 1999). This unique sensitivity for MS abnormalities suggests that post-mortem NMR imaging could be used to guide sampling of CNS tissue at autopsy. Also, with NMR imaging it is possible to obtain a quick survey of the distribution of lesions, something that would be impossible using routine sectioning at autopsy.

3. GADOLINIUM ENHANCEMENT: MARKER OF BLOOD–BRAIN BARRIER INTEGRITY

Chelated gadolinium can be used as a paramagnetic contrast agent for NMR imaging. Gadolinium has a strong paramagnetic effect and effectively shortens the T1 relaxation rate. It is a so-called extracellular contrast agent, and is not able to cross the intact blood–brain barrier. Gadolinium enhancement thus is a marker of blood–brain barrier breakdown, and histologically correlates with the inflammatory phase of lesion development. Both in experimental allergic encephalomyelitis (EAE) and MS, gadolinium enhancement correlates with the presence of active macrophages in relation to demyelination (Hawkins *et al.* 1990; Katz *et al.* 1993; Nesbit *et al.* 1991; Bruck *et al.* 1997). In MS, virtually all new (NMR-visible) lesions go through a phase of enhancement persisting for two to eight weeks.

The number of enhancing lesions correlates with a number of clinical measures, of which the occurrence of a relapse is the most evident. Several studies have clearly that the number and volume of enhancing tissue predict the onset and severity of relapses (Khoury *et al.* 1994; Smith *et al.* 1993). This is also reflected in differences between clinical subgroups, with fewer enhancing lesions present in clinically benign disease than in patients with relapsing–remitting (RR) disease (Thompson *et al.* 1990). Furthermore, secondary progressive (SP) patients show more extensive lesions on MRI than RR ones (Lycklama à Nijeholt *et al.* 1998). In patients with RR disease, the expanded disability status scale score correlates with the number of gadolinium-enhancing lesions (Stone *et al.* 1995). The number of enhancing lesions correlates with the level of myelin basic protein in the cerebrospinal fluid (CSF), which is a marker of myelin destruction. Following steroid treatment there is a strong suppression of enhancing lesions, which correlates with a decrease in myelin breakdown products in CSF, as well as with clinical remission (Miller *et al.* 1992; Barkhof *et al.* 1992*a*). A fine example of the clinical relevance of gadolinium enhancement is the optic nerve, in which the presence of enhancement predicts clinical signs and symptoms, and in which resolution of enhancement parallels the functional recovery (Youl *et al.* 1991). Similarly in the spinal cord, virtually every new enhancing lesion is accompanied by new symptoms (Kidd *et al.* 1996).

4. HYPOINTENSE T1 LESIONS ('BLACK HOLES'): MARKER OF TISSUE DESTRUCTION

Almost any alteration in brain tissue composition in MS will increase the T2 relaxation time, but prolongation of T1 relaxation time occurs only in part of the lesions to

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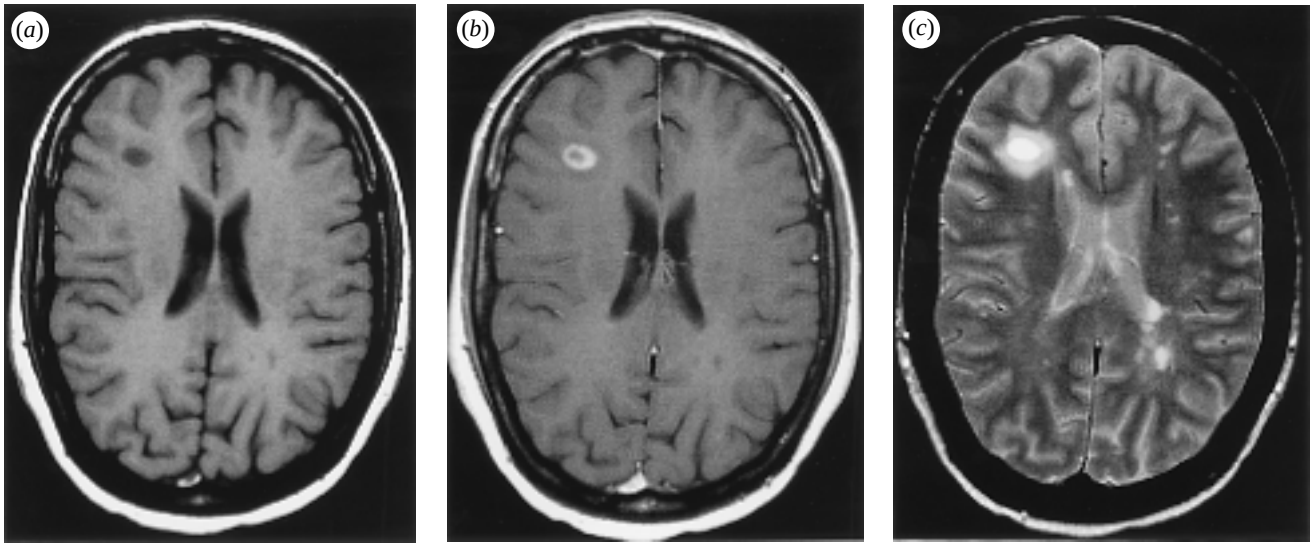

Figure 2. Unenhanced T1-weighted (*a*) versus contrast-enhanced T1-weighted (*b*) and T2-weighted (*c*) spin-echo MR images of an MS patient. The enhancing lesion visible on (*b*) and (*c*) appears severely hypointense on the precontrast T1-weighted MR image. Dependent on the histopathology of this active lesion, this (acute) hypointense lesion may revert to isointensity at follow-up (resolution of oedema and inflammation, remyelination) or the hypointense signal may persist, reflecting a severe loss of myelin and axons.

such an extent that a marked decrease in signal intensity on (moderately) T1-weighted images occurs (see figure 1). These hypointense lesions (or 'black holes') show a better correlation with clinical disability than has been observed for T2 lesions (Van Walderveen *et al.* 1995; Truyen *et al.* 1996), which indicate that a more destructive histopathological process might be present. Biopsy (Bruck *et al.* 1997) and autopsy (Van Walderveen *et al.* 1998, Van Waesberghe *et al.* 1999) studies have indeed shown that the degree of hypointensity on moderately T1-weighted images correlates strongly with the degree of matrix tissue (widening of the extracellular space) and with loss of axons. The occurrence of hypointense lesions is more pronounced in SP patients compared with RR ones (Lycklama à Nijeholt *et al.* 1998; Truyen *et al.* 1996). This may indicate that at a certain time point in the RR phase of the disease, repair mechanisms start to fall short and progressive tissue destruction with concomitant clinical disability develops (Truyen *et al.* 1996).

The majority of hypointense lesions represent chronic black holes, in which irreversible tissue destruction has occurred. However, most active lesions which show gadolinium enhancement also temporarily appear hypointense on precontrast T1-weighted images (Van Waesberghe *et al.* 1998*b*) (see figure 2). This phenomenon probably relates to the presence of oedema, inflammatory cells and (incomplete) demyelination. As a result, approximately half of these acute hypointense lesions are no longer visible on T1-weighted MR images at follow-up; it is speculated that this recovery relates not only to resolution of oedema and inflammation, but also to remyelination (Van Waesberghe *et al.* 1998*b*). A persistent black hole at follow-up probably relates to persistent and complete loss of myelin, and more importantly, to (early) axonal loss in the acute phase of lesion development (Van Waesberghe *et al.* 1998*b*), similar to what has been observed histopathologically (Trapp *et al.* 1998; Ferguson *et al.* 1997).

5. RELAXATION TIME MEASUREMENTS: FROM EXPERIMENTAL RESULTS TO *IN VIVO* APPLICATION

The measurement of T1 relaxation times can be performed using several NMR techniques, which do not necessarily yield exactly the same results. Most techniques are quite time-consuming, and can only be performed in a single-slice mode. With the advent of 'echo-planar imaging', this problem can be partially circumvented, allowing T1 to be measured in a multislice data set in the order of a few minutes (see figure 3). *In vivo* measurement of T1 and T2 relaxation times of protons provides information about the tissue water environment. In normal brain tissue, the longitudinal relaxation decay curve follows a mono-exponential function. Although earlier studies identified a mono-exponential T2 behaviour for brain white matter, more recent studies showed that the transverse relaxation decay curve of brain white matter is multi-exponential in nature. This probably results from improved multi-echo MR techniques, allowing the use of a sufficiently short echo time to identify a very short T2 component, and the ability to decompose the decay curve into an arbitrary number of exponentials. The different T2 times of this multi-exponential decay curve correspond to three different water reservoirs: (i) a minor fraction with a very short T2 between 10 and 50 ms due to water compartmentalized in myelin membranes, so-called myelin water; (ii) a major fraction (approximately 80% of the water in normal brain) with T2 between 70 and 95 ms due to water in cytoplasmic and extracellular spaces; and (iii) a small fraction with T2 values of 1 s or more, consistent with CSF (for example in perivascular spaces) (Stewart *et al.* 1993; MacKay *et al.* 1994; Whittall *et al.* 1997).

Previous studies have shown that, while (changes in) T1 and T2 relaxation times tend to correlate, the degree

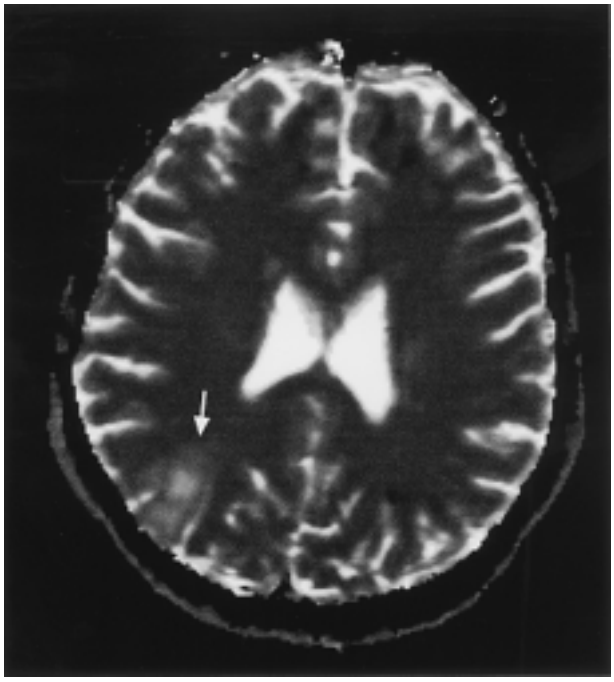

Figure 3. T1 relaxation time map of an MS patient. The T1 relaxation time was measured using an inversion recovery spin-echo echo-planar imaging sequence, with varying inversion times to obtain differences in T1 weighting in successive images. By using a hill-climbing algorithm, images were fitted on a pixel-by-pixel basis to calculate T1 relaxation time maps. In this T1 relaxation time map a large lesion is visible (see arrow) with increased signal intensity, representing a prolongation of T1 relaxation time (which is especially evident in the centre of the lesion)

to which they do depends on the kind of pathology at hand. One should keep in mind that these studies, given the problems related to measuring very short T2 relaxation times, do not distinguish a myelin water component or even a CSF component in analysing the T2 decay curve, and therefore typically report a bi-exponential or monoexponential curve, respectively. For example, experimental (triethyltin-induced) cerebral oedema affects the brain white matter diffusely, without enlargement of the extracellular space or astrocyte swelling (Barnes *et al.* 1986). This results in a lengthening of T2 relaxation time, which is almost twice that of the T1 relaxation time. In contrast, in vasogenic oedema, the extracellular space enlarges with accumulation of protein-rich fluid, this time with a proportional increase of T2 and T1. Further, the T2 decay now follows a bi-exponential behaviour, reflecting the separate pools of intracellular water and extracellular oedema (Barnes *et al.* 1987). A biexponential T2 decay, reflecting enlargement of the extracellular space, also occurs in the case of axonal loss. This contrasts with findings in experimental gliosis in cats, where T1 relaxation time is increased without a corresponding increase in T2 relaxation time and the T2 magnetization decay remains mono-exponential (Barnes *et al.* 1988).

In animals with EAE, a mild prolongation of T1 and T2 is observed, reflecting the oedematous nature of the lesions. As in patients with MS, NMR changes can often be observed before onset of clinical symptoms or before onset of pathological changes (O'Brien *et al.* 1987; Stewart *et al.* 1991). In primate EAE, prolonged T1 and T2 values are associated with the presence of inflammation, demyelination and haemorrhagic necrosis (Stewart *et al.* 1991). In guinea-pigs, prolongation of T1 was observed during meningeal and perivascular inflammation, while T2 increased with demyelination (Karlik *et al.* 1986). In EAE lesions in the spinal cord and brain of guinea-pigs, the short T2 component, assigned to myelin water, was smaller or absent in demyelinated lesions (Stewart *et al.* 1993).

In MS lesions, both T1 and T2 values are increased to a variable degree (Larsson *et al.* 1988), with a large overlap between acute and chronic plaques (Larsson *et al.* 1989). In acute plaques, T1 relaxation time is mono-exponentially prolonged and decreases over time, while T2 relaxation process becomes bi-exponential initially, and reverses to mono-exponential decay in some cases. The bi-exponential T2 decay curve reflects myelin loss, with enlargement of the extracellular space, and gliosis. After resolution of oedema, only one component (gliosis) can be detected. Using the short T2 component between 10 and 55 ms, it is possible to map the myelin water component (MacKay *et al.* 1994), while the very long T2 component can be used to map expansion of the extracellular space due matrix degeneration (including axonal loss) (Kidd *et al.* 1997; Barnes *et al.* 1991). Similarly, severe prolongation in chronic MS lesions reflects expansion of the extracellular space, and relates to severe matrix destruction and axonal loss (Van Walderveen *et al.* 1998; Van Waesberghe *et al.* 1999).

6. MAGNETIZATION TRANSFER IMAGING: MEASURING CHEMICAL EXCHANGE

Conventional MR imaging is dominated by the contribution of free water protons. The protons which are bound to macromolecules do not directly contribute to the NMR image, but their contribution can be visualized using magnetization transfer (MT) imaging. MT depends on interaction between mobile water protons and immobile protons in structures such as proteins and lipids at the hydrophilic macromolecular surface (Wolff & Balaban 1989). The phenomena of chemical exchange and cross-relaxation are characteristic of normal brain tissue, where the presence of multiple (myelin) membranes compartmentalizes the water protons, resulting in multiple possible sites of MT. The amount of signal suppression following off-resonance irradiation is characterized by the MT ratio (MTR), which reflects several characteristics of the macromolecular environment and allows a semiquantitative characterization of the integrity or the damage of the macromolecular structure of cell membranes.

In acute EAE, oedematous lesions were found to have only slightly decreased MTR values, while in MS patients a wide range of MTR values were observed (Dousset *et al.* 1992). Based on the assumption that the main difference between acute EAE and MS is demyelination, it was initially suggested that MT imaging may provide an NMR tool to differentiate oedema from demyelination; such observations are supported by the finding of markedly reduced MTR values in patients with progressive

multifocal leucencephalopathy, a primary demyelinating disorder (Ernst *et al.* 1999). However, over time it has become apparent that a decrease in MTR values closely correlates with prolongation of T1 relaxation time (figure 1), and that both MR parameters correlate in the same manner with matrix destruction and axonal loss histopathologically (Dousset *et al.* 1995; Van Waesberghe *et al.* 1999). Actually, the correlation between MTR and the amount of myelin water (which resembles the degree of demyelination) is limited (Vavasour *et al.* 1998), which also suggests that other factors apart from demyelination may contribute to reduced MTR values.

7. DIFFUSION-WEIGHTED IMAGING: BROWNIAN MOTION VISUALIZED

Diffusion-weighted imaging (DWI) is a novel NMR technique which allows the detection of the random movements of water protons. DWI is based on the application of MR gradient pulses which results in dephasing of the signal intensity due to the Brownian motion of water protons. Randomly moving spins (in contrast to stationary spins) will not completely refocus and therefore attenuate the signal. Since water protons diffuse faster along myelinated fibres than across them, the apparent diffusion coefficient is directionally restricted, or anisotropic. Therefore, there is a marked anisotropy in brain white matter and minimal anisotropy in grey matter (Le Bihan *et al.* 1995). DWI can therefore be used to map directional restriction (anisotropy), related to the limited freedom of mobility of water along the direction of intact myelinated fibres.

With the advent of echo-planar-based sequences and strong gradients, DWI has become clinically feasible, with great promise in acute ischaemic disease, due to an early restriction of water self diffusion within minutes after onset of ischaemia (Moseley *et al.* 1990; Knight *et al.* 1994). In animals with EAE, an increase in diffusion in lesions and even normal-appearing white matter (NAWM) has been observed, correlating with the clinical severity score (Verhoye *et al.* 1996; Richards *et al.* 1995). In MS patients, an increase in diffusion has been reported in lesions (Horsfield *et al.* 1996; Christiansen *et al.* 1993). This increase is thought to reflect oedema and expanded extracellular space. At the same time, anisotropy is lost indicating that the fibre orientation is lost, compatible with demyelination. Little is known about acute lesions, although restricted diffusion has been reported in gadolinium-enhancing lesions and lesions in the optic nerve during acute optic neuritis (Iwasawa *et al.* 1997).

8. MAGNETIC RESONANCE SPECTROSCOPY: *IN VIVO* METABOLIC TISSUE CHARACTERIZATION

Even before the application of NMR to image biological tissues, NMR spectroscopy (MRS) had been used to characterize tissue metabolites (with limited spatial resolution). In contrast to NMR imaging, which visualizes differences in free water protons between tissues, in proton MRS the protons of the free water pool are selectively suppressed. This allows separation of resonances from various brain metabolites, including *N*-acetyl aspartate (NAA), creatine (Cr), myoinositol, lactate, choline (Cho)-containing compounds, and mobile lipids. NAA is virtually exclusively present in neurons and axons and is therefore used as an *in vivo* marker for neuro-axonal loss or as an index for neuronal viability (Nakano *et al.* 1998). Cr is present in all cell types, although in higher concentrations in astrocytes and oligodendrocytes than in neurons (Urenjak *et al.* 1993). Myoinositol is present in various cell types, including glia (Brand *et al.* 1993). Cho is a turnover product of cell membranes, with increased levels indicating increased membrane turnover—for example demyelination—or an increased number of (inflammatory) cells. Lactate does not occur in brain tissue with normal aerobic glycolysis, but can be observed in macrophages. Mobile lipids do not normally occur in the brain, but are found in demyelination.

Proton MRS studies in MS patients have shown that in active lesions, showing gadolinium enhancement, depressed NAA levels are observed (which may reverse after cessation of enhancement) (Davie *et al.* 1994; Narayana *et al.* 1998). Further, active lesions show increased levels of lactate (indicating the invasion of macrophages), mobile lipid peaks (myelin breakdown products) and increased levels of Cho (demyelination) (Davie *et al.* 1994; Landtblom *et al.* 1996; De Stefano *et al.* 1995), in contrast to 'stable' lesions with reduced Cho levels. In established MS lesions, decreased levels of NAA are observed, which is in agreement with the observation of a decrease in axonal density observed histopathologically (Matthews *et al.* 1991; Arnold *et al.* 1990, 1992; Davie *et al.* 1995; Van Walderveen *et al.* 1999*b*) (figure 4). The concentration of NAA correlates strongly with T1 relaxation time (Van Walderveen *et al.* 1999*b*) (table 2). Since NAA is a well-accepted marker for neuro-axonal density or viability, whereas T1 relaxation time measurements can be used to assess information about enlargement of the extracellular space, this strong correlation indicates that both MR markers may be used interchangeably to monitor disease progression. Lactate is found transiently in acute lesions, indicating active phagocytosis, while mobile lipids can be found for many months after a demyelinating event (Davie *et al.* 1994).

9. THE NORMAL APPEARING WHITE MATTER IN MULTIPLE SCLEROSIS IS NOT SO NORMAL ON NMR

All of the aforementioned quantifiable techniques have been used to study the white matter in between the focal MS lesions seen on conventional imaging. In this so-called NAWM, clear abnormalities can be observed, confirming the histopathological data about widespread subtle abnormalities in the NAWM (consisting of small areas of reactive astrocytes, oedema and perivascular cellular infiltration). In MS patients, T1 relaxation time of NAWM is prolonged compared with that of control white matter, and is most evident in patients with longer disease duration and increasing disability (Lacomis *et al.* 1986; Haughton *et al.* 1992). In pixel maps of T1 relaxation, abnormalities are one or two pixels in size, but given their widespread nature, this 'invisible' lesion load may constitute a significant proportion of the total lesion load (Barbosa *et al.* 1994).

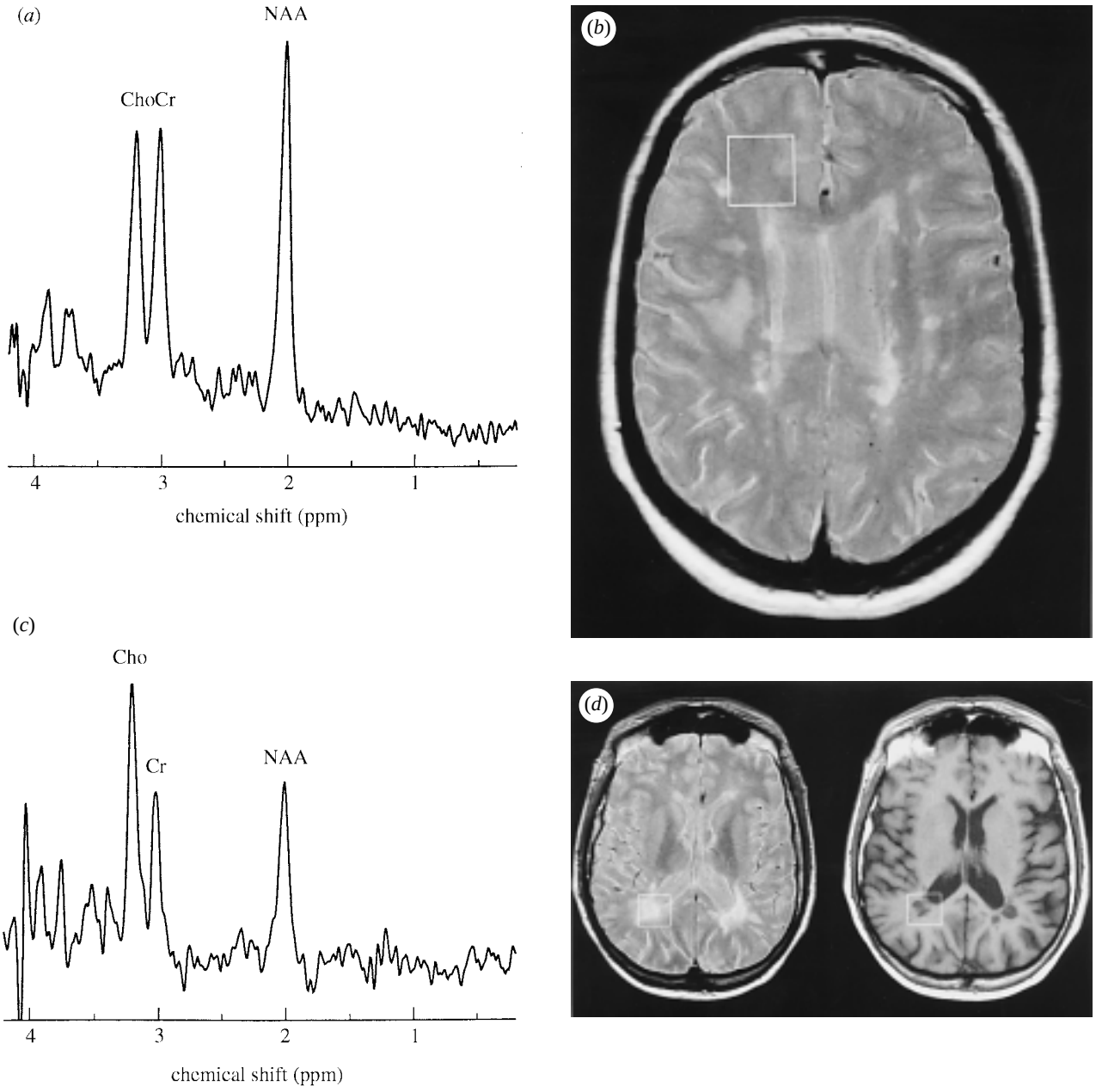

Figure 4. Localized proton magnetic resonance spectra (repetition time 2500 ms, echo time 135 ms, 128 scans) originated from an 8 ml volume of interest obtained from NAWM (*a*), corresponding T2-weighted image (*b*) and a severely hypointense MS lesion (*c*), corresponding T2- and T1-weighted images (*d*). The *y* range is scaled similarly for all spectra. In the severely hypointense MS lesion a lower concentration of NAA and Cr is present compared with the spectra obtained from NAWM, which indicates a combined loss of axons (NAA) and glial (Cr) cells.

Compared with white matter of controls, the MTR of NAWM is significantly reduced in MS patients (Dousset *et al.* 1992; Van Waesberghe *et al.* 1998*a*; Filippi *et al.* 1995), although no significant difference is present between the NAWM of RR and that of SP MS (Loevner *et al.* 1995; Filippi *et al.* 1995). The reduction in MTR values is especially apparent in the NAWM adjacent to focal T2 lesions, which suggests that secondary axonal degeneration might contribute to this phenomenon (Filippi *et al.* 1995). In several recent reports, minor reductions in MTR have been reported prior to the occurrence of new enhancing lesion (Filippi *et al.* 1998*b*; Goodkin *et al.* 1998). This indicates that before the occurrence of a demyelinating event, tissue alterations are already apparent. Since such MTR changes are subtle, and comparable with what can be found in the NAWM in other patients, it may herald the infiltration of brain tissue by inflammatory cells.

Using proton MRS, consistent findings of a decrease in the NAA–Cr concentration ratio have been found in NAWM of MS patients (Rooney *et al.* 1997; Husted *et al.* 1994; Fu *et al.* 1998), which was shown to be more extensive in the NAWM of SP MS patients compared with RR MS patients (Fu *et al.* 1998). This decrease in NAA–Cr ratio is a result of a decrease in the concentration of NAA, which is paralleled by an increase in Cr (Lai *et al.* 1997). These spectroscopic findings indicate that involvement of NAWM in MS patients consists of a proliferation

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Table 2. *Concentration of NAA and T1 relaxation time for normal white matter (NWM) in controls, NAWM in MS patients and in MS lesions*

(The MS lesions are divided according to degree of hypointensity on unenhanced T1-weighted spin-echo MR images into iso- to mildly hypointense (not discernible or iso- to hyperintense to grey matter), severely hypointense (hypointense compared with grey matter) and acute hypointense (per definition less than six months of age). The concentration of NAA is lower in NAWM compared with NWM, and decreases further in MS lesions, this decrease being most apparent in severely hypointense lesions. Compared with NAWM, the acute MS lesions show a substantial decrease in the concentration of NAA; this may be indicative of axonal damage or loss in the early phase of lesion development, which may persist at follow-up. Note that the decrease in concentration of NAA is paralleled by a simultaneous increase in T1 relaxation time, which is most apparent in severely hypointense lesions.)

	NAA (mmol l^{-1} volume of interest ± s.d.)	T1 relaxation time (ms, median ± s.d.)
NWM ($n = 13$)	6.93 ± 0.63	595 ± 19
NAWM ($n = 14$)	6.15 ± 0.56	710 ± 87
iso- to mildly hypointense ($n = 9$)	5.78 ± 1.13	848 ± 64
acute hypointense ($n = 2$)	4.63 ± 0.23	985 ± 64
severely hypointense ($n = 15$)	4.39 ± 1.28	1043 ± 186

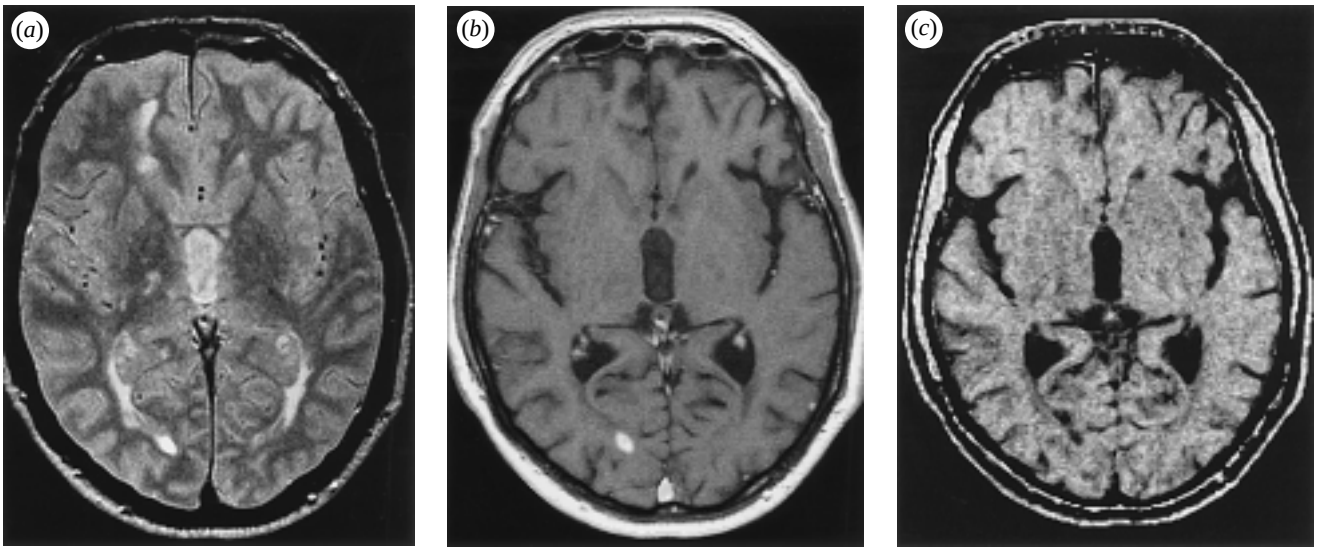

Figure 5. T2-weighted (*a*) and contrast-enhanced T1-weighted (*b*) MTR image (*c*). Illustrative example, showing an enhancing lesion on (*a*) and (*b*) with a decreased MTR on (*c*) (decreased signal); reflecting a loss of tissue structure in the active phase of lesion development.

of glial cells (increase in Cr). The decreased concentration of NAA may be either the consequence of neuro-axonal pathology or of axonal degeneration extending from focal lesions.

10. CAN WE PREDICT LESION FATE AND DEVELOPMENT OF AXONAL LOSS USING NMR?

While classical teaching tells us that axonal damage is limited in MS, recent studies have reinforced the notion that axonal loss actually occurs frequently, even in early demyelinating lesions (Trapp *et al.* 1998; Ferguson *et al.* 1997; Lassmann, this issue; Perry & Anthony, this issue), and is the main determinant of progressive neurological deterioration (Smith & McDonald, this issue). In chronic MS lesions, the degree of axonal loss varies from 0 to 100% and is strongly correlated with loss of MT and increase in T1 relaxation time (Van Walderveen *et al.* 1998; Van Waesberghe *et al.* 1999). In acute, enhancing demyelinating lesions, axonal density can already be markedly reduced. The degree of hypointensity on T1-weighted images was shown to be affected mainly by two factors, the extent of axonal reduction and the amount of extracellular oedema (Bruck *et al.* 1997). Interestingly, even in actively demyelinating lesions, the degree of loss of MT and the prolongation of T1 relaxation time is more linked to axonal loss than to the degree of myelin loss (Van Waesberghe *et al.* 1999). In contrast to histopathological sampling, NMR provides an opportunity to sequentially measure tissue changes *in vivo*, in an attempt to follow the development of (irreversible) tissue damage, and to predict the outcome of new lesions.

Following the enhancing stage, an abnormality on T2-weighted images usually persists, the reason why NMR imaging is such a sensitive representation of past disease activity or disease burden. In the acute phase, most (80%) enhancing lesions appear hypointense on unenhanced T1-weighted images (Van Waesberghe *et al.*

1998*b*), probably reflecting a mixture of oedema, inflammation and demyelination. Approximately half will reverse to isointensity at follow-up, while the other half will remain hypointense. Factors predicting the evolution of individual lesions are largely unknown. Hypointense appearance at six months of follow-up is in part determined by the MTR value at the time of initial enhancement and by the duration of enhancement. Also, ring-enhancing lesions are persistently hypointense in all cases, in contrast to nodular enhancing lesions. The MTR value at the time of initial enhancement predicts the persistent hypointense appearance (indicating axonal loss) at follow-up, although in individual lesions this is difficult to predict, since MTR values are generally decreased during the phase of enhancement (Lai *et al.* 1997; Silver *et al.* 1998; Filippi *et al.* 1998*a*) (figure 5). But in a large number of cases, they too tend to reverse to subnormal or normal values after enhancement ceases (Van Waesberghe *et al.* 1998*b*; Dousset *et al.* 1998). This is similar to the observation of reversible decreases of NAA in acute lesions (Narayana *et al.* 1998; Davie *et al.* 1994) and normalization of T1 relaxation time at follow-up in acute lesions (Larsson *et al.* 1989). At this time, therefore, factors that predict persistent black holes, loss of MT or NAA, indicating axonal loss, are largely undefined. For individual patients, the percentage of enhancing lesions that evolve into black holes is only weakly related to the rate of enhancing lesions, and more strongly determined by the presence of black holes at the initial scan (Van Walderveen *et al.* 1999*a*). Therefore, apart from the amount of new inflammatory activity, other factors, possibly genetic, appear to determine the development of axonal loss in MS lesions (Van Walderveen *et al.* 1999*a*).

11. WILL THE CLINICO-RADIOLOGICAL PARADOX EVER BE FULLY RESOLVED?

Several factors contribute to the clinico-radiological paradox. These include limitations of the clinical scoring, lack of longitudinal data, and the histopathological heterogeneity of lesions seen on conventional T2-weighted NMR images. In this paper several NMR parameters with allegedly higher tissue specificity have been discussed, which are partly related to each other. Several markers have been described that relate to axonal loss in chronic MS lesions, such as loss of NAA, reduction in MTR and prolongation of T1-relaxation time. More should be learned about lesional fate with regard to axonal loss, which is presently difficult to predict in the early inflammatory phase of lesion development. On top of that, several mechanisms for clinical recovery exist, which are not mutually exclusive, and may operate simultaneously; remyelination occurs frequently, and is likely to be associated with persistent T2 abnormalities (Van Walderveen *et al.* 1998; Van Waesberghe *et al.* 1999; Barkhof 1997; Lassmann, this issue; Perry & Anthony, this issue; Smith & McDonald, this issue).

Further recovery mechanisms include upregulation of sodium channels, axonal sprouting, collateral connections–pathways, and cortical adaptation; all of these are probably difficult to assess with (structural) NMR. Therefore, a perfect correlation between NMR findings and clinical outcome is extremely unlikely. On the other hand, NMR is a sensitive biological marker, picking up tissue changes before clinical symptoms occur. The fact that (partial) correlations with clinical parameters are consistently found indicates the clinical relevance of these changes. Final clinical outcome is influenced by a multitude of modifying factors, which are poorly understood at present. Nevertheless, while the clinical effect of a given new lesion may be difficult to ascertain, the absence of new NMR-visible lesions, or the absence of severe tissue damage within such lesions, is bound to be prognostically favourable, as will be the degree to which such changes are prevented by treatment.

The MR Centre for MS Research is kindly supported by the Dutch Foundation for MS Research, the Vrije Universiteit, and the University Hospital of the Vrije Universiteit. Marianne van Walderveen is recipient of a research grant on 'Black holes in MS' by the Dutch Foundation for MS Research (95–245).

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