

MRI LESION ACTIVITY IN RELAPSING-REMITTING PATIENTS WITH MULTIPLE SCLEROSIS

by

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Abstract

Multiple sclerosis (MS) is a disease of the central nervous system seen mainly in young adults in which demyelination is the most specific pathological change. Magnetic resonance imaging (MRI) is the most sensitive method for imaging MS lesions. Serial MRI can detect brain lesion activity in MS and provide an important tool for monitoring therapeutic trials. Fifty relapsing-remitting MS patients, as part of a beta-interferon trial, were examined every 6 weeks with MRI for a 2 year period in this study. The patients were randomized into three treatment arms: placebo (N=17), 1.6 mIU (N=17), and 8 mIU (N=16) beta-interferon self-administered subcutaneously every other day. Activity of MS lesions on MRI was defined as new (first appearance), enlarging (increase in size of preexisting lesion), recurrent (reappearance of lesions that had disappeared), and enhancing (lesion enhancement on T1 weighted MRI scan after a contrast agent, gadolinium, injection). Lesion activity as detected in serial MRI examination is much more common than the clinical relapse rate. Study of lesion activity is helpful in understanding MS. This thesis includes serial studies of lesion activity on MRI. The studies are (1) to detect the activity of the individual lesion in order to see if there was any characteristics that distinguished active lesions from each other and from stable ones; (2) to identify the features of enhancing and non-enhancing lesions in shape, size, and location to learn the difference between morphologically active and gadolinium enhancing lesions due to their pathological difference; (3) to describe the growth pattern of enlarging MS lesions as we believe that enlarging lesions differ pathologically from new ones; (4) to compare the sensitivity of gadolinium MRI with unenhanced serial MRI

in detecting activity of lesions to see which one is the more sensitive technique for detecting active lesions. The findings from the studies disclose features of MS active lesions that provide more information in understanding the natural history of MS. To determine how much MS lesion activity information, as detected with frequent serial MRI, is lost if a less frequent scan interval is used, a study of effects of scanning frequency was carried out. Lesion activity was assessed in a blinded fashion at scan intervals of 6 weeks, 3 months, 6 months, and 1 year. We found that scanning in less than 6 week intervals detects fewer active MS lesions. Active lesions located in the corticospinal tract were studied to see any relationship between MRI activity and clinical course. The study found that there was a positive relationship between active lesions in the corticospinal tract and clinical relapses. To monitor lesion activity as an outcome to measure therapeutic trials, a study of 50 Vancouver patients was conducted. The result provides further evidence that lesion activity measurement is a useful and sensitive tool in a therapeutic trial even with small group of patients.

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LIST OF ABBREVIATIONS

AO	albino oxford
BBB	blood-brain barrier
CNS	central nervous system
CP	chronic progressive
CT	computered tomography
CSF	cerebrospinal fluid
CST	corticospinal tract
DA	dark august
EAE	experimental allergic encephalomyelitis
EC	endothelial cell
EDSS	expanded disability status scale
5-HT	serotonin
Gd	gadolinium
Gd-DTPA	gadopentetate dimeglumine
GPC	glycero-3-phosphorylcholine
HRP	horseradise peroxidase
ICAM-1	intercellular adhesion molecule-1
IFNB	interferon beta-1 b
IL-2	interleukin-2
IR	inversion recovery
MBP	myelin basic protein
MIU	million international unit
MRI	magnetic resonance imaging

MRS	magnetic resonance spectroscopy
MS	multiple sclerosis
MTI	magnetic transfer imaging
NAA	<i>N</i> -acetylaspartate
NMR	nuclear magnetic resonance
NRS	neurological rating scale
PNS	peripheral nervous system
PP	primary progressive
RF	radiofrequency
RR	relapsing-remitting
SD	standard deviation
SE	spin echo
SP	secondary progressive
TE	echo time
TR	repetition time

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Chapter 1

Multiple Sclerosis

To understand the active lesions detected in magnetic resonance imaging (MRI), one should be acquainted with the basic properties of myelin, demyelination, gadolinium (Gd) enhancement in MRI and multiple sclerosis (MS). Based on this knowledge it is possible to discuss features of active lesions and the relationship between lesion activity and clinical status. Verification of these relationships can be obtained by postmortem MRI of MS brains. Serial MRI also can provide much information, which will be discussed in this thesis.

1.1. General introduction

Definition and Classification

Nerve conduction depends on adequate axonal sheathing in order to maintain nerve saltatory conduction. In the peripheral nervous system (PNS) sheathing is performed by the Schwann cells while in the central nervous system (CNS) sheathing is performed by oligodendrocytes. In both cases nerve sheaths consist of myelin bi-layers. Insufficient myelination is associated with incapacitated nerve conduction. A shortage or abnormal function of myelin can have a primary (congenital) cause (dysmyelination) or can have a secondary (acquired) cause (demyelination). Demyelination of the peripheral nervous system occurs in chronic inflammatory demyelinating polyneuropathy and Guillain-Barre syndrome. Several demyelinating diseases of the CNS are known, of which MS is the most common. By definition MS is a demyelinating disease of the CNS characterized by various plaque formation.

Clinical Presentation

MS is clinically characterized by episodes of focal disorder of the optic nerves, spinal cord, and brain, which remit to a varying extent and recur over a period of many years. The clinical manifestations are protean, being determined by the varied location and extent of the foci of demyelination; nevertheless, the lesions tend to have a predilection for certain portions of the CNS, resulting in characteristic complexes of symptoms and signs that can often be readily recognized.

Approximately 95 percent of cases of MS begin between the ages of 10 to 50 years and 5 to 6 percent of cases start between ages 50 and 60 years [Broman et al., 1981]. In general, patients who develop MS while young tend to have more intracranial lesions and a higher frequency of exacerbations, whereas patients with late-onset MS are more likely to have a progressive spinal cord disease [Andersen et al., 1980]. The mean age of onset of MS is about 30 years in most studies. The reason for this age susceptibility is unknown. The female to male ratio of cases is 2:1. The highest ratios noted in prevalence studies may be due to a greater mortality rate in males with MS [Sibley and Paty, 1981].

Genetic factors have a causal role in MS. Several twin studies have been conducted to estimate the MS concordance rates between mono- and dizygotic twins [MacKay and Myrianthopoulos, 1966; Cendrowski, 1968; Bobovick et al., 1978; Williams et al., 1980; Currier and Eldridge 1982]. Their conflicting results may be explained, at least in some studies, by biases such as public appeals, which lead to an excess of monozygotic pairs. In a large Canadian population-based study [Ebers et al., 1986] (where biases were minimized), higher concordance rates in monozygotic twins (25.9%, 27 pairs) than in dizygotic twins (2.3%, 43 pairs) were observed. A 7.5 years follow-up study [Sadovnick et al., 1993] of the population-based series provided further evidence. The survey in the British Isles had similar results [Mumford et al., 1994]. Conversely, a French study of 54 twin pairs (17 monozygotic and 37 dizygotic pairs) revealed no significant difference, and

the observed clinical concordance rate was low in both groups [French research group on MS, 1992]. These discrepancies might be explained by differences in the population risk according to the geographical area on population susceptibility.

Relatives of MS patients have an increased risk of MS. 10-20% of MS patients have an affected relative. In high-risk areas, siblings of MS patients have an estimated risk of 1.2% compared to 0.1% in the general population. Subclinical demyelinating lesions may occur in the brains of asymptomatic individuals, and the first-degree relatives of MS patients are at particular risk. In recent studies using MRI, there is an indication that subclinical MS may be present in apparently normal members of multiplex families [Lynch et al., 1990; Tienari et al., 1992].

MS is unequally distributed over the world. There are geographic differences in incidence and prevalence that cannot be genetically explained. Overall the disease is more common in northern America, northern Europe, and New Zealand. In British Columbia, Canada, MS is the most common neurological disorder in young adults; its prevalence is 117.2 per 100,000 population [Sweeney et al, 1986]. Prevalence studies for migrants from high to low risk areas indicate the age of adolescence to be critical for risk retention: those migrating under age 15 years acquire the lower risk of their new residence. The migrant data, defines MS as an acquired exogenous (environmental) disease whose acquisition in ordinary circumstance takes place years before clinical onset.

The most common initial clinical manifestations are sensory (40%) and visual (35%), followed by motor (21%), brainstem (16%), cerebellar (15%) and bladder (4%) symptoms [de Graaf, 1988]. Signs and symptoms in MS patients include fatigue, optic neuritis, internuclear ophthalmoplegia, Lhermitte's sign, chronic myelopathies and urge-incontinence. The frequent occurrence of psychiatric and cognitive defects in MS has recently been recognized [Ron, 1992; Stenager et al., 1990; Minden and Schiffer, 1990]. Fixed disability usually occurs at a given moment in time and is frequently expressed as a disability score. The most widely used disability score is the Expanded Disability Status

Scale (EDSS) score [Kurtzke, 1983] [Appendix A]. On this 10-point scale, 0 means no abnormalities, 6 or more the inability to walk without aid, and 10 deceased. The interrater variability in assessing the EDSS is 0.5 point and subsequently a change of 1 point or more is needed to demonstrate a significant change in disability; this variation is even more marked at the lower of the scale. Severity of exacerbations can be classified by the Neurological Rating Scale (NRS) [Sipe et al., 1984]. The NRS is based on assessment of each component of the neurologic examination and reflects overall neurologic function. The assignment of points in the NRS receives the full "normal" point value, with a progressive loss in points for mild (-1; 1+), moderate (-2; 2+), or severe (-3, -4; 3+, 4+) involvement. Severe (-3; 3+) and maximal (-4; 4+) deficits are scored as severe on the NRS.

Several disease courses can be seen in MS. The initial course usually is a relapsing-remitting (RR) one, followed in 2 out of 3 patients by a secondary progressive (SP) course. The progression has been defined as a development of steadily increasing disability over a period of at least 12 months. One third of the patients with a RR disease course, still have an EDSS score of 3 or less after 10 or more years, and their condition is referred to as benign MS. A small percentage of the patients (5 - 10%) will follow a progressive course from the onset without relapse or remissions, referred to as primary progressive (PP) MS. A single episode of clinical disease activity is referred to as isolated syndromes.

1.2. Pathology of multiple sclerosis

The presence of multiple gray sclerotic plaques throughout the brain and spinal cord of patients dying with longstanding multiple sclerosis is so striking that the pathological appearances have endowed the disease with its commonly used name. Demyelination

refers to the removal of apparently normal myelin from axons of the CNS, usually against a background of perivenular infiltration by small lymphocytes, plasma cells, and large mononuclear cells. Biochemically, myelin stands apart from other membranes in both possessing its unique proteins and its unusually high percentage (70%) of lipids. Thirty percent of the membrane is made up of protein, one component in particular, myelin basic protein (MBP), has characteristic properties that render the sheath highly susceptible to immunologic attack. MBP, an 18,000 molecular weight, 170 amino acid molecule, is cross-reactive and highly encephalitogenic when inoculated into most species. Even short sequences of this molecule are encephalitogenic, and it has been demonstrated that different polypeptides along the molecule influence cell-mediated and antibody-mediated immunity to the membrane.

In normal white matter, myelin is the major structural element, and related to its biochemistry, a wide variety of lipid stains exist which clearly differentiate white from gray matter. Ultrastructurally, white matter is made up of densely packed, electron-dense, lipid-rich myelin sheaths surrounding pale-staining axons. Each sheath has the characteristic spiral lamellar pattern. The sheath of one fiber is often contiguous with and separated from its neighbor by a single myelin period. Groups of unmyelinated axons are sometimes seen. Nerve fibers often lie in groups separated by fibrous astrocytic septae and interfascicular oligodendrocytes. Blood vessels in normal white matter display little or no space between the closely apposed basal laminae of the endothelial cells and the glia limitans, except for that occupied by an occasional pericyte, macrophage, smooth muscle cell, fibroblast, or collagen pocket.

In the majority of cases, the MS brain displays no outward abnormalities. The brain weight is usually within normal limits. The optic nerves are frequently atrophic and display gray zones from which myelin has been depleted. Coronal section of the brain reveals large lesions which vary in appearance, texture, size, shape, number, and topography (figure 1). Variations in appearance and texture reflect the age and activity of

the lesions. Pink, soft lesions indicate areas of recent activity, while older lesions are gray, sometimes rather translucent, gelatinous, and firm to the touch. Lesions vary in size from less than a millimeter to several centimeters across. Lesions may unite and arborize throughout the white matter, presumably along the vasculature. Some cases of MS will display large confluent lesions. The location of MS lesions usually defies clinical and anatomical explanation. The entire central neuraxis is vulnerable, and in the vast majority of cases the cerebrum is involved. The heaviest concentration of periventricular lesions appears to occur in relationship to subependymal vasculature. One commonly involved area is the angle between the caudate nucleus and the corpus callosum. Smaller lesions commonly occur in the white matter at the tips of gyri where they may spill over into gray matter. Lesions may also be found in the striatum, pallidum, and the thalamus. Lesions also occur around the margins of the third ventricle and hypothalamic pathways.

The lesions in disseminated sclerosis, wherever they are situated, are distributed evidently without any relation to nerve tracts. Within the cerebrum the veins pass towards the wall of the ventricles and the choroid plexus towards the veins of Galen, and have in this way a distribution altogether different from that of the arteries. It is known that the lesions are deposited in relation to the distribution of the veins and to the walls of the ventricles. The lesions of relapsing remitting MS fall into four categories: 1) chronic, inactive plaques; 2) chronic, active plaques; 3) shadow plaques, and 4) acute plaques.

The most common location of inactive chronic plaques are periventricular, the optic nerves and chiasm, and the spinal cord. Myelin stains will reveal demyelinated areas sharply demarcated from the adjacent myelinated white matter, imparting upon them a punched-out appearance. Axon stains will disclose a moderate decrease in the density of axons toward the periphery of the plaque, but deeper areas will be more depleted. The loss of myelin from a fiber is abrupt at the lesion edge and is visualized as a myelinated

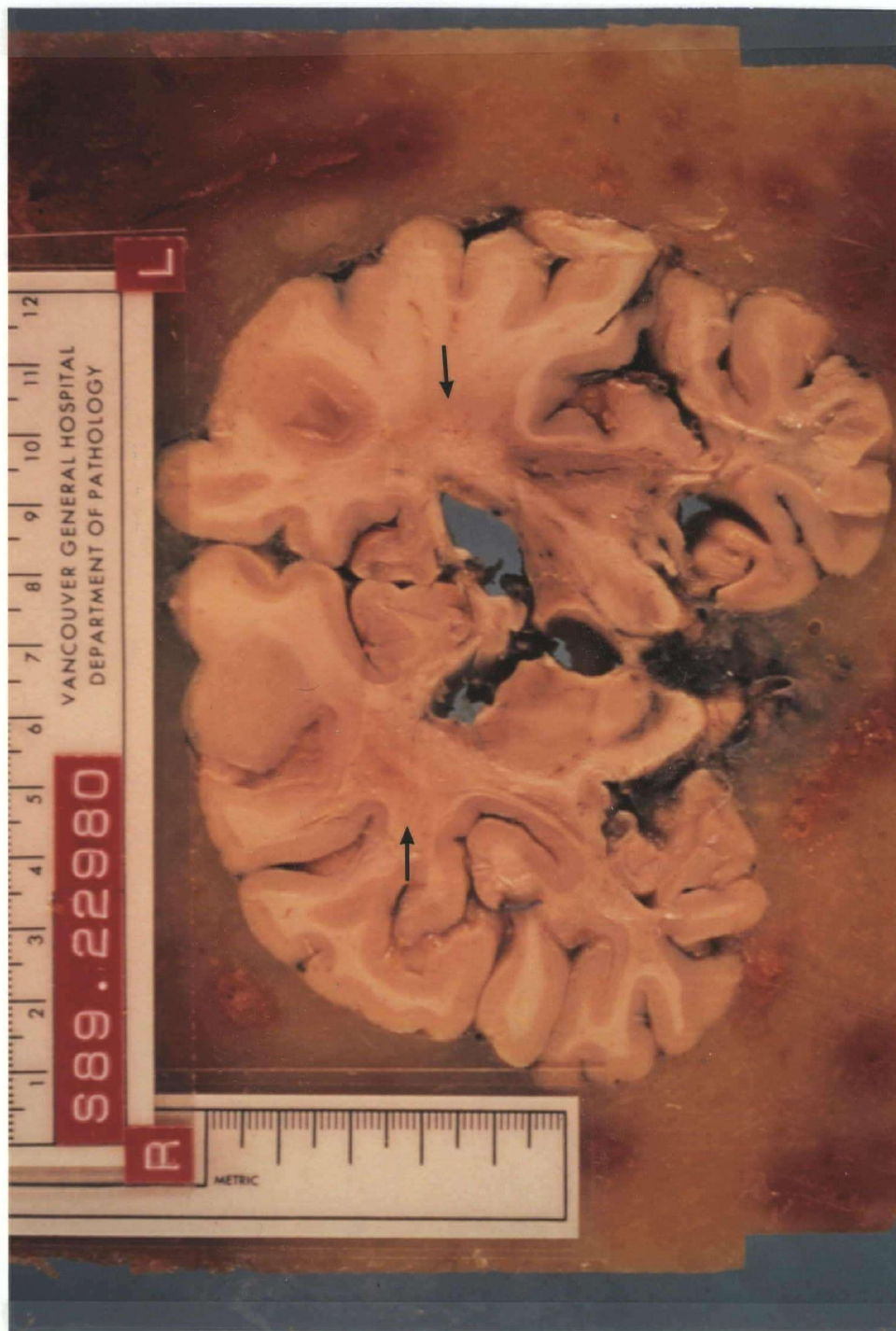


Figure 1. Pathological appearance of MS lesion. Arrows show large MS plaques.

segment becoming naked and continuing into the lesion as a demyelinated fiber. Frequently, even in silent lesions, foamy macrophages can be seen. As a result of loss of parenchymal elements and intense gliosis, considerable atrophy of affected regions is not uncommon. Histologically, most inactive lesions have a rather acellular appearance, consisting of a fibrillary astroglial feltwork that is responsible for the sclerosis, color, and texture of the tissue. The stroma of most chronic lesions contains a few sudanophilic, lipid-laden macrophages, fat granule cells, compound granular corpuscles, foamy cells, and histiocytes, which are randomly scattered throughout the parenchyma or exist in small collections around blood vessels. Inflammatory cells are usually rare within silent lesions. Plasma cells are the most common non-gliotic cellular element, and small lymphocytes are rare. Oligodendroglial depletion is severe in chronic lesions of MS.

A chronic, active MS lesion is regarded as an established lesion along the edges of which is a broad zone of perivascular and parenchymal inflammation together with diffuse ongoing demyelination. Macrophage activity, glial hypertrophy, hypercellularity, and some edema are in evidence. On gross examination, such a lesion would appear to have a pink or white tinge about its margins, corresponding to inflammation and macrophage activity, respectively. Chronic active lesions have a less well demarcated margin on myelin staining. Small lesions can often be found centered on small veins within the white matter proper. Presumably such small demyelinating foci will eventually coalesce to form larger lesions. The adjacent myelinated white matter is often hypercellular and contains an increased number of proliferated oligodendrocytes. Foamy macrophages are invariably found throughout the normal white matter far away from lesions. Such a phenomenon might be representative of persistent low-level activity. Within the center of active lesions, only a few T cells and many Ia-positive macrophages and B cells are present. Throughout active lesions, astrocytic hypertrophy is common, particular in the white matter adjacent to the lesion. Hypertrophic astrocytes extend for some distance into the surrounding white matter. It was seen that despite the presence of

ongoing demyelination, inflammation, and macrophages containing recognizable myelin debris, surviving and proliferating oligodendroglia are common.

Shadow plaques contain diffusely distributed, thinly myelinated fibers. The consensus is that they represent areas of CNS remyelination. This conclusion is based on a preponderance of large diameter axons with thinner than normal myelin sheaths, attenuated sheaths containing aberrant collections of oligodendroglial cytoplasm, and a modest increase in the number of oligodendrocytes. Evidence of previous damage can be found in the form of a moderate background gliosis, an abundance of nonspecific corpora amylacea, a few fibrotic blood vessels, and the occasional foamy macrophage. Inflammation cells are absent, as are prominent fibrous astroglial and macrophage responses.

The major pattern of myelin disruption in acute lesion is extensive extracellular vesicular dissolution, a change seen light microscopically in myelin-stained sections as thickening and pallor of the sheath leading to fragmentation. Oligodendrocytes may survive in large numbers in acute plaques. Oligodendrocytes loss in MS is probably secondary to myelin damage which is a proliferative response which follows some weeks after acute destruction of both myelin and oligodendrocytes [Prineas, 1985; 1994]. Inflammation is usually seen in acute lesions. Plasma cells are most numerous in fresh lesions. In acute lesions in the acute form of disease, extensive remyelination can begin within a few days or weeks. It possibly contributes to clinical remission, and may even result in disappearance of the plaque.

As the cause of MS remains unclear, the exact sequence of events in the evolution of MS lesions can only be hinted at (Table 1). Prineas [1985] summarizes the following concepts: 1) both myelin and oligodendrocytes are targets of 2) macrophages and their proteolytic enzymes, 3) after being recognized by oligoclonal IgG antigens or T-lymphocytes, and 4) remyelination occurs in acute and chronic plaques.

Table 1: Sequence of events in the evolution of MS lesions

T-cell activation with production of cytokines
Blood-brain barrier breakdown
Inflammation
Edema
Demyelination
Gliosis
astrocytosis, proliferation of microglia (foam cells) and oligodendroglia
Remyelination
Axonal loss

1.3. Blood-brain barrier in multiple sclerosis

The brain requires a stable internal environment different from that of other organs of the body in order to function at its highest integrative level. Small fluctuations in extracellular fluid concentrations of hormones, amino acids, nutrients, vitamins, and ions are tolerated by the peripheral organs. However, the CNS has a unique microenvironment, a disturbance of which would interfere with the processes of the CNS integration. This unique microenvironment of the CNS demands a dynamic homeostasis in order to insure that individual neurons, synapses, and neuronal systems may receive, process, store, transfer, integrate, retrieve, and utilize the billions of bits of information essential to normal neurological function. Furthermore, the entire internal environment of

the CNS, including its supportive tissues, must be maintained in isolation from that of the blood. The evolution in parallel of the highest integrative level of the CNS function and the most efficient blood-brain barrier (BBB) is supportive of the hypothesis that the homeostasis and isolation of the extracellular fluid microenvironment of central neurons facilitates brain integrative functions [Cserr and Bundgaard, 1986]. Thus far, all species that have a BBB are able to achieve and maintain both isolation and homeostasis of the internal environment of the brain. The BBB integrity is essential to normal brain function. It has been noted that there is striking local damage to the BBB in MS. The damage to the BBB may allow the cellular and humoral components in blood to penetrate the endothelium and induce the demyelinating changes in the CNS. MRI, as one of the modern techniques for brain imaging can easily detect the presence or absence of a focal disturbance in the BBB.

1.3.1 The structure of the normal BBB

Brain capillaries are relatively impermeable as compared with capillaries of most other regions of the body. Characteristically, typical cerebral capillaries are composed of a single layer of endothelial cells (ECs). A narrow, uninterrupted basal membrane surrounds the outer surface of ECs. The end-feet of astrocytes may lie directly upon the perivascular basal membrane. These processes are frequently separated from the lamina by a narrow intercellular space. The astrocytic ensheathment of the perivascular basal membrane is discontinuous because of the frequent presence of gaps or space between adjacent astrocytic end-feet [Pollay and Robert, 1980].

The crucial roles of the BBB are contributed by the ECs and their continuous tight intercellular junctions. The electron microscopic studies [Reese and Karnovsky, 1967; Brightman and Reese, 1969] clearly showed overlapping lateral edges of endothelial cells

sealed together by tight junctions that formed a continuous belt around the capillary at each interendothelial junction. On the luminal side of the tight junction complex molecules of an electron dense marker could be visualized after intravenous injection in the proximal portion of the cleft, however their passage is usually stopped abruptly at the first tight junction. Also, following administration, molecules of the marker could be visualized in the abluminal basal lamina and into the first abluminal portion of the interendothelial cleft; again, the electron dense marker molecules were abruptly blocked at the first tight junction. The width of the tight junction along the interendothelial cleft, as measured in electron micrographs, is less than twice the width of the endothelial cell luminal or abluminal membrane, which suggests that fusion of apposing membranes was complete and permanent.

The capillary ECs do not show transendothelial channels and have a very low frequency of pinocytotic vesicles under normal conditions. Transcapillary exchange of vascular endothelium in cardiac and skeletal muscle had been shown to transport material by vesicular filling on the capillary luminal side and vesicular discharging on the abluminal side of the capillary. However, there is no evidence of discharge of Horseradish peroxidase (HRP) from the luminal to the abluminal side of brain endothelium [Reese and Karnovsky, 1967]. Bundgaard [1986] has shown electron microscopically, by three-dimensional reconstructions based on consecutive ultrathin sections, that vesicular structures observed in brain endothelium are points of invagination on the cell membrane.

The BBB prevents the free diffusion of circulating molecules and cells into brain interstitial space [Pardridge et al., 1986]. The rate that substances penetrate the BBB is found to be related to molecular size, lipid solubility, and the presence of a specific carrier-mediated transport system. However, the barrier function of the BBB may be damaged under some diseases, such as, in MS.

1.3.2 The BBB breakdown in MS

The early lesions of MS usually developed around small cerebral blood vessels. The observation has long been considered to be an important clue to the pathogenesis of MS disease [Adams, 1978]. Using trypan blue as a indicator, Broman [1964] demonstrated that the trypan blue staining of the sclerotic plaque of brain appeared uniform but actually included a staining of veins but not of arteries. Leakage of plasma proteins had been found [Broman, 1964; Gay and Esiri, 1991]. The leakage was most marked around capillaries and the small veins of the venocapillary junctions. The ECs of the vessels within acute MS plaques frequently contained many vesicles.

Albumin is a non-CNS protein synthesized only in the liver. The concentration of albumin in the CSF, corrected for intravascular albumin, is used to help evaluate the BBB in MS. Tourtellotte and Ma [1978] revealed that albumin could diffuse through the extracellular space and sink into the CSF once the albumin had penetrated the disrupted BBB. Hence, a significantly elevated CSF albumin concentration reflects generalized damage of the BBB. Some have observed, the frequency of the BBB alteration to be 23% for MS patients [Tourtellotte and Ma, 1978; Eickhoff et al., 1977]. This frequency differs from the study of Annunziata and co-workers [1988] who show BBB impairment in 35% of patients with MS and blood-retina barrier impairment in 45% of MS patients.

The function of the BBB to some degree influences the IgG content of CSF [Pardridge et al., 1986]. The elevation of IgG in CSF may be influenced by the concentration of IgG in serum, by damage of the BBB, and by IgG synthesis within the CNS. Therefore, a simultaneous protein analysis of serum and CSF is necessary to differentiate among these three conditions. BBB permeability to radiotracers of different molecular sizes has been studied in experimental allergic encephalomyelitis (EAE), an animal model of MS, sensitized using a tissue sampling technique [Juhler et al., 1984]. It has been found that

the BBB permeability to small molecules (Na^+ and Cl^-) increases significantly, preceding clinical symptoms by one day in the lumbar spinal cord and to coincide with the onset of clinical disease in other regions. In all regions, increased BBB permeability preceded the occurrence of histological lesions. The increase of the BBB permeability to sucrose was found to be only slight, but no permeability increase to insulin could be demonstrated [Juhler et al., 1984]. This study can not only confirm the BBB damage in EAE, but also demonstrate evidence of the relationship between the clinical picture and BBB permeability.

1.3.3. The possible mechanism of the BBB breakdown

The mechanism of the BBB damage in MS and EAE is at present unclear. There are some suggestions on how to induce disruption of the BBB. These suggestions include mannitol, vasoactive amines and/or other humoral or cellular components in the immune response which can affect the BBB.

Serotonin (5-HT) and histamine have shown evidence of increasing the permeability of the BBB. After 5-HT is perfused through the cerebral ventricular system, the permeability across cerebral vessels is found to be increased [Westergard, 1978]. However, the ECs are intact. HRP which is employed as a marker of any increase of the BBB alteration does not form a continuous line between ECs, from the vessel lumen to the subendothelial BM. Nevertheless, several vesicles, filled with HRP have been observed in the cytoplasm of ECs. Freely situated HRP-containing vesicles are also found. Exposure of conscious young rats to 4 h heat stress at 38 °C is associated with increased BBB permeability and with increased plasma and brain 5-HT level [Sharma and Dey, 1987]. Histamine in the regulation of the BBB was studied [Gross et al, 1981; Gulati et al, 1985]. Intra-arterial administration of histamine produced increases in permeability of the BBB in rat cerebral cortex. The response was present in many regions

of cortex and was mediated by H₂ receptors. Histamine may produce parenchyma edema which is concentration-dependent. However, these studies are not on EAE or MS. The histamine level in plasma or CSF in EAE animal model or MS patients is unknown. That is, vasoactive aminic mechanisms which are employed to explain the BBB damage on either EAE or MS are not completely understood.

While immune reactions against specific endothelial autoantigens may in some instances contribute to vascular damage, cytokines and other inflammatory factors are the central mediators in the regulation of endothelial permeability for cells and macromolecules [Lassmann et al., 1991]. Interleukin-2 (IL-2) may be produced by activated T cells. It has been found that levels of IL-2 in the blood and brain of EAE are increased. Other studies [Damle et al., 1987; Alexander et al., 1989; Vukmanovic et al., 1989; Ellison et al., 1987] showed that IL-2 may induce BBB dysfunction. When adoptive immunotherapeutic trials in patients with brain tumors with IL-2-activated killer cells were studied, Damle, et al. [1987] found that infusion of high doses of IL-2 caused systemic toxicity in patients with tumor and experimental animals resulting in the development of a vascular leakage syndrome. In a dose-dependent manner, IL-2 caused lymphocytes to strongly adhere to ECs and IL-2-activated killer cells may cause lysis of ECs. Ellison and co-workers [1987] used cats who were infused with recombinant IL-2 (rIL-2) and showed the BBB alteration in multiple foci throughout the brain, being most prominent within white matter regions. In contrast, Alexander and associates [1989] demonstrated that parenteral injection of IL-2 increased BBB disruption in tumor-bearing rat brain but did not increase the vascular permeability of normal brain after comparing rats with intracerebral 9L gliosarcomas to control group who were administrated high-dose parenteral IL-2. Using two strains of rats, Albino Oxford (AO) and Dark August (DA) which have a different capacity to produce IL-2 (AO is lower than DA), it was shown [Hickey and Kimura, 1988; Kinrichs et al., 1987] that AO rats exhibited lower susceptibility to the induction of EAE, and IL-2 production by their spleen and lymph

node cells was also significantly lower. Hence, we believe that IL-2 in the blood is one of the factors which can induce BBB disruption. The mechanism of IL-2 on ECs is still to be elucidated.

Anti-EC antibodies may be present in patients with MS and rheumatoid arthritis [Tanaka et al., 1987; Tsukada et al., 1989; Heurkeas et al., 1989]. In the sera of patients with MS, the concentrations of IgG which bind to cerebral ECs and of immune complexes were significantly increased using cultured brain ECs. The levels of IgG binding to ECs were still increased in the sera of exacerbated MS patients after blocking Fc receptors compared to these of controls. The antibodies to each protein fraction extracted from the rat cerebral endothelial cell membrane were studied in patients with MS [Tsukada et al., 1989]. The patients with active relapsing MS displayed significantly higher levels of IgG binding to the EC membrane fraction than did the controls. IgG antibodies reactive with ECs were also found in patients with rheumatoid arthritis [Heurkeas et al., 1989]. The results showed that anti-EC antibodies and immune complexes were present in cases of MS and that they may play a pathogenetic role in the BBB damage.

The extent to which antigen-specific recognition events may take place on the luminal surface of cerebral vascular endothelium *in vivo* remains controversial. All encephalitogenic T lymphocyte lines recognize their target autoantigens, MBP, in the molecular context of class II products of the MHC, Ia determinants. Thus, in order to immunogenically present protein (auto-)-antigens, ECs must be inducible to express Ia determinants. After stimulation with γ -interferon, BBB endothelia and astrocytes express class I at least in some *in vitro* models class II MHC antigens [Traugott et al., 1988; Lassmann et al., 1991; Frohman et al., 1988]. Some [Lassmann et al., 1991; Wekerle, 1988] suggested that antigen presentation by ECs may be incomplete and may lead to T-cell anergy and/or to actual lysis of the endothelium.

It has been observed in EAE that T cells in the circulating blood can cross the BBB under electron microscopy [Dorovini-Zis et al., 1992], but, the mechanism by which T cells and macrophages in the peripheral blood initially enter the brain is still unclear. Several studies have hypothesized [Frohman et al., 1989; Fontana and Fierz, 1985] over the mechanisms in which T cells induced BBB damage, and then crossed the barrier. Some authors [Wong and Dorovini-Zis, 1992; Wilcox et al., 1990; Frohman et al., 1989] suggested that because T cells had the cell surface receptor for intercellular adhesion molecule-1(ICAM-1), and ECs can be induced to express ICAM-1, the adhesion interaction between these two cell types may regulate the margination of T cells before they cross the BBB and entered the brain. Astrocytes can play a regulatory role connecting the brain with the immune response during the BBB disruption. Astrocytes may transfer information because the astrocytes can induce the BBB properties in ECs. Initially, astrocytes could be involved in bidirectional transport of antigens between ECs and brain parenchyma. This can lead to exposure of antigens on the surface of ECs which can be recognized by T cells. The interaction between T cells and ECs could lead to the disruption of the BBB.

Lymphocyte transmigration through the BBB is not related to antigen presentation and specificity but critically depends on whether or not the BBB is damaged. After BBB disruption, using hyperosmotic mannitol, lymphocytes can be detected in the brain parenchyma of normal rats [Kajiwara et al., 1990]. MBP-specific T cells did not require MHC-compatible ECs in inducibility of EAE in the model of bone marrow chimeras [Hickey and Kimura, 1988; Kinrichs et al., 1987], but compatibility with the transferred bone marrow progeny was essential. On the other hand, the activated state of T cells is important for their transmigration through the BBB. Using antigen-specific activated T cell lines which are labelled in vitro with [¹⁴C]thymidine, encephalitogenic MBP-specific T cells can cross the BBB within 24 h after i.v. injection. Therefore, this finding is evidence that lymphocytes can open the BBB themselves when they adhere the ECs with

or without Ia antigen. Lymphocytes, monocytes, and some cytokines can enter the brain parenchyma through the damaged BBB to produce local inflammation. Eventually demyelination occurs.

The BBB damage in MS and EAE is due to a complicated process and is a result of interactions between multiple factors. MHC class II antigen on the ECs and astrocytes and humoral and cellular components in the peripheral blood can inevitably take part in BBB disruption, but, Ia antigens on the ECs and astrocytes, anti-EC antibodies, and T cells probably play a more important role. MS is probably a kind of BBB disease. The occurrence of MS may be a consequence of the BBB disruption caused by some factors located outside the brain. Burnham, et al. [1991] using high-dose steroids in MS patients, provided evidence that steroids may reduce the BBB disruption in a more specific manner. Enhancement on MRI can detect the BBB disruption and the improvement in the BBB disruption is correlated with clinical improvement. More studies on the relationship between the BBB breakdown and clinical aspects of MS should be done. It is important to identify drugs which can maintain BBB integrity therefore improving the symptoms of MS in the hope of eventually finding a complete cure.

Chapter 2

MRI of Multiple sclerosis

2.1. Introduction of MRI

MRI is the latest in a long and imaginative series of technological advances which have been put to work in the service of medical diagnosis. Although the images produced by this technique are superficially similar to computered tomography (CT), the physical and biological principles involved are very different. MRI is based on the physical phenomenon called nuclear magnetic resonance (NMR). NMR was described in 1946 by Bloch and Purcell working independently [Bloch and Hansen, 1946; Purcell et al. 1946]. They discovered that certain atomic nuclei, when placed in a uniform magnetic field and subjected to a brief pulse of radiofrequency, emit a pulse of radiofrequency (RF) in response. This resonance can be measured and contains information about the stimulated nuclei. In the early seventies, Damadian [1971], Lauterbur [1973] and other researchers [Mansfield and Grannell, 1973] described methods of using these physical concepts to produce images representing body tissues in a manner similar to that of CT.

The patient is placed inside a powerful magnet and a series of radiofrequency pulses are initiated. The body tissues contain atomic nuclei which behave as 'small magnets' and respond at resonance to produce a RF signal which is detected and stored. By varying the magnetic field and the RF pulse all the resultant data can be processed to produce images of the body tissues. Since the hydrogen nucleus (proton) produces the strongest signal, and is found in all body tissues, this is the nucleus currently used for mist imaging. It is

possible to detect other nuclei, such as phosphorus, in the body, but imaging from these nuclei is still only in the research phase.

2.1.1. Basic physical and biological principles

MRI is based on the phenomenon that nuclei of certain atoms respond to a magnetic field by aligning with or against it. This process is called magnetization. The hydrogen nuclei in water and short-chain fatty acids, which are abundant in living tissue, undergo magnetization. The number of nuclei aligned with the magnetic field is slightly greater than that aligned against it, thus, a net magnetization is created that can be represented as a vector in the direction of the magnetic field. The magnitude of the vector is related to the proton density.

The magnetization vector is oriented in the direction of the magnetic field, which is designated the longitudinal direction. The direction of the magnetization vector may be altered by the addition of energy in the form of an RF pulse of appropriate frequency. In the most common type of MRI, a RF pulse with enough energy to rotate the magnetization vector 90° from the static magnetic field is applied. This "90° pulse" changes the direction of the vector from the longitudinal plane into the transverse plane. When the RF pulse is terminated, the hydrogen nuclei will begin to realign in the direction of the external magnetic field. The rate at which the realignment is reestablished depends on the rate that the added energy is dissipated to the surrounding environment. The dissipation mechanism is referred to as longitudinal relaxation, and the time required for 63% of the magnetization vector to return to the longitudinal direction from the transverse plane is designated T_1 , the longitudinal relaxation time.

While the magnetization vector is in the transverse plane, it rotates around the axis of the magnetic field and induces a current in a receiver coil. The amount of current generated initially is directly related to the size of the magnetization vector in the

transverse plane. Since the magnetization vector is the sum of many nuclei rotating at slightly different frequencies, the magnitude of the vector and the signal strength diminishes as the nuclei lose coherence or fan out within the transverse plane. The rate at which coherence is lost is designated transverse relaxation. The time at which 63% of the transverse magnetization dissipates is defined as T_2 , the transverse relaxation time. The loss of coherence is accelerated by inhomogeneities in the external magnetic field. This accelerated loss can be compensated for by using a spin-echo (SE) pulse sequence. In the SE sequence coherence is reestablished by using a pulse of radio waves, with twice the energy of the 90° pulse, called a refocusing, or 180° pulse. This pulse flips the nuclei 180° in the transverse plane and subsequently refocuses the transverse magnetization vector [Osborn, 1988].

2.1.2. Pulse sequences

A pulse sequence consists of one or more pulses of radio waves. A pulse sequence is repeated typically 128 or 256 times or more depending on the desired pixel size and number of signal averages to produce enough signals for an image.

The most commonly used pulse sequence is the SE sequence, which includes a 90° pulse that rotates the magnetization vector 90° from the magnetic field and a 180° pulse that restores coherence. The time between the 90° pulses is called the repetition time (TR); the time between the 90° pulse and the restoration of coherence is called the echo time (TE).

Another pulse sequence used in MR imaging is inversion recovery (IR). In this sequence a 180° pulse followed by a 90° pulse is applied at regular intervals (TR). IR was not used in this study.

2.1.3. Signal intensity

The signal intensity in MRI is dependent upon at least four parameters intrinsic to tissue: T_1 relaxation time, proton density, T_2 relaxation time, and flow.

To acquire T_1 -weighted images a SE pulse sequence with a short TR (200-1000 msec) and a short TE (20-25 msec) is used. The longer the TR, the greater the percentage of the magnetization vector that returns to the longitudinal plane before the next pulse and therefore the more signal that is generated. Tissues with a short T_1 have a greater signal intensity than tissues with a long T_1 at a given TR value. As TR decreases from 1,000 to 200 msec, the signal from cerebral tissues diminishes and contrast due to differences in T_1 decreases.

To obtain an image in which the proton density is the major determinant of intensity, the TR in a pulse sequence must exceed four times the T_1 of the tissue being studied to allow nearly complete remagnetization and the TE must be as short as possible to minimize the loss of signal intensity due to T_2 relaxation. A proton density image is typically acquired in a SE pulse sequence with a long TR (2000-2500 msec) and a short TE (20 - 25 msec), allowing near-complete T_1 relaxation. In a proton density image the signal intensity of fat is greater than gray matter, which in turn is greater than white matter. The signal intensity of CSF is less than the other tissues because its T_1 relaxation time is longer, allowing for a smaller percentage of protons to become magnetized prior to execution of the next pulse sequence.

T_2 -weighted images may be acquired at the same time as proton density image by using a SE pulse sequence with a long TR combined with a long TE. To understand the differences between proton density and T_2 -weighted images, it is necessary to know the relationship of signal intensity to TE. As the TE lengthens, the signal intensity decreases exponentially because of loss of coherence in the transverse plane. The long T_2 of CSF

causes minimal signal decay, such that with long TE values CSF has a relatively greater signal intensity than cerebral tissue.

2.1.4. Gadolinium (Gd) enhancement in MRI

Paramagnetic compounds have at least one unpaired electron. That electron has a magnetic moment approximately 1000 times stronger than the magnetic moment of a proton. As a result of motion (diffusion, rotation, etc), the paramagnetic compounds act at the atomic level to produce a rapid fluctuation in the local magnetic field. This process facilitates energy transfer among the excited protons and also from the protons to their environment, and the magnetic moments of the hydrogen nuclei deflected by the RF pulse return more rapidly to their initial state. Paramagnetic compounds can only shorten relaxation times; they cannot prolong them. Moreover, they always affect both T_1 and T_2 relaxation times, usually to an equal degree. In some circumstances T_2 may be shortened to a much greater degree than T_1 ; the reverse is never true.

As a paramagnetic agent Gd has become available for clinical use. Gd is a rare-earth element, the ion of which has 7 unpaired electrons. Because it is poorly tolerated in its pure form, Gd has been detoxified by complexation into stable chelates. One of the Gd chelates, DTPA (diethylenetriaminepentaaceticacid dimeglumine), is widely used. Although Gd-DTPA enhances both T_1 and T_2 relaxation in vivo, with the clinical dose used in vivo the T_1 effect is very marked and the T_2 effect virtually absent [Weinman et al., 1990].

Gd-DTPA strongly enhances relaxation in vivo at a dose of 0.1 - 0.2 mmol/kg, while the lethal doses in 50% of rats (LD50) is 10 mmol/kg [Weinmann, et al., 1984]. There is no evidence of dissociation of the Gd-DTPA complex in vivo, and more than 90% of the Gd-DTPA is excreted through the kidneys within 24 hours [Weinmann, et al., 1984]. Gd-DTPA dimeglumine is well tolerated in vivo, and adverse drug reactions occur in 1.46%

of the patients [Niendorf et al., 1991], and in the majority of cases consist of minor side effects, such as nausea, local warmth/pain, headache, paraesthesia, etc. Severe side effects are very rare.

2.2. MRI of multiple sclerosis

Traditionally, the role of radiographic imaging studies in the diagnosis of MS has been predominantly indirect (to rule out a space-occupying lesion). Although computerized tomography (CT) has demonstrated abnormal findings in patients with MS, the incidence of positive CT findings in patients with MS varied between 9% and 80%. The CT abnormalities were often nonspecific areas of low density, atrophy, and/or contrast enhancement. Thus CT was used primarily to eliminate the possibility of other intracranial lesions, such as a mass or arteriovenous malformation. Similarly, myelography was used to evaluate patients with spinal cord symptoms to exclude an intrinsic spinal cord mass or spinal cord compression.

Before MRI, patients with clinical symptoms of MS had to undergo CT and/or myelography, a battery of paraclinical studies (including visual and auditory evoked responses and urodynamic studies), and CSF studies (CSF electrophoresis for detection of oligoclonal bands) to establish a diagnosis. Now in many cases, MRI and an appropriate clinical history allow the neurologist to make the diagnosis of MS based on clear cut dissemination in space with a high degree of confidence, thereby obliterating the need for other ancillary studies.

Since the initial report by Young et al [1981], describing the increased sensitivity of MRI compared with CT in the diagnosis of MS, many reports, including some from our research group, have documented the usefulness of MRI [Li et al, 1984; Miller et al., 1988; Grossman et al., 1986; Barnes et al., 1988; Poser et al., 1987]. MRI is easier to perform and less invasive than previous techniques to diagnose MS. Furthermore, unlike

paraclinical tests or CSF tests, MRI is site-specific in defining demyelinating plaques. This information potentially enables correlation with clinical symptoms, has prognostic significance, and permits MRI to be used for follow-up to determine regression or progression of lesions. This last advantage has proven important in evaluating new experimental methods of therapy [Paty, 1987; Paty et al., 1993b].

Although the MRI appearance may vary slightly from patient to patient or even among lesions in the same patient, certain characteristic findings are common to most MS lesions. MS lesions are generally detected as areas of increased T_1 and T_2 relaxation times relative to white matter of the brain. Since MS lesions have a prolonged T_1 relaxation time, they are well defined on IR scans. Indeed, in many of the initial MR reports, MS lesions were demonstrated predominantly with this technique. Using this technique, MS lesions are visualized as areas of diminished signal intensity within the white matter of the brain. A major disadvantage of IR scanning is its relative insensitivity for detecting lesions that lie immediately adjacent to the ventricles or subarachnoid spaces, which are very common in the MS brain, because of partial volume averaging with the low intensity of the adjacent CSF. Some lesions may also be missed if they are contiguous with the gray matter of the brain, which also has a lower intensity than white matter on IR scans.

The spin echo (SE) technique is currently the best method for screening patients with suspected MS. In contrast to IR scans, long TR SE sequences demonstrate MS lesions as areas of high signal intensity. SE scanning sequences with a long TR of 2500 to 3000 msec. and dual echos using short and long TEs of 30 and 80 msec. are preferred. A TR of at least 3000 msec. is preferred when scanning at high field strengths of 1.5 T. The pathologic lesions demonstrate moderately increased signal intensity compared with brain or CSF on spin-density or mildly T_2 -weighted images (TE of 30 msec.) and increase further in signal intensity on heavily T_2 -weighted images (TE of 80 msec.). Periventricular lesions can also be obscured by partial volume effects on heavily T_2 -weighted spin-echo images, on which both CSF and MS lesions are displayed as very

high signal intensity. However, these periventricular MS lesions can easily be distinguished from adjacent CSF spaces by comparison with proton density images on which the lesions will have a greater signal intensity than does the adjacent CSF.

Generally, lesions in the periventricular regions are best seen on 30 msec. TE images, whereas posterior fossa and deep white matter lesions are often best displayed on 80 msec TE images. Dual-echo sequences are particularly useful whenever there are subtle lesions that may be equivocal on one echo, since they can be confirmed as a real finding when also seen on the other echo. Since the T_2 relaxation time of demyelinating plaques is less than that of CSF, the lesions may decline in relative signal intensity compared with the ventricles or subarachnoid space when TE times are extended beyond 80 msec. Thus SE sequences with TE times that are greater than 80 to 100 msec may obscure some lesions.

Minimum possible slice thickness should be used to decrease partial volume averaging from the surrounding low-intensity white matter. A 5 mm slice with a 0.5 - 1 mm gap is typically used, although the gap is primarily determined by cross-slice contamination or "cross talk." When routine imaging fails to demonstrate disease, some investigators include T_2 -weighted 2 mm slices with increased lesion detection [Schima, et al. 1993]

Although gradient-echo sequences have many applications in cranial and spinal imaging, the relatively decreased contrast resolution in comparison to spin-echo sequences, as well as the increased susceptibility artifacts of gradient-echo sequences, are significant disadvantages in imaging MS. Rarely if ever will gradient-echo sequences provide additional information in cases of MS.

Characteristic findings of MS consist of multiple, usually small, lesions within the white matter of the brain that have long T_1 and long T_2 relaxation times, as well as increased proton density, in comparison with normal white matter. The cause for the increased T_1 and T_2 relaxation times seen with MS lesions relates in part to the gliosis that occurs in chronic plaque formation. In acute lesions, edema resulting from the BBB

breakdown results in prolonged relaxation times and may also produce an area of abnormality considerably larger than the actual demyelinated area. Demyelination itself, with the loss of fatty myelin around the axonal sheaths within the MS lesion, does not contribute significantly to prolonged T_2 changes, since the amount of lipid lost is not great enough to cause the magnitude of change demonstrated on MRI and because the lipid protons of myelin have an extremely short T_2 relaxation time and are effectively invisible on MRI. The loss of myelin lipid does, however, provide a more hydrophilic environment. The subsequent increase in water content leads to the observed increases in proton density, T_1 , and T_2 times [Ormerod, 1989].

The individual lesion of MS are usually less than 10 mm in size, most often between 1 and 5 mm. Confluent plaque formation from merging of multiple small individual demyelinating plaques that are contiguous with each other or secondary to an acute, active demyelinating plaque can occur. In either case, these types of lesions can sometimes become quite large simulating tumors.

The most common location of lesions seen by MRI is in the periventricular region adjacent to the superolateral angles of the lateral ventricles. This distribution corresponds with pathologic descriptions [Steward, et al.1986]. Lesions in the corona radiata often appear oval or elongated in configuration, with the long axis of the demyelinating lesion oriented along the subependymal veins, perpendicular to the walls of the ventricles. This orientation corresponds to the pathologically described periventricular, perivenular location of MS lesions. The ovoid shape corresponds to the perivenular inflammation seen pathologically. This appearance is highly characteristic appearance for MS lesions and helps to distinguish MS from other white matter diseases, most notably deep white matter gliosis and infarction in older patients. Lesions are also often seen within the body of the corpus callosum, a site rarely affected by microinfarcts, further allowing these two entities to be distinguished. In addition, MS lesions tend to be more focal than deep white matter ischemic changes.

Additional common sites of involvement include the walls of ventricles adjacent to the atrial trigones and occipital horns, the white matter of the centrum semiovale, the forceps major and minor, and the temporal lobes.

Demyelinating plaques are also often demonstrated in the brainstem and cerebellum on MRI. There, they often abut the CSF spaces. Brainin et al. [1987] reported that among clinically definite cases of MS, pontine lesions were identified in 71%, Medullary lesions were identified in 50%, and midbrain lesions were identified in 25%. Lesions are also frequently seen within the cerebellum, although less commonly than in the brainstem. The middle cerebellar peduncles and the white matter of the corpus medullaris are other preferred locations.

The acute lesions of MS have a characteristic temporal course. Acute lesions reach a maximum size in approximately 4 weeks and subsequently regress and decrease in size, leaving a smaller residual gliotic lesion demonstrating a prolonged relaxation time [Koopmans et al, 1988, Willoughby et al., 1989]. Acute MS plaques can demonstrate enhancement on MRI after intravenous injection of the MR contrast agent, Gd-DTPA. Since this pattern of enhancement is consistent and can be seen even with clinically silent acute demyelinating lesions, MRI has proven much more sensitive in detecting new disease activity than is the clinical examination. Because of this great sensitivity, MRI promises to be useful in monitoring the results of therapy, and potentially distinguishing between benign and chronic progressive forms of MS [Paty, 1987].

Although MS is generally considered a white matter disease, it must be realized that approximately 5% to 10 % of lesions occur in gray matter of the brain, such as the cerebral cortex or within basal ganglia nuclei.

2.3. The BBB and MRI

Using CT scan after the intravenous injection of a contrast medium, regions of abnormal enhancement, reflecting extravasation of iodine through a damaged BBB, have frequently been reported in MS patients [Hreshey, 1979]. Compared to CT, MRI is more sensitive in detecting the MS plaques in the CNS. To provide contrast enhancement in MRI, Gd-DTPA is used as a marker for the BBB breakdown [Runge, 1985]. Using mannitol to reversibly open the BBB in dogs, Runge and colleagues [1985] showed that gadolinium may significantly detect an alteration of the BBB.

Gadolinium enhanced MRI (G-MRI) appears more sensitive in the detection of active lesions than clinical examination alone. Some studies [Grossman, 1988; Miller, 1988; Grossman, 1986] showed that more patients had enhanced lesions than were considered to have clinically active lesions. The BBB impairment is a consistent finding in new lesions detected with MRI, but, this impairment can also develop in older previously nonenhancing plaques without evidence of an increase in size. The enhancing regions were often less extensive than that on the corresponding high signal on T₂-weighted images. Maximum intensity of enhancement occurs from 4 to 120 minutes after Gd-DTPA injection. In the great majority of lesions peaking around 29 minutes [Kermode et al., 1990]. The enhancement can persist for 3 to 5 weeks. Our study [Zhao et al., 1993] found that G-MRI is sensitive for detecting lesion activity. Some lesions can be detected on G-MRI which may not be seen on standard MRI on the same scan. Those lesions can be either new or enlarging lesions that appear stable on follow up standard MRI. The BBB disturbance may precede other MRI signs of MS lesions (figure 2).

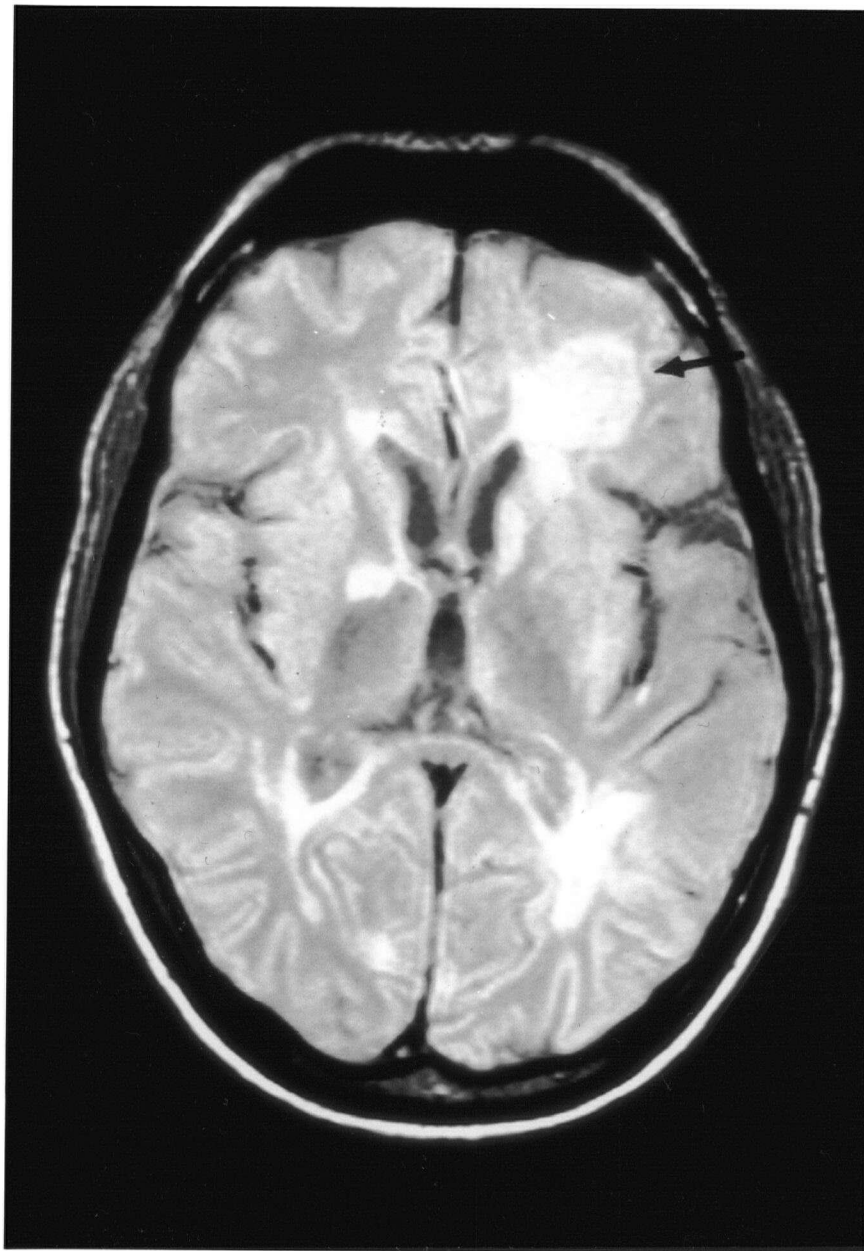


Figure 2a. Proton density imaging. TR 2844/TE 20. Arrow shows one of MS lesions.

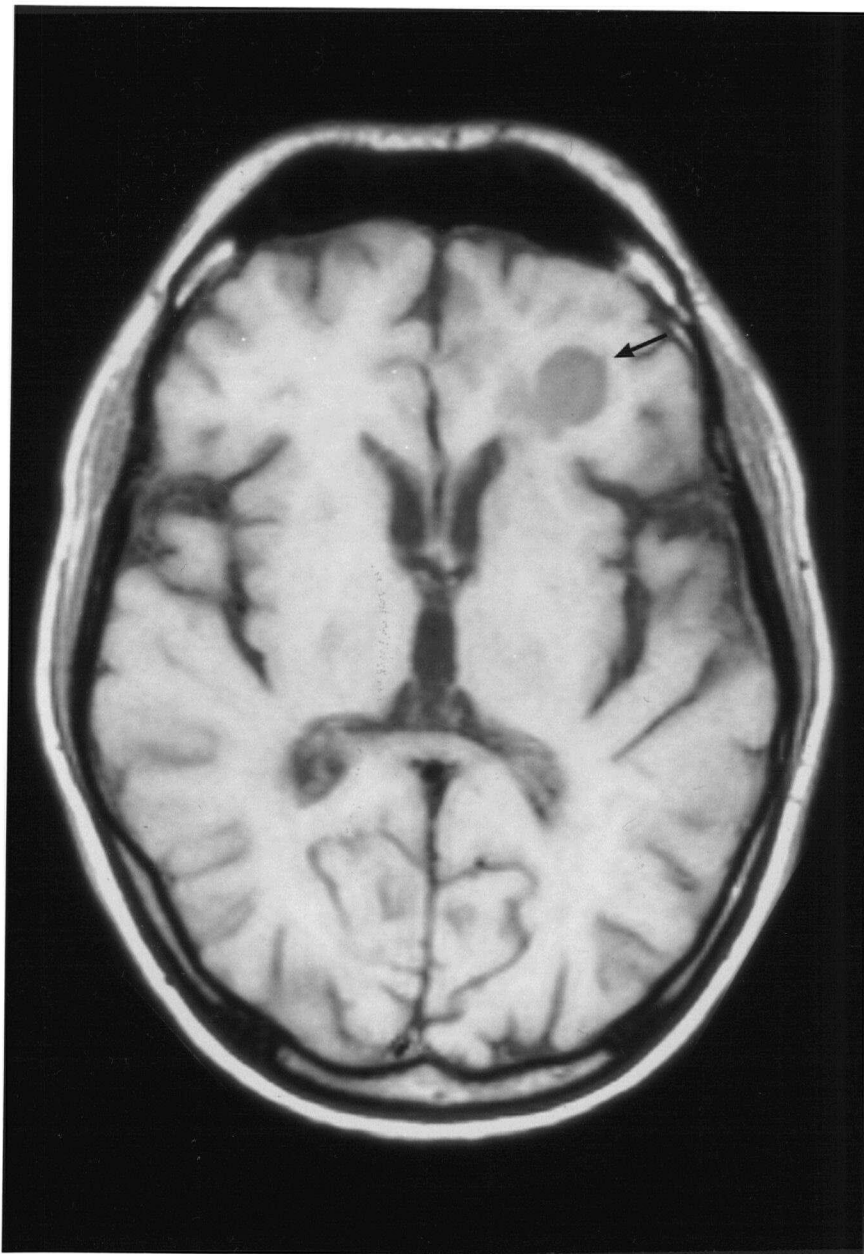


Figure 2b. T1-weighted image pre-Gd injection. TR 720/TE 15. Arrow shows MS lesion with hypointensity.

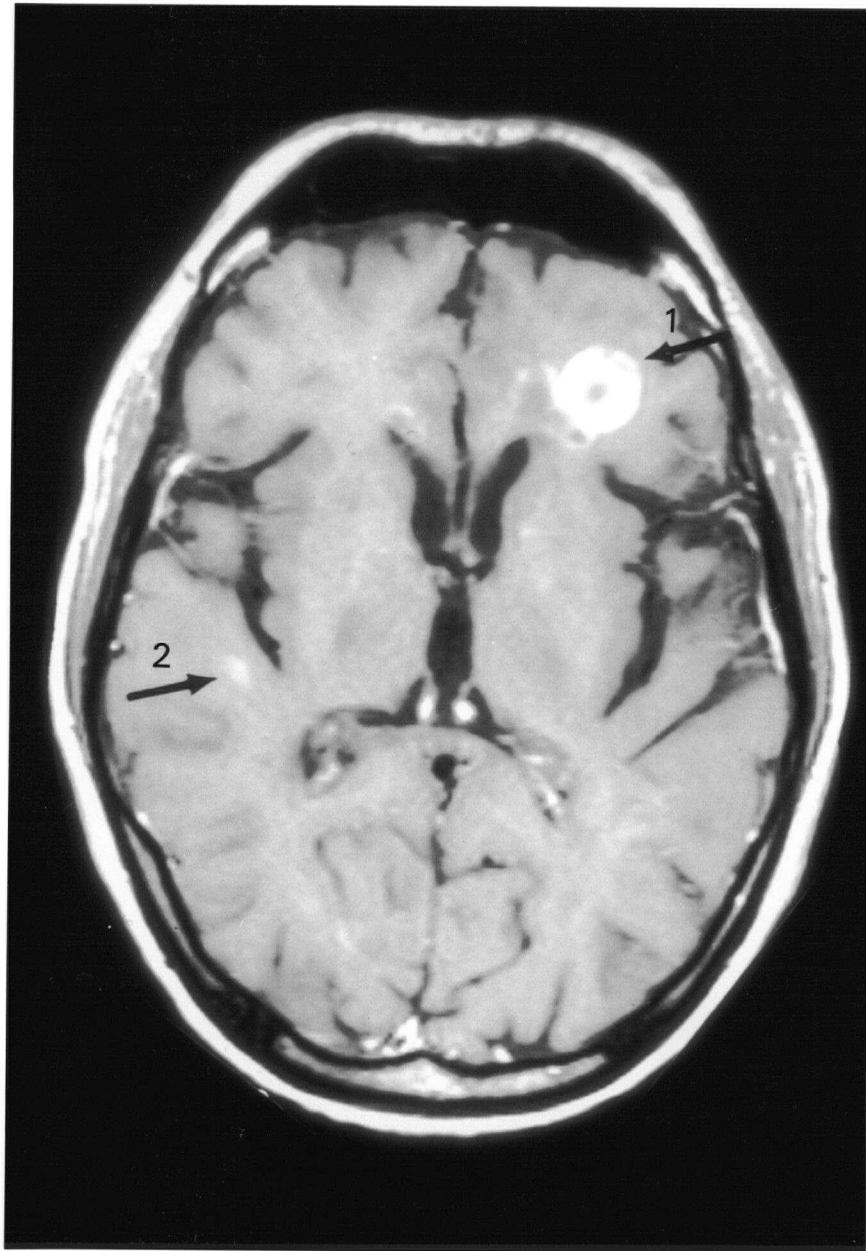


Figure 2c. T1-weighted image, TR 720/TE 15. Arrow 2 shows an enhancing lesion which was not detected in proton density image.

2.4. Serial MRI as a tool in monitoring therapeutic trials in multiple sclerosis

In early experience with MRI, intermittent scans showed that chronic lesions could be seen to increase in size and asymptomatic lesions could be seen to come and go [Li et al., 1984; Johnson et al., 1984]. Using serial MRI combined with frequent neurological examinations, it quickly became apparent that disease activity as measured by MRI could be quite dramatic and often subclinical.

Several serial studies were carried out in our center. Seven relapsing-remitting patients [Isaac et al., 1988] entered into the first study. The patients were examined by monthly MRI scans, physical examination, and immunological testing over six months. Neurological histories were taken at each visit. Five clinical relapses occurred in three patients. There were 17 new or enlarging MRI lesions seen during the study. Seventeen out of the thirty-six follow-up MRI examinations (48%) showed evidence of new and/or increasing disease activity. The mean clinical relapse rate was 1.4 relapses per patient per year. The rate of the appearance of new MRI lesions was 8.0 activity events per patient per year.

The second study included 9 patients with minimally disabling but active relapsing disease [Willoughby et al., 1989]. Each patient had a careful interim history along with neurological and MRI examinations done once every two weeks for an average of five months. Clinically detected activity was minimal with only three findings. One patient had two minor spinal cord sensory relapses. MRI examinations showed that there were ten instances in which new MRI lesions appeared, and two instances in which there was enlargement of pre-existing lesions. All of the MRI activity was asymptomatic. The clinical relapse rate was 0.4 relapses per patient per year. The frequency of MRI activity

was 2.6 positive MRI examinations per patient per year. There were eighty-three follow-up MRI examinations, and ten of those examinations (12%) showed evidence of increasing disease activity. This study, along with the first study, showed that the degree of disease activity in relapsing patients as detected by MRI could be as high as five times the clinical relapse rate.

Eight severely disabled patients in the progressive phase of MS were included in the third study [Koopmans et al., 1989]. Seven of the eight patients had begun with relapsing disease and could be considered RP or secondarily progressive. The other patient had chronic progressive (CP) or primarily progressive disease from outset. As in the second study, all patients had histories, physical examinations and MRI examinations, in addition to immunological tests, once every two weeks over a period of six months. In this study there were ninety-eight follow-up MRI examinations, during which time no clinical relapses were seen. However, twenty-five new MRI lesions were seen. There were also sixty-one instances in which previously seen stable MRI lesions increased in size. There were thus eighty-six MRI activity events during the study. Forty-seven of the ninety-eight follow-up scans (48%) showed evidence of increasing disease activity. These studies provide the evidence that serial MRI is more sensitive in detecting disease activity than clinical exacerbation.

Using serial MRI to monitor disease activity, three therapeutic trials in the past 5 years, all in our center, have been done. In the first study, MRI was used to evaluate the efficacy of systemic lymphoblastoid interferon therapy in chronic progressive MS [Koopmans et al., 1993]. Thirty-six patients with chronic progressive MS were treated with lymphoblastoid interferon daily for 6 months and 27 received placebo. Patients had MRI at the outset of the study and after 6 and 24 months. Lesion activity was evaluated by visual analysis. A semiautomated computer-assisted quantification of overall lesion load was used to determine the change of overall lesion burden. We found that both the interferon- and placebo-treated groups developed more active lesions as the study

progressed. There was no difference in lesion activity between the two groups. Comparison of lesion load, however, showed a trend toward improvement after 6 months for the interferon-treated group. This difference between the two groups had disappeared by the end of the study. However, the average overall increase in lesion load (burden of disease) was 10% per year. The conclusion in this study was that lymphoblastoid interferon was not effective in decreasing active MRI-detected lesions or in decreasing MRI lesion load in patients with chronic progressive MS, but that a yearly predictable increase in burden of disease occurred overtime.

One hundred fifty seven CP MS patients from 6 centers in USA and Canada, randomized for a 2 year cyclosporine therapeutic trial, were included in our second study [Koopmans et al., 1992]. Seventy-seven patients received cyclosporine and 80 placebo. Entry and exit MRI scans were obtained for each patient. Pooled MRI data from all centers again showed an increase in lesion load overtime. However, there was no significant difference between placebo and cyclosporine groups.

Our third study was an interferon beta-1b (IFNB) therapeutic trial in 372 ambulatory patients with RR MS, [Paty et al., 1993; The IFNB Multiple Sclerosis Study Group, 1993; Arnason, 1993]. In the multicenter, randomized, double-blind, placebo-controlled trial, one-third of the patients received placebo, one-third 1.6 million international unit (MIU) of IFNB, and one-third 8 MIU of IFNB. Yearly MRI was used to monitor the disease activity. In the serial MRIs, MS activity was significantly less in the high-dose IFNB group. Clinical and MRI results support that IFNB has made a significant impact on the natural history of MS in these patients.

Other MRI serial studies had been reported, some using gadolinium [Miller, 1989, Kermode AG 1990, Bastianello S 1990, Wiebe 1992, Harris 1991, Thompson AJ 1990, Smith 1993]. Up to 90 percent of new MRI lesions enhanced with gadolinium, and occasionally an enhancing area was seen before the standard MRI lesion was seen [Kermode 1990]. Spinal cord MRI added about 20 percent to the activity seen on the

standard head scans [Wiebe 1992]. In general, two-thirds of gadolinium enhancing lesions showed enhancement on only one scan [Miller, 1989]. Miller and associates found that twenty minutes after Gd-DTPA injection was the optimum time to see enhancement. Repeat Gd-DTPA injections were well tolerated by their patients.

There are some profound differences among several clinical categories of patients [Thompson 1990]. Primary progressive patients had the lowest rate of development of new lesions at 3.3 new lesions per patient per year. The next highest rate was for benign patients who had 8.8 new lesions per patient per year. Typical RR and RP patients had 17.2 and 18.2 new lesions per patient per year respectively.

The data from serial studies have shown that the rate of lesion activity varies widely among individual patients. Unfortunately, the rate of development of new and/or otherwise active lesions also varies considerably over time in the same patient. Some studies showed [Harris et al, 1991, Smith, 1993] that there were "burst" activity periods in some patients. Some patients can be active over a three month period of time and then be totally inactive over the next two to three months. However, "burst" periods of activity can be seen in our yearly MRI study [Zhao et al, 1995 (in press)]. The yearly MRI study showed that the more active lesions detected in the first year in an individual patient, the more likely one sees higher activity rates in follow up scans. Our other studies [Paty et al, 1993; Zhao et al, 1991] showed that the rate of lesion activity varied widely among patients. Some patients did not have any MRI lesion activity over 2 or more years, but some patients had more than dozens of new or enlarging lesions. This variability implies that a "run in" period of scanning prior to a clinical therapeutic study may predict the subsequent MRI activity in groups of MS patients making it helpful for selecting patients in designing new therapeutic trials. Nevertheless, the "run in" period of time could be a key in determining MRI activity in individual patients which could let researchers know how long and how many patients required before a new study starts.

Miller and his colleagues [1988] have seen high rates of asymptomatic changes in their serial Gd enhanced MRI studies in relapsing progressive patients. New lesions reached a maximum size in two to four weeks and then faded over the subsequent six to eight weeks. In contrast to the pattern seen in RP MS by our group and by Queen Square group, Thompson et al. [1992] found primary CP MS to have a different pattern. Their primary CP patients had a low relative burden of disease and had a low tendency to develop new MRI lesions. Additionally, only one of the twenty small new MRI lesions that they saw enhanced.

The optimum frequency of scanning, considering the information available, has been once every two weeks. Assuming no lesion lasts less than two weeks, 100 percent of new and active lesions are detectable at two weeks. Based on our experience only 67% percent of that lesion activity is seen with scans performed every four weeks and 40 percent of the lesion activity is seen with scans every six weeks. Conversely, 36 percent of active lesions had a duration of activity of less than four weeks, another 28 percent between four and six weeks, and another 7 percent between six and eight weeks. However, scanning at yearly intervals allows most of the new lesions to be seen.

This data supports serial MRI's usefulness as a tool in monitoring therapeutic trials in MS. On the use of MRI to monitor clinical trials, some suggestions had been made [Miller, 1991]. (1) MRI monitoring of treatment trials is appropriate. (2) Such monitoring studies can be done in periods as short as six months. (3) Gd-DTPA enhancement adds 10 percent or more to the activity determined by enhanced scanning and should be done routinely. (4) Groups of patients should be stratified into a). early relapsing-remitting MS; b). benign MS; c). Secondarily progressive MS; d). Primary chronic progressive MS.

Chapter 3

MRI Active Lesions

3.1. Definition of active lesions

"Active" lesions were identified as follows:

1. New lesion: A lesion that had not previously been seen (figure 3).
2. Enlarging lesion: Enlargement of a previously seen stable lesion (figure 4).
3. Recurrent Lesion: A lesion that develops at the same site where a previous lesion had been seen (figure 5).
4. Enhancing lesion: A lesion that enhances in the T_1 -weighted image post Gd-DTPA injection (figure 6).

A continuously enlarging lesion over several examinations was counted as active only once. Each significant change indicating increased activity that was not a continuation of a previous change was noted as an "MRI event". There could be one or several MRI events in each active scan. One lesion could also have several MRI events during a study.

In patients with RR MS there are many more preexisting lesions seen and the pattern of lesion change is great.

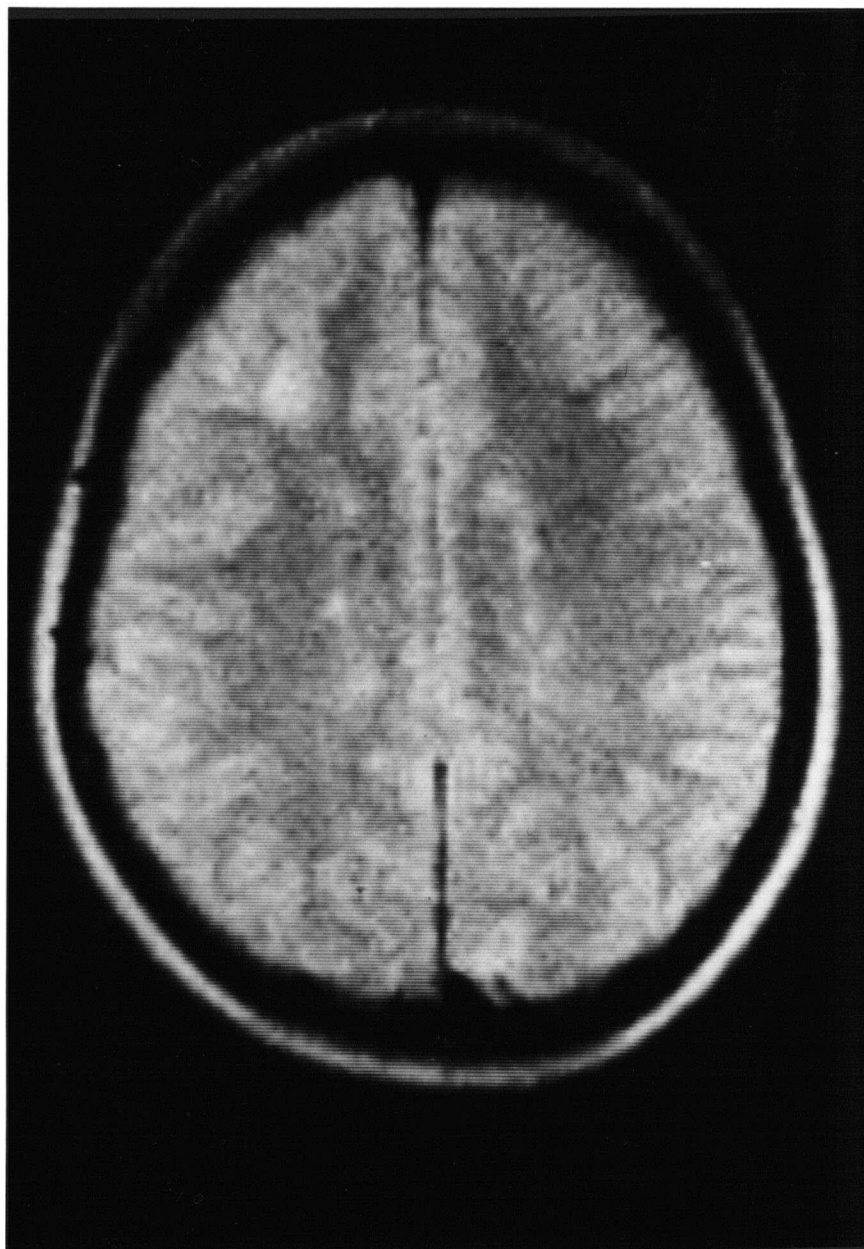


Figure 3a. Proton density image, TR 2133/TE 60.

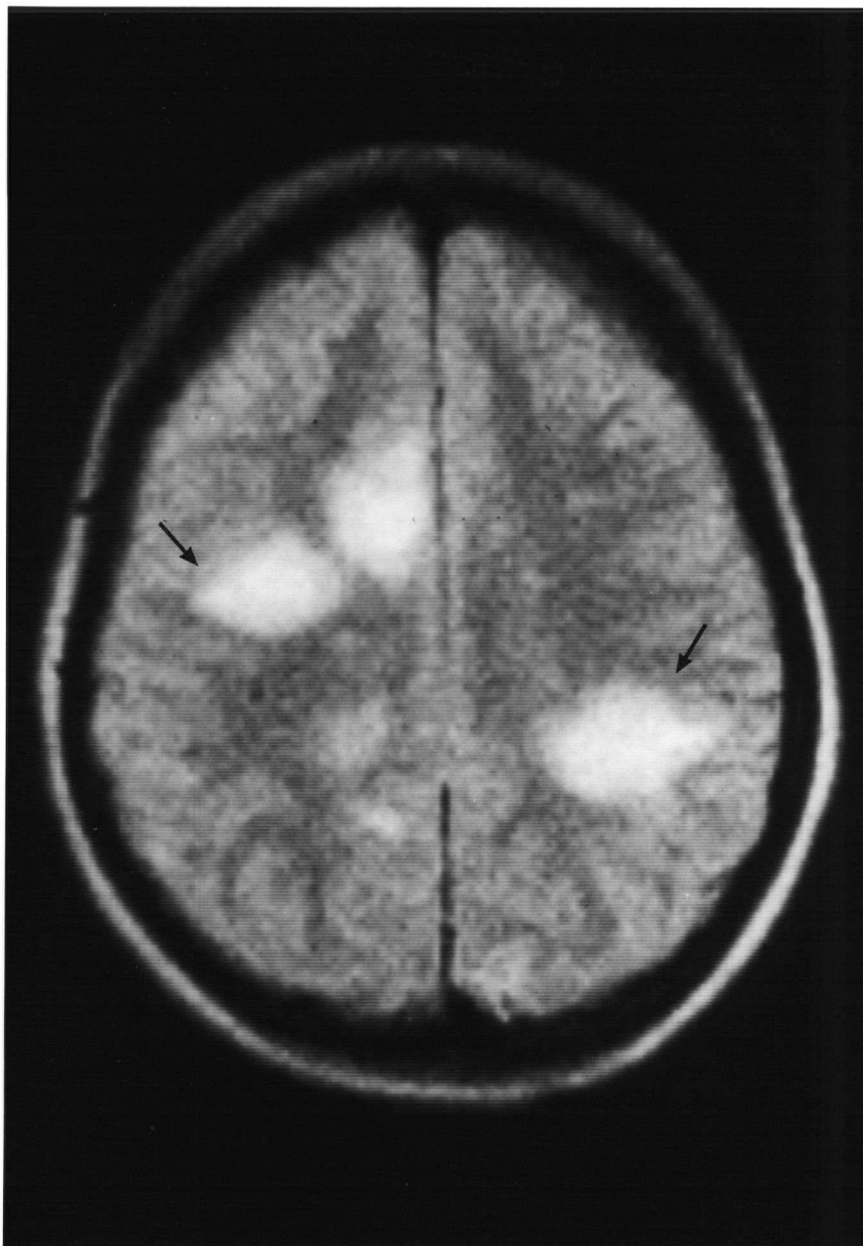


Figure 3b. Proton density image, TR 2133/TE 60. Arrows show new lesions



Figure 4a. Proton density image, TR 2133/TE 60. Arrow shows one of many MS lesions



Figure 4b. Proton density image, TR 2133/TE 60, 6 weeks later. Arrow shows the lesion enlargement. The lesion also merges with a neighbor lesion.

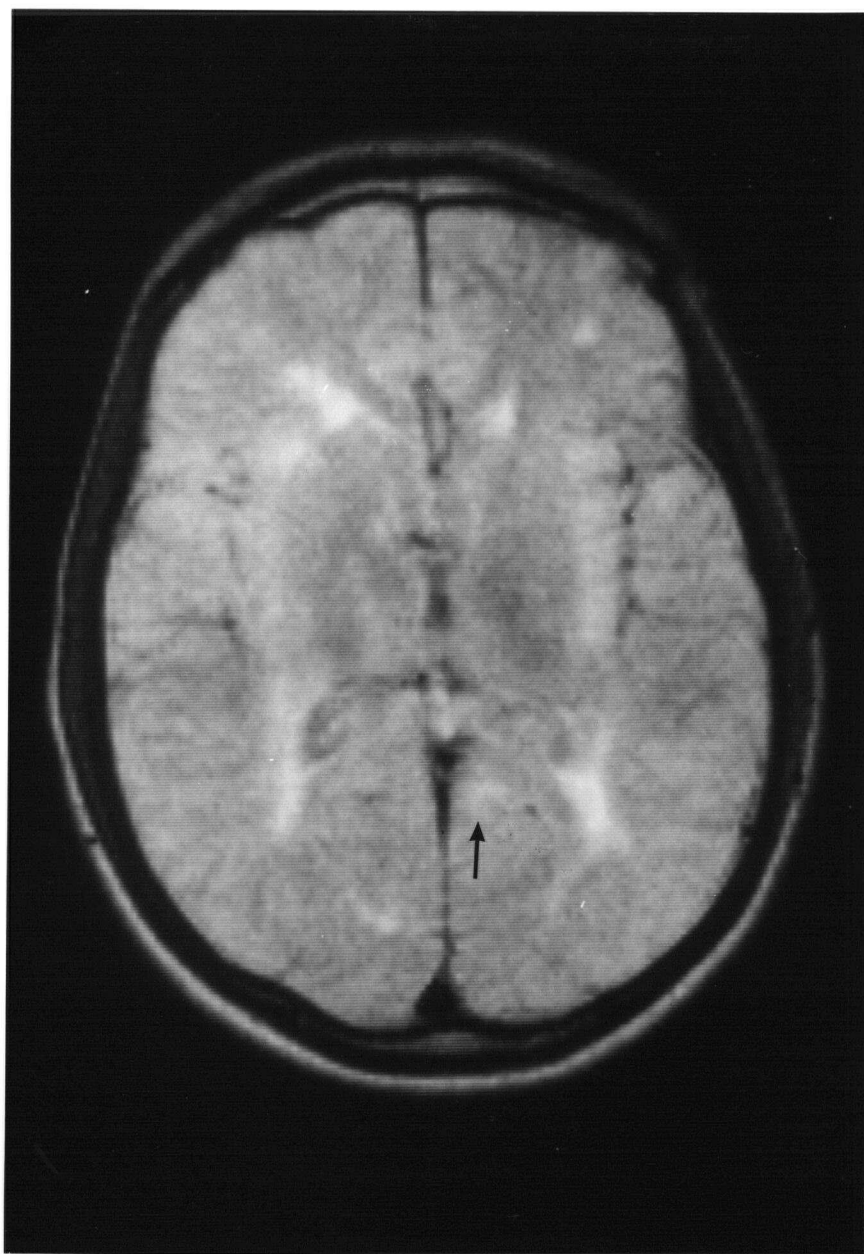


Figure 5a. Proton density image, TR 2133/TE 60. Arrow shows one of several MS lesions

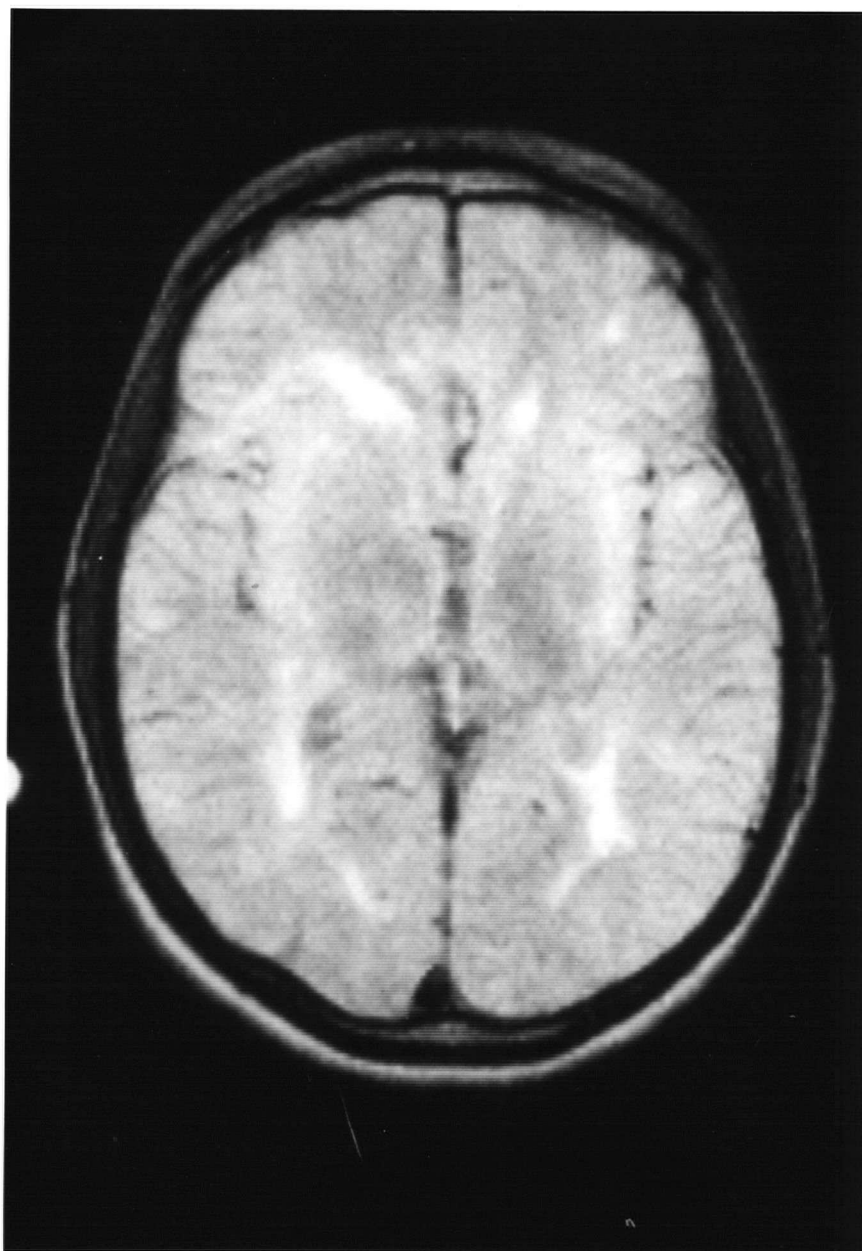


Figure 5b. Proton density image, TR 2133/TE 60, 6 weeks later.

The lesion marked by the arrow in the previous scan has disappeared.

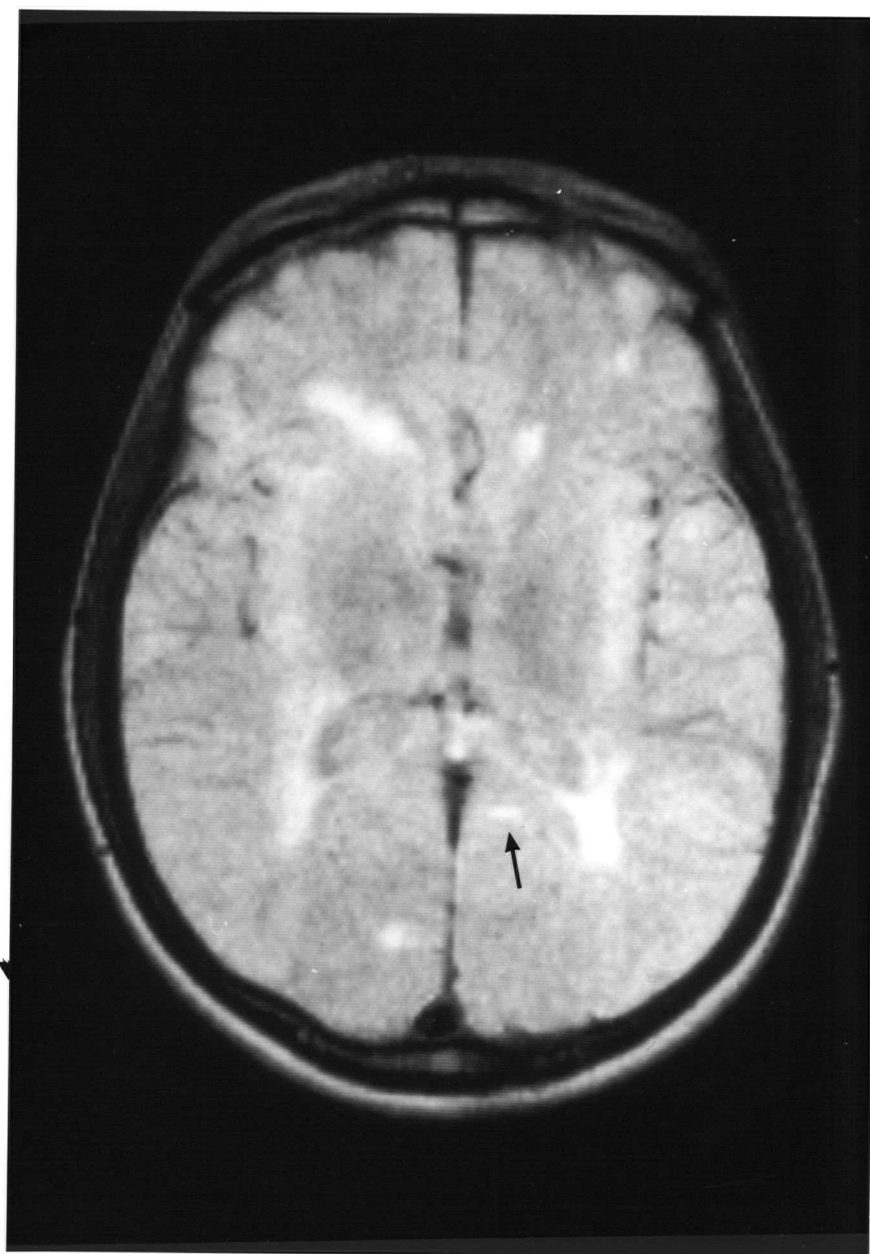


Figure 5c. Proton density image, TR 2133/TE 60. 12 weeks later.

The lesion that disappeared in the previous scan has reappeared (arrow). Careful examination of the rest of the image shows that the changes in this lesion were not artefactual.

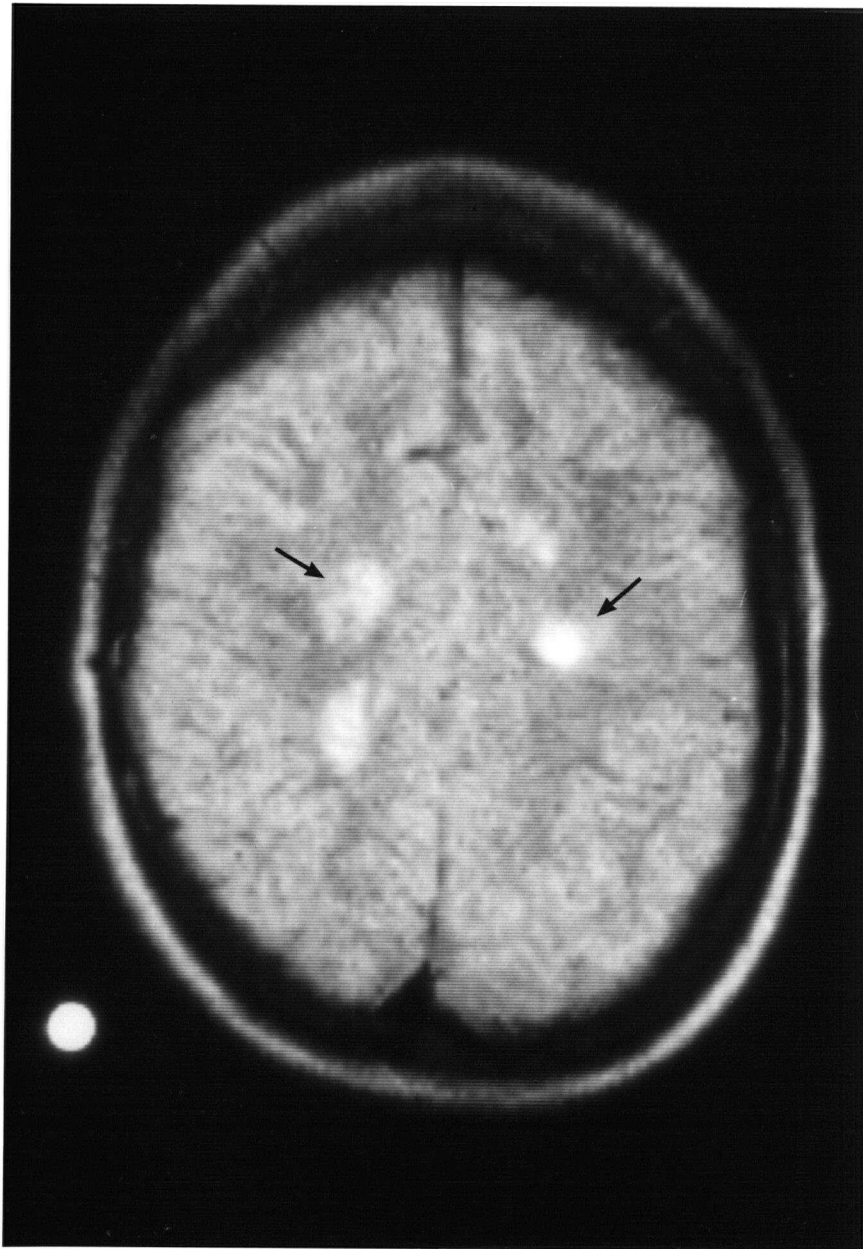


Figure 6a. Proton density image, TR 2133/TE 60. The arrows identify two of several lesions

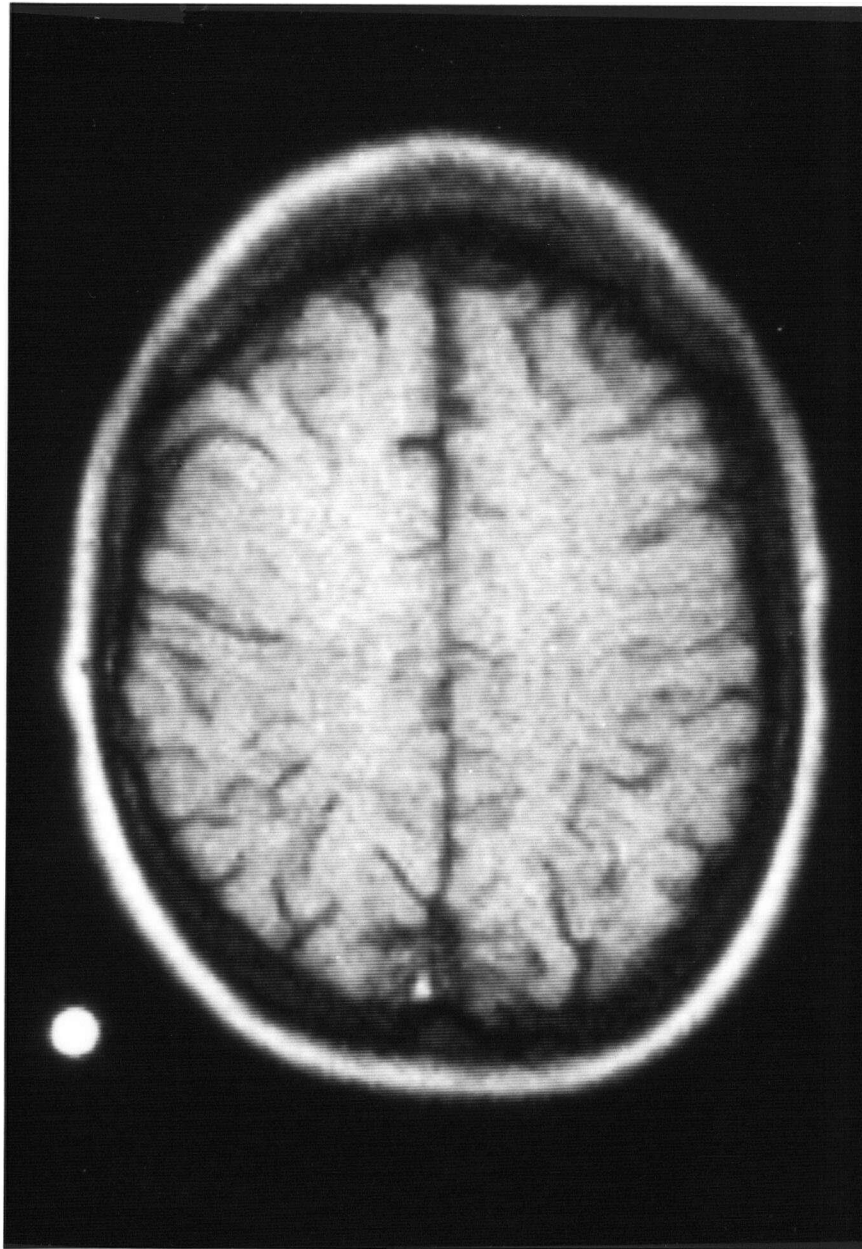


Figure 6b. T₁-weighted image, TR 683/TE 26, on the same day as fig. 6a
Pre - Gadolinium injection. No lesion was detected.

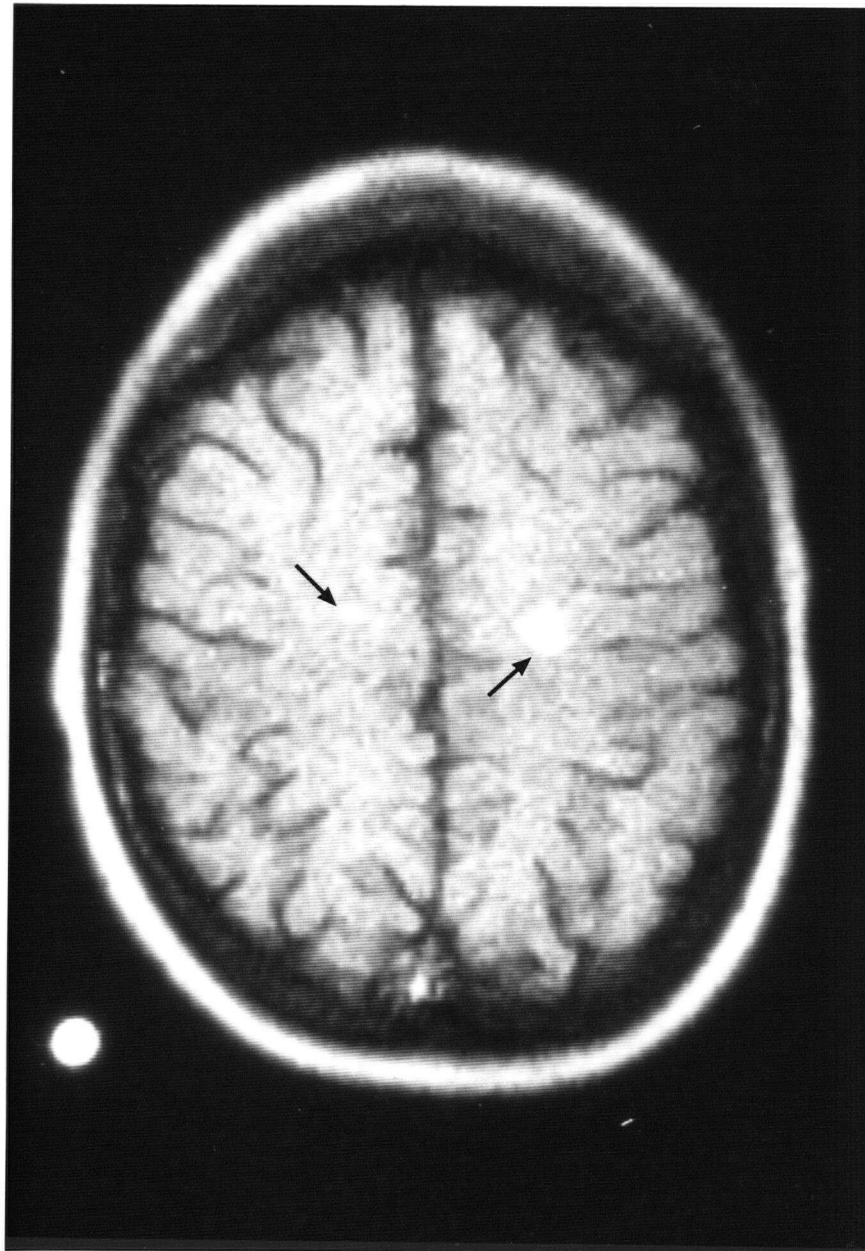


Figure 6c. T₁-weighted image, TR 683/TE 26, post gadolinium injection on the same day. Two enhancing lesions were found (arrows). These lesions correspond to two of the stable looking lesions marked by arrows on figure 6a.

3.2. Materials and methods in this study

3.2.1. Patient population

We studied 50 patients with clinically definite relapsing remitting MS who had been entered into a therapeutic trial using beta-interferon. There were 12 men and 38 women whose ages ranged between 22 and 53 years (mean, 38.6 years). Mean duration of disease was 8.2 years (range, 1.1 to 20.0 years; SD = 5.0 years). Disability of patients was between 0 and 3.5 (mean, 2.0, SD = 1.0) on the Kurtzke Expanded Disability Status Scale (EDSS). All patients had an abnormal cerebral MRI characteristic of MS, with scattered areas of increased intensity on the spin-echo (SE) sequence. Patients were randomized into three treatment arms: Placebo (N=17), 1.6 mIU (N=17), 8 mIU (N=16) IFNB self-administered subcutaneously every other day.

Each patient had a cerebral MR scan every 6 weeks for 2 years. Clinical examination was also done on patients every 6 weeks.

3.2.2. MR scan technique

MR scans were performed using a Picker International Cryogenic MR 2000 scanner with a superconducting magnet operating at a static magnetic field strength of 0.15 Tesla and a 30 cm diameter receiver coil in the Vancouver Hospital and Health Science Center, University of British Columbia (UBC) Site. Twelve contiguous 10 mm thick axial slices were obtained through the brain from the upper cerebral hemisphere to the medulla. The inplane resolution was 1 mm. A dual echo SE sequence was used with echo delay times (TE) of 60 and 120 msec and a repetition time (TR) of 2133 msec.

MRI serial studies require very careful repositioning. Careful attention is the key to the repositioning process. The repositioning error was minimized through alignment of

external and internal landmarks. Initial repositioning was based on two angles from external landmarks (the canthomeatal line and the nasion-tragal line). A midline sagittal slice (pilot) was then obtained and the head position verified using an internal angle (angle between top of the cerebellum and the anterior sphenoid sinus). If this angle differed by more than 2 degrees from the baseline study, the patient was repositioned and the pilot scan repeated. Slices for the actual scans were then programmed so that the middle slice of a simultaneous 12-slice series was centered on the top of the cerebellum. Halfway through an individual scanning session, the pilot scan was repeated to ensure that positioning was still adequate. If movement occurred, the patient was repositioned and the appropriate sequence repeated.

3.2.3. Quantitative analysis of MRI

An interactive computer program was designed to display MR images and permit manual tracing of lesions [Koopmans, et al. 1993]. The lesion borders were outlined on a computer monitor. The area of the individual lesions could then be determined and analyzed.

3.2.4. Statistics

The differences in MRI data were evaluated by means of the chi-square test for categorical variable and by Student's *t* test or the ANOVA for quantitative variables.

3.3. Assessment of the individual lesions in RR MS on MRI

3.3.1. Introduction

Activity of MS lesions as detected on MRI can be determined by performing serial examinations over time. Active lesions include new, enlarging, and recurrent lesions (see sect. 3.1). Some new lesions had totally resolved in 4 - 8 weeks. On pathology, chronic active plaques have a different pattern from that of chronic inactive ones [Adams, 1983]. A chronic active MS plaque has a broad zone of perivascular and parenchymal inflammation along the edges together with diffuse demyelination. However, the pathogenesis of the developing new lesion is still not known. The rate of MRI active lesions using this method of analysis was 2.4 per patient per year in one study [Willoughby et al., 1989] while the clinical relapse frequency was 0.4 per patient per year. Many active lesions are asymptomatic causing a higher frequency of MRI activity. Understanding the dynamics of these active lesions may in part explain the pathogenesis of MS. However, the identification of lesion activity using both standard and enhanced MRI involves extra time, cost, and invasiveness. One objective of this study was to observe any characteristics that could allow active lesions to be distinguished from stable ones on systematic standard unenhanced scans, thus avoiding the extra time, cost and invasiveness of Gd imaging.

3.3.2 Materials and methods

Lesion Analysis of Magnetic Resonance Scans

Researchers, experienced in MRI and masked to the clinical course of the patients, identified lesions for activity. Changes in lesion size and number between scans were determined by comparative examination of previous scans. Only changes in lesions that

were independently agreed to by all observers were recorded. A lesion enlarging continuously over several examinations was counted as being active only once. A lesion seen in adjacent slices was also counted only as one active lesion. Lesions that did not change over at least 9 months were recorded as stable. A total of 150 active lesions (50 new, 50 recurrent, and 50 enlarging) and 50 stable lesions were analyzed. The first 50 of each category of lesions were identified and analyzed as to their margin (well defined vs. ill defined), shape (round/oval vs. irregular), and location (periventricular vs. nonperiventricular; cerebral, cerebellum, and brainstem; and white matter vs. gray-white matter junction). Periventricular lesion was defined as any part of lesion which contacted with ventricle and non-periventricular lesion was defined as the lesion was away from ventricle.

Quantitative Analysis of Magnetic Resonance Images

The lesion borders were outlined on a computer monitor by a technician. The area of the individual lesions could then be determined and analyzed. Lesions were also grouped as small when the area was less than 30 mm², medium when the area was between 30 and 100 mm², and large when the area was greater than 100 mm².

3.3.3 Results

MRI morphological findings in the margin and shape of the lesions are summarized in table 2. The margins of most lesions were well defined. When they were ill defined, they were more commonly new or recurrent lesions rather than stable or enlarging ones. New lesions were also more likely to be round and oval in shape.

The location of lesions is summarized in Table 3. Enlarging and stable lesions were slightly more common in a periventricular rather than a non periventricular location. On the other hand, new and recurrent lesions were more commonly located away from the

Table 2: Margin and shape of lesions (n = 50 for each group)

	<u>New</u>		<u>Enlarging</u>		<u>Recurrent</u>		<u>Stable</u>	
	No.	%	No.	%	No.	%	No.	%
Margin well-defined	42	84	49	98	42	84	50	100
Margin ill-defined	8	16	1	2	8	16	0	0
Round and Oval	36	72	22	44	30	60	17	34
Irregular	14	28	28	56	20	40	33	66

* Enl = enlarging lesions, Rec = recurrent lesions, Sta = stable lesions.

Table 3: Location of lesions (n = 50 for each group)

Location	<u>New</u>		<u>Enlarging</u>		<u>Recurrent</u>		<u>Stable</u>	
	No.	%	No.	%	No.	%	No.	%
periventricular	9	18	29	58	12	24	27	54
non-periventricular	41	82	21	42	38	76	23	46
cerebrum	41	82	48	96	42	84	48	96
cerebellum	3	6	0	0	4	8	0	0
brainstem	6	12	2	4	4	8	2	4
white matter	25	50	41	82	36	72	42	84
GW junction*	25	50	9	18	14	28	8	16

* GW junction = junction of gray and white matter

Table 4: Mean area of lesions (mm², n = 50 for each group)

	<u>New</u>		<u>Enlarging</u>		<u>Recurrent</u>		<u>Stable</u>	
	No.	%	No.	%	No.	%	No.	%
Small	20	40	2	4	16	36	20	40
Medium	19	38	16	32	30	60	15	30
Large	11	22	32	64	4	8	15	30
Mean Area (mm ²)	79		370		48		107	

ventricles (for periventricular and non-periventricular location, new lesions vs. enlarging lesions, $p < 0.005$). While more stable, enlarging, and recurrent lesions were found in the white matter, new lesions were just as likely to be at the gray white matter junction as in the white matter.

Table 4 summarizes the size and the mean maximum area of each type of active lesions. As a group, enlarging and stable lesions are larger than new and recurrent ones. Most of the enlarging lesions were of the large category. Recurrent lesions tended to be of the medium or small category (mean area, new lesions vs. enlarging lesions, $p < 0.0003$).

3.3.4 Discussion

Lesion activity as detected by serial MRI examinations is much more common than is the clinical relapse rate. Our previous studies showed 2.3 new lesions/patient/year in a relapsing remitting group of MS patients and 6.0 new lesions/patient/year in a secondary progressive group. There were 2.9 total active events/patient/year in the RR-MS group and 21.8 total active events/patient/year in the RP-MS group [Koopmans et al., 1989; Willoughby et al., 1989; Paty et al., 1988]. On the other hand, the clinical relapsing rate was only 0.3 to 0.4 attacks/patient/year [Adams, 1989]. The very high rate of activity as detected on MRI indicates that most of these active lesions are asymptomatic.

Active lesions can be detected in both serial unenhanced MRI and gadolinium enhanced MRI [Koopmans et al., 1989b; Bastianello et al., 1990; Miller et al., 1988]. Gadolinium enhancement indicates blood-brain barrier breakdown in the sites of inflammatory demyelination while findings in serial MRI showed morphologic change in plaques in which inflammation may be an important factor [Raine et al., 1991; Hawkins et al., 1990; Kermode et al., 1990]. In this study, new and recurrent lesions tended to have well-defined margins and were round and oval in shape while enlarging and stable lesions

tend to have ill-defined margins and were irregular. New lesions probably represent acute inflammation where demyelination may or may not be seen. Nevertheless, Raine suggested that these new lesions represent not acute lesions but merely growing fingers from active chronic plaques [Raine et al., 1991]. Our findings show that the majority of new lesions were non-periventricular (new vs. enlarging lesions, $p < 0.005$). These data suggest that new and recurrent lesions are rarely fingers of active chronic plaques. However, detecting a new lesion on a conventional MRI scan can be difficult.

Chronic, active plaques constitute a less demarcated margin and are centered on small veins within the white matter proper on pathology [Raine et al., 1991]. Foamy macrophages can be invariably found throughout the normal white matter far away from lesions. Most of enlarging lesions we saw (82%) were originally from stable lesions. Compared to other kinds of lesions, enlarging lesions were larger in size and were irregular in shape, similar to their original stable lesions in size and shape.

The location of active lesions showed new and recurrent lesions were non-periventricular (82% and 76%) contrasting to enlarging and stable lesions that were periventricular (42% + 46%) in location. The results were similar to findings by Koopmans and colleagues [1989a]. To date, it is not clear whether new lesions can be distinguished from stable ones in location only or also in their pathogenesis. In contrast to the other groups, new lesion also tended to be detected at the junction of gray and white matter (50%). Gray matter has a higher blood supply than white matter. The junction of gray and white matter which is more vascular may influence, the greater chance of new lesion formation.

This study provides evidence that there are some differences that distinguish new and recurrent lesions from enlarging and stable ones. New and recurrent lesions were smaller, more likely to be round or oval, often distant from ventricles, with a more ill-defined margin when compared with stable ones. Enlarging lesions were much like stable lesions

in shape, size, location, and area. However, it may be difficult to separate active from stable lesions without serial MRI and gadolinium enhancement.

3.4. Features of enhancing and non-enhancing lesions of MS on MRI

3.4.1. Introduction

Conventional standard serial T₂ and PD MRI and gadolinium-enhanced MRI are now established as the most useful markers of disease activity in MS. Their role in monitoring therapeutic trials is currently being clarified. One of the many factors requiring consideration in establishing a research protocol is the clinical pattern of disease activity.

Gd-DTPA enhanced MRI can detect BBB breakdown. What this breach in the normal BBB to Gd-DTPA means in histological terms is of considerable interest because of its implications for understanding the development and evolution of the plaque as well as its potential use in monitoring treatment. As expected, lesion enhancement is not always seen in morphologically active lesions as seen on MRI, conversely a stable lesion on serial standard MRI can be enhanced by Gd-DTPA as its only indication of activity. In this study we examined the morphological features of enhancing and nonenhancing active lesions.

3.4.2. Materials and methods

Enhanced scans were performed on entry into the trial and whenever morphologically active lesions were identified on serial standard MRI. Pre and post contrast scans were obtained using a TR of 683 msec and TE of 26 msec. Enhanced scans were performed 3 and 15 minutes after Gd-DTPA administration (Magnevist, 0.1 mmol/kg). The repositioning error on follow-up scans and gadolinium enhancement scans was minimized by use of our previously described procedure (see section 3.2.2).

Analysis of Magnetic Resonance Scans

Three of us (GJ Zhao., DKB Li., BL Tanton.), experienced in MRI and masked to the clinical course of patients, identified lesions for activity and enhancement. Changes in lesion size and number between scans revealing morphological activity were determined by comparative examination of previous scans. We only recorded changes in lesions that were independently agreed on by all observers. A lesion enlarging continuously over several examinations was counted as active only once. A lesion seen in adjacent slices was also counted as one active lesion only. Active lesions were defined as (1) new; (2) enlarging; (3) recurrent; and (4) stable (see section 3.1). An enhancing lesion seen on study entry was defined as active if morphological change was seen in follow-up. The degree of enhancement after Gd-DTPA was graded as follows: 0. no enhancement, 1. questionable enhancement, 2. faint but definite enhancement, 3. moderate enhancement, and 4. intense enhancement. All lesions were examined as to their margin (well defined vs. ill defined), shape (round/oval vs. irregular), and location (periventricular vs. non-periventricular).

3.4.3. Results

Of the 308 morphologically active lesions that were identified on serial MRI, 33 had a concurrent gadolinium enhanced scan of which 14 showed enhancement and 19 did not. Twenty nine (29) other enhancing lesions were identified at entry for a total of 43 enhancing lesions. Fifty (50) morphologically stable lesions identified consecutively from the first 7 patients were studied as a control group.

The location, margin, and shape of lesions were summarized (table 5). In enhancing lesions, 29 of 43 were both enhancing and morphologically active in which 23 (79%) of 29 lesions had a clear margin. Seventeen [17/29 (59%)] lesions were round or oval in shape, and 6 of 29 (21%) were periventricular in location. In contrast, 14 of 43 (33%)

were enhancing but morphologically stable in which 11 of 14 (79%) were clear in margin, 6 of 14 (43%) were round or oval in shape, and 9 of 14 (64%) were periventricular in location. 54% of the stable lesion were periventricular in location. Lesion location was interesting in that morphologically active lesions, whether or not they were non-enhancing or enhancing, presented a similar proportion to be periventricular. Morphologically stable lesions, whether enhancing or non-enhancing, were also seen in almost the same periventricular proportion. There was no significant difference in periventricular versus non-periventricular location of enhancing lesions versus non-enhancing lesions ($p > 0.2$).

Of 43 enhancing lesions, 29 were identified at entry scan. Twelve (12) of 29 (41%) were periventricular and 17 of 29 (59%) were non-periventricular. In 14 enhancing but morphologically stable lesions, 10 (72%) were identified on the entry scan. In other words, of 29 enhancing lesion detected on the entry scan, 14 were morphologically stable in follow up.

Signal intensity of enhancing lesions was studied 3 minutes and 15 minutes post-injection (table 6). Twelve (12) of 43 (28%) lesions had not been detected after 3 minutes but were seen in 15 minutes. The total number of lesions that were in grade 3 and 4 was obviously higher in 15 minutes (20 lesions) than in 3 minutes (10 lesions).

Fourteen (14) of the 43 enhancing lesions (33%) remained stable on follow up by serial MRI. Eight (8) of 19 (42%) non-enhancing lesions disappeared at the 3 month follow up compared to only 7 of 43 (16%) enhancing lesions. The area of lesions was summarized in Table 7. Most non-enhancing lesions were less than 30 mm² in size. Twenty three (23) of 29 (79%) enhancing and morphological active lesions and 13 of 14 (93%) of the enhancing but morphologically stable lesions were greater than 30 mm² in size. In contrast, only 9 of 19 (47%) non-enhancing morphologically active lesions and 30 of 50 (60%) of the non-enhancing morphologically stable lesions were greater than 30

Table 5. Location, margin, and shape of lesions

		<u>Enhanced</u> (n=43)		<u>Non-enhanced</u> (n=19)		<u>Stable</u> (n=50)	
		<u>Active</u> (n=29)		<u>Active</u> (n=12)		<u>Non-active</u> (n=7)	
		No.	%	No.	%	No.	%
Periventricular	6	21	64	2	17	2	29
Deep white matter	16	55	36	3	25	3	43
GW junction*	7	24	0	7	58	2	28
Margin clear	23	79	11	8	67	5	71
Margin unclear	6	21	3	4	33	2	29
Round and oval	17	59	6	11	92	5	71
Irregular in shape	12	41	8	1	8	2	29
						33	66

* GW junction = junction of gray and white matter

Table 6: Signal intensity of enhancing lesions (n = 43)

Grade	<u>Time Post-injection</u>			
	3 min	(%)	15 min	(%)
0	12	27.9	0	0.0
1	8	18.6	11	25.6
2	13	30.2	12	27.9
3	7	16.3	13	30.2
4	3	7.0	7	16.3

Table 7: Lesion size in 3 groups of lesions

	<30(mm ²) (%)		30--100(mm ²) (%)		>100(mm ²) (%)	
Non-enhanced (n=19)	10	52.6	4	21.1	5	26.3
Enhanced (n=43)	8	18.6	26	60.5	9	20.9
Stable (n=50)	20	40	15	30	15	30

mm² in size. Compared non-enhanced lesions with enhanced lesions, The difference was stastically significant in size less than 30 mm² versus greater than 30 mm² ($p < 0.01$).

3.4.4. Discussion

The findings of this study show that enhancing lesions can be larger although enhancing and nonenhancing lesions usually cannot be distinguished on morphological features alone. The majority of enhancing lesions, 34 of 43 (81%) were greater than 30 mm². Obviously, larger lesions can be easily detected especially if the lesion was just in the enlargement phase when the MR was done. If the MR scan was performed in the phase before the lesion's peak, the lesion would then be enhanced. In other words, a morphologically active but nonenhancing lesion could have been scanned just in the shrinking phase. This point can be indirectly confirmed in this study in which the rate of lesion disappearance was higher (42%) in morphologically active but non-enhancing lesions than with morphologically active and enhancing lesions (16%) on scans followed up to three months.

The frequency of clinically silent MS has been estimated at the 25% rate of those diagnosed clinically [Engell, 1989]. In the silent group, the MS plaques were located mainly in the periventricular areas. In a study by Brownell and Hughes [1962], 40% of MS plaques in the cerebrum were found in the periventricular location in pathology. In contrast, 80 percent of pathologically active lesions were in the deep cerebral white matter or at the gray-white matter junction. On MRI only 20% of the active lesions were periventricular [Koopmans et al., 1989a]. Koopmans and co-workers [1989b] found that 77% of enhancing CT lesions were in the deep white matter or at the gray-white matter junction and only 23% were periventricular. The findings were in accordance with those seen in this study in which only 21% of enhancing and morphological active lesions were periventricular compared with 64% of enhancing but morphological stable ones. On the

other hand, most of the stable and morphologically non-active but enhancing lesions tended to be located in the periventricular region. It was not surprising that 41% of 29 enhancing lesions that were identified on the entry scan were periventricular since the majority (72%) of enhancing but morphological stable lesions were seen on the entry scan. Therefore, enhanced lesions identified on the entry scan may be similar to stable lesions in location and in duration. It is suggested that there may be some differences in pathogenesis and a linking process between the two groups of morphological stable and morphologically active lesions.

Gadolinium enhancement seen on MRI indicated BBB breakdown at the site of inflammatory demyelination. Serial standard MRI showed morphological changes in plaques in which inflammation may play an important role [Hawkins et al., 1990; Kermode et al., 1990]. Some studies [Kato et al., 1989; Juhler et al., 1985; Lossinsky et al., 1989] showed the ultrastructure of the BBB in chronic relapsing EAE in the inactive stage with gliosis and perivascular fibrosis. The basement membrane of the perivascular processes of astrocytes in the demyelinating lesion sites was only partially formed, and neural parenchyma has not fully separated from the perivascular mesenchymal tissues by the EM of astrocytic processes. However, the most important finding was that vesicular transport is increased and mitochondrial content decreased in endothelial cells; tight junctions were opened between endothelial cells; and the interendothelial space was widened when the BBB was disrupted [Claudio et al., 1990; Claudio et al., 1989]. The mechanism of BBB damage in EAE is similar to that in MS. Gay and Esiri [1991] showed a considerable leakage of large plasma proteins from the capillary beds and venocapillary junctions around all the acute plaques examined. Moreover, this process takes the form of an annulus around the plaque edge, a feature of enhancing lesions in which gadolinium leakage is maximal at the plaque periphery and reduced in the plaque center [Kermode et al., 1988]. The appearance of considerable protein leakage in normally myelinated areas around lesions led Kermode, et al to believe that the leakage

per se was not necessarily demyelinating. This finding would suggest that MR images may occur in the absence of any demyelinating lesion. In other words, some morphologically enhancing active lesions can be purely inflammatory and disappear totally and not leaving any trace in MR imaging and be with not demyelination.

The time course of intensity changes was noted in this study. More enhancing lesions were detected when imaged 15 minutes than 3 minutes post-gadolinium. Kermode, et al. [1990a] found that maximum intensity of enhancement was variable occurring from 4 to 120 minutes after contrast injection, the great majority of lesions reaching maximum around 29 minutes. Our finding provides further evidence that scan time post contrast injection not only affects the intensity of enhancement, but also affects number of enhancing lesions detected which is important in designing serial gadolinium studies.

What lesion can be called an enhancing or morphologically active lesion? The enhancing lesion indicates the status of the BBB breakdown which allows cytokines and immune factors to enter and leave the brain freely. If a patient is currently suffering a dramatic change of pathology in a brain lesion, an enhancing lesion can be detected in T₁-weighted imaging post Gd-DTPA. On the other hand, morphologically active lesions, which may or may not be enhanced, suggests that breakdown and recovery of the BBB has occurred. The study of enhancing lesions is important for therapeutic trials because increasing the rate of BBB repair and therefore reducing the number of enhancing lesions may decrease the chance of further loss of neurological function in MS patients. In addition, morphologically stable unenhanced lesions can not be resolved as active. Morphologically active lesions seen on serial MRI can provide information on lesion activity even though the pathological changes are not known.

This study provides evidence of the features of enhancing and non-enhancing lesions on MRI which include larger size lesions, such as more round and oval shape in non-enhancing than that of enhancing lesions. However, distinguishing enhancing from non-enhancing lesions using conventional serial MRI is not possible using only morphological

features. Only enhanced MRI is capable of making this distinction, but may not be necessary in some therapeutic trials (the IFNB Multiple Sclerosis Study Group, 1993).

3.5. The growth pattern of enlarging lesions in MS: observations in serial MRI

3.5.1. Introduction

There are three kinds of active lesions detected in serial T₂-weighted MRI scans: new lesions that had not previously been seen; enlarging lesions that increase in size from a preexisting stable lesion or active lesion; and recurrent lesions that develop at the same site where a lesion had been previously seen. Stable lesions do not change morphologically at follow-up. We had shown in a previous study that enlarging lesions are more similar in size and shape to stable lesions than are new and recurrent ones. Therefore, this study of enlarging lesions can provide new information on both pathological and morphological aspects of MS.

3.5.2. Materials and methods

Analysis of Magnetic Resonance Scans

Researchers, experienced in MRI and masked to the clinical course, identified lesion enlargement. Enlarging lesions were grouped in terms of (1) growth direction of the enlarging lesions, (2) size of the lesion before and after enlargement, (3) the speed of change during the enlargement.

In this study, lesion size before and after enlargement was defined as follows: (1) small lesion: less than 80 mm² in diameter; (2) moderate size lesion: 80 - 300 mm²; (3) large lesion: greater than 300 mm²; (4) confluent lesion: two or more lesions merged together.

3.5.3. Results

Sixty-three enlarging lesions were identified. Before enlargement, 94% (59/63) were stable and 6% (4/63) were new, 63% (40/63) were small, 27% (17/63) medium, and 10% (6/63) were large lesions. After enlargement, 20% (13/63) were still small, 48% (30/63) medium, and 32% (20/63) large. The time from original size to maximum size was 6 weeks in 83%, (47 of the total 63 lesions), 12 weeks in 13% (8/63), 24 weeks in 1% (1/63), and 30 weeks in 3% (2/63). All but 4 were seen in the final scan, 75% (44/59) were smaller on the study subsequent (6 weeks) to the scan in which maximal size was seen. Fading time from maximum size to minimum was variable. 34 (20/59) reached smallest size in 6 weeks, 31% (18/59) in 12 weeks, 23% (14/59) in 18 weeks, 9% (5/59) in 24 weeks, and 3% (2/59) in 30 weeks. In 44% (26/59) the lesions returned to their original size; in 49% (29/59) the lesions were smaller but still larger than were originally seen.

The direction of lesion growth was analyzed. Of the 24 periventricular lesions, 11 (46%) enlarged predominantly away from the ventricle, 3 anteriorly, 2 rostrally, 2 caudally, and 4 equally in all directions. Of the 39 non-periventricular lesions, 11 (28%) enlarged away from ventricles, 8 towards the ventricles, 2 posteriorly, 7 rostrally, 4 caudally, and 7 in all directions. The lesions located in the internal capsules enlarged only rostrally or caudally.

3.5.4. Discussion

Using MRI, active MS lesions were often seen. In general, new lesions reached their peak in 2 - 4 weeks, either receded in 4 - 8 weeks or remained for relatively long periods. New lesions are probably due to BBB breakdown at the site of inflammation. The

inflammatory disturbance may or may not result in demyelination. We believe that the lesions which totally disappear may be only inflammatory in nature.

Enlarging lesions differ from new lesions because they originate from previously stable ones and therefore represent re-activation. The majority of morphologically stable lesions are inactive, however they can enhance as their only sign of activity. It is unclear what signals a stable lesion to become active. New lesions were often seen in the non-periventricular region, round or oval in shape, and small or medium in size. In contrast, enlarging lesions are often seen in the periventricular region, irregular in shape, and larger in size. These characteristics of enlarging lesions are similar to those of stable ones.

The factors that control the pattern of growth in MS lesions are unclear. The process may spread along the course of small veins and venules by direct expansion at the edge of active plaques. Our study provides evidence that MS lesions enlarge asymmetrically more often than concentrically. The enlargement may be along the course of venules or along the projections of white matter tracts as we saw in the internal capsules [Zhao, 1993].

3.6. Sensitivity of gadolinium enhanced MRI and serial MRI in detecting activity of lesions in relapsing-remitting MS

3.6.1. Introduction

MS lesions can be seen to be active in serial standard MRI (S-MRI) studies and with gadolinium enhanced MRI (G-MRI). Active lesions are indistinguishable in appearance, distribution, shape, and size from stable ones if imaged only once using a standard MR scan. Gd enhanced MRI activity is due to blood-brain barrier (BBB) impairment. However, both S-MRI and G-MRI are time consuming and costly. Therefore, finding not only the most sensitive technique but adequate technique for detecting active lesions is important.

3.6.2. Methods

MRI Protocol

The enhanced scans were performed at 3 and 15 minutes after Gd-DTPA administration (0.1 mmol/kg). Pre and post contrast scans were obtained using a TR of 683 msec and TE of 26 msec.

Analysis of Magnetic Resonance Scans

Two of us (G.J.Z. and B.L.T.), experienced in MRI and masked to the clinical course, identified lesions for activity. We identified active lesions as (1) new; (2) enlarging; and (3) recurrent lesions. The grade of enhancement after Gd-DTPA was rated as follows: 0. no enhancement, 1. suspected vague enhancement, 2. faint but definite enhancement, 3. moderate enhancement, and 4. intense enhancement.

3.6.3. Results

308 active lesions were identified on S-MRI. 33 were studied with gadolinium. 14 of 33 (42%) were enhancing and 19 of 33 (58%) were non-enhancing. 29 other lesions were enhancing at entry for a total of 43 enhancing lesions.

Table 8: Location of lesions

Location	Enhanced		Non-enhanced	
	(n = 43)	%	(n = 19)	%
Periventricular	15	34.9	4	21.1
White matter	21	48.8	6	31.6
GW junction ¹	7	16.3	9	47.3

Enhanced vs. non-enhanced

in periventricular vs. non-periventricular² location: $p > 0.2$

1. GW junction = junction of gray and white matter

2. Non-periventricular location = white matter and GW junction

Table 9: Signal intensity of enhancing lesions (n = 43)

Grade	Time Post-injection			
	3 min	(%)	15 min	(%)
0	12	27.9	0	0.0
1	8	18.6	11	25.6
2	13	30.2	12	27.9
3	7	16.3	13	30.2
4	3	7.0	7	16.3

Table 10: Mean area of lesions

	<30(mm ²) (%)		30--100(mm ²) (%)		>100(mm ²) (%)	
Non-enhanced (n=19)	10	52.6	4	21.1	5	26.3
Enhanced (n=43)	8	18.6	26	60.5	9	20.9

Non-enhanced vs. Enhanced in

size of < 30 mm² vs. > 30 mm²: $p < 0.01$

Fourteen of the 43 enhancing lesions (33%) remained stable on follow up by S-MRI. 8 of 19 (42%) non-enhancing lesions disappeared at 3 months follow up compared to only 7 of 43 (16%) enhancing lesions. We found 3 lesions that enhanced when there was no morphological evidence of activity by S-MRI. Morphological enlargement was seen in all lesions at 6 weeks follow up by S-MRI.

3.6.4. Discussion

Gadolinium enhanced MRI can be used as a marker of disease activity, notably breakdown in the BBB and inflammation. Several lines of evidence (pathological, animal experimental and immunocytochemical studies) [Kermode et al., 1991; Thompson et al., 1991] suggest that gadolinium enhancement (BBB disruption) of MS lesions indicates the presence of inflammation. Indirect evidence that gadolinium enhancement indicates inflammation is provided by longitudinal MRI studies [Barkhof et al., 1992], showing that the vast majority of MS lesions display an initial phase of gadolinium enhancement, while traditional pathology tells us that new, active lesions are characterized by inflammation.

Gadolinium enhancement is a time limited feature of MS lesions [Grossman et al., 1988; Miller et al., 1988; Bastianello et al., 1990]. The evolution of gadolinium enhancement in MS lesions in several studies indicates a rather uniform time-frame of BBB disruption and reintegration, despite differences among the studies in patient selection and scanning protocol. Using monthly follow-up MR scans 22% [Miller et al., 1988] to 33% [Harris et al., 1991] of gadolinium enhancing lesions still enhance after 4 weeks. Some of the variance in persistence of gadolinium enhancement reported can be explained by variance in study design.

This study showed that both G-MRI and S-MRI can detect lesion activity, but neither G-MRI nor S-MRI can show all active lesions. S-MRI is sensitive for seeing lesions

change in size, particularly smaller lesions. G-MRI is sensitive for monitoring pathological alteration in the lesion itself, even though there may be no change in morphological criteria. Therefore, S-MRI and G-MRI are complementary methods for detecting active lesions.

3.7. MR detection of multiple sclerosis lesion activity: effects of scanning frequency

3.7.1. Introduction

Clinical evaluation of outcome in therapeutic trials in MS is difficult. The spontaneously variable natural history of MS requires clinical trials to be large and of long duration. Clinical scoring methods are relatively crude and do not accurately reflect the extent of underlying pathology. Serial MRI can detect brain lesion extent and activity in MS, including that of asymptomatic lesions.

Unenhanced serial T₂ and PD MRI is now generally accepted as an important tool for monitoring therapeutic trials. However, the optimum scanning frequency interval is not yet universally agreed upon. The expense of frequent scanning, patient compliance and the natural history of the evolution of MS lesions are all factors that need to be considered when determining the optimum scanning frequency.

In this study we attempted to determine (a) how much MS lesion activity information, as detected with frequent serial MRI (6 weekly) over a 2 year period, is lost if increased scan intervals are used and (b) at what point the treatment effect is lost if scan intervals are increased.

3.7.2. Materials and methods

Patient Population

Forty five of the 50 patients had a cerebral MR scan every 6 weeks for a total of 18 examinations each. Two patients exited from the study after 14 examinations, two patients after 15 examinations, and one patient after 16 examinations.

Analysis of Magnetic Resonance Scans and Patients

Lesion activity was assessed by observers, experienced in MRI and masked to the clinical course of patients, at scan intervals of 6 weeks. Changes in lesion size and number between scans were determined by comparative examination of previous scans. Only changes in lesions that were independently agreed on by all observers were recorded. A lesion enlarging continuously over several examinations was counted as active only once. A lesion seen in adjacent slices was also counted as one active lesion only.

The evaluation was then repeated using only selected scans simulating scan intervals of 12 weeks, 24 weeks, and 48 weeks. We also determined the total number of patients with one or more active lesions for scan intervals of 6 weeks, 24 weeks, and 48 weeks.

Statistical Methods

For continuous variables, treatment-group differences were analyzed using an analysis of variance (ANOVA) based on ranked data. Percent differences were tested by Chi-square.

3.7.3 Results

Thirty eight of 45 patients (84%) had a total 247 active MS lesions. There were 141 new, 77 enlarging and 43 recurrent lesions. Seven patients (16%) did not have any active lesions.

Figure 7 shows the number of total active, new, enlarging and recurrent lesions that were seen with increased scan intervals. Overall a greater number of previously detected enlarging and recurrent lesions had disappeared as compared to the detection of new lesions ($p < 0.001$).

Fig. 7. Percentage of Active Lesions Versus Scan Interval

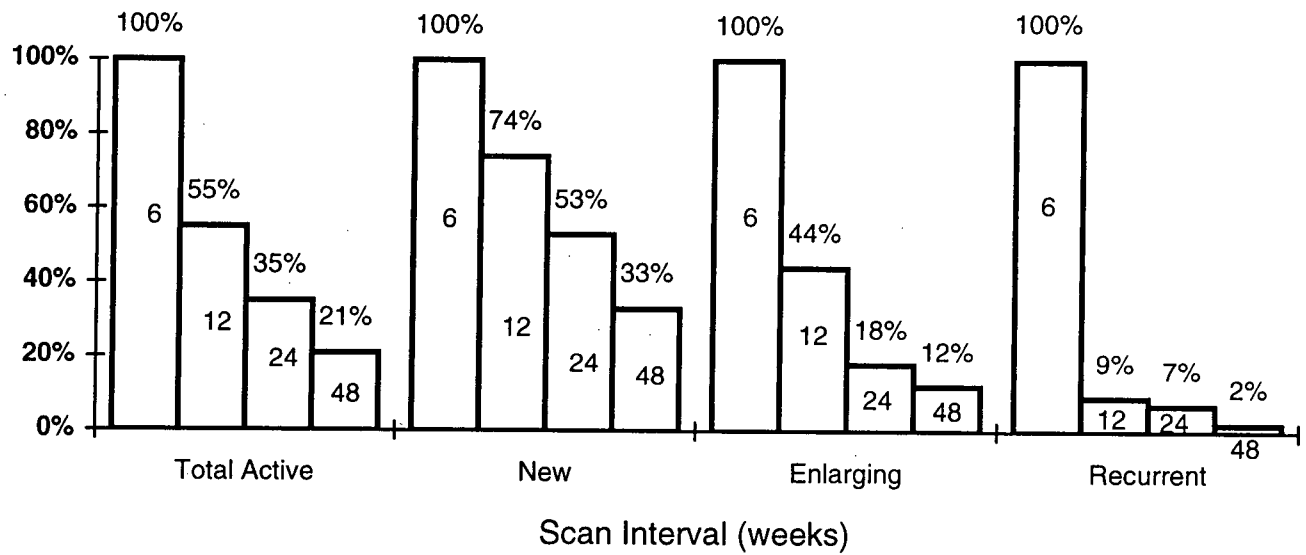
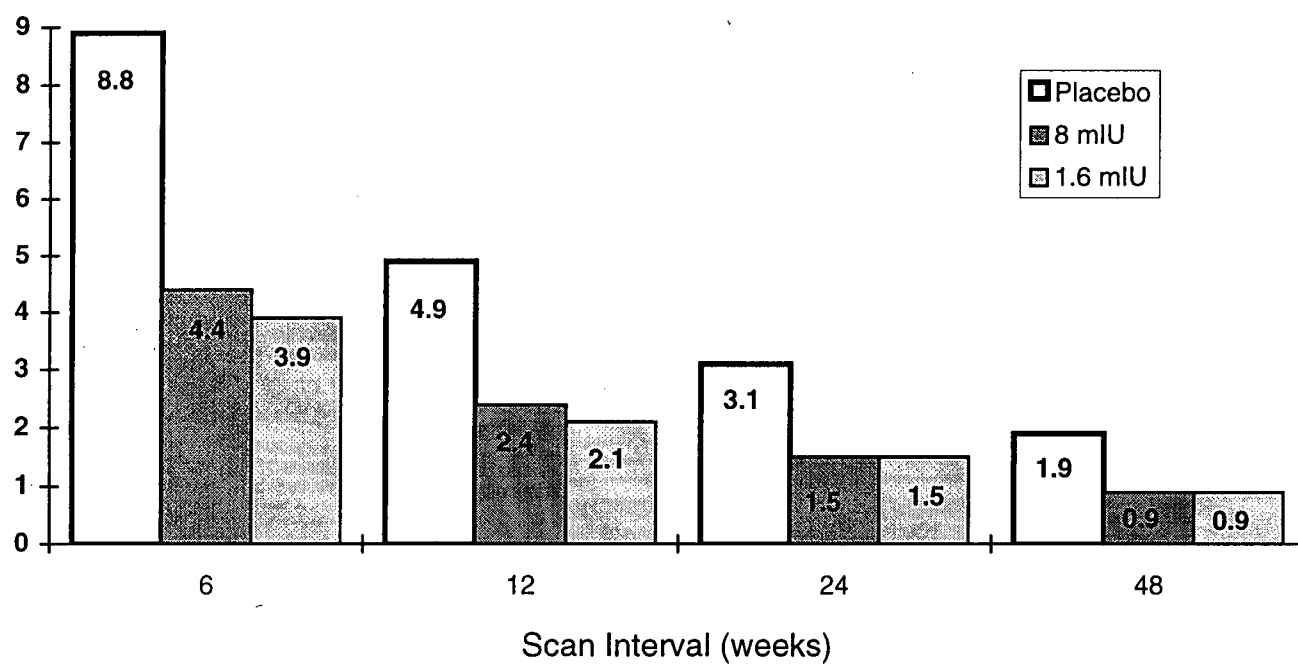


Fig. 8. Mean Number of Active Lesions in Placebo Versus Treated Patients



As previously reported [Paty, et al. 1993], over the 2 years both high and low dose IFNB showed a steady reduction of lesion activity compared to placebo ($p < 0.001$). With longer scan intervals this treatment effect remains, however, it lost statistical significance with a scan interval longer than 48 weeks. (6 week interval, $p = 0.0294$; 12 week interval, $p = 0.0324$; 24 week interval, $p = 0.0302$; 48 week interval, $p = 0.1103$) (figure 8).

3.7.4. Discussion

Previous longitudinal MRI scanning studies of MS show that disease activity patterns vary according to the clinical course. Isaac et al [1988] and Willoughby and coworkers [1989] reported disease activity in relapsing remitting MS, defined as the presence of new and/or enlarging lesions on biweekly follow-up non-enhanced MRI scans in 17 RR patients. Most of the MRI activity in that study was due to new lesions (83%). Subsequently in a serial MR study of the chronic progressive phase of MS, Koopmans and coworkers [1989a] reported a much more complex pattern of disease activity in 8 patients. In these patients most of the lesion activity (71%) was due to enlarging and recurring lesions and only 29% was due to new lesions.

New lesions typically enlarged over 2 to 4 weeks with subsequent decrease in size over 4 to 6 weeks. The lesions usually left a small residual abnormality, but some of the smaller lesions faded entirely, leaving no definite trace. This is probably because the residual lesions are too small to be identified (beyond the resolution of the scanner). There is also a suggestion that more lesions disappear when thick (1 mm) slices are used on low field strength machines. The pattern of change in enlarging lesions was similar with a rapid increase to maximum size in about 4 weeks followed by a more gradual decline over a further 4 to 8 weeks leaving a residual abnormality. Recurrent lesions developed from previously stable, new, or enlarging lesions. Their changing pattern was similar to enlarging lesions.

Serial unenhanced MRI can play an important role for monitoring therapeutic trials of MS. Enhanced MRI will show additional activity events as well. When evaluating new therapies for MS it is important to state the MRI outcome measure to be used. The expected outcome of a beneficial drug therapy for MS may include a decrease in the overall number of active lesions and less active scans or fewer patients with active disease in the treatment group.

The major findings of this study were as follows: 1). Scanning at greater than 6 week intervals reduced the detection of active MS lesions. This decrease in activity was disproportionately more for enlarging and recurrent lesions than it is for new lesions. 2). The reduction in number of active lesions caused by increased scanning intervals was seen in all three groups (placebo, 1.6 MIU and 8 MIU IFNB) and was not significantly different among the groups. The treatment effect significance was lost when the scan interval was longer than 48 weeks. It is remarkable that a significant treatment effect could be seen with such a small sample size, using unenhanced MRI, at a scanning interval of 24 weeks.

Our results have important implications for designing future therapeutic trials in which serial MRI is used to measure treatment outcome. For instance, if one is studying patients with early RR-MS (in whom new lesions is most important for assessing disease activity) one may obtain adequate activity levels at scanning interval of 24 weeks. However, in RP MS patients (in whom enlarging and recurring lesions are important for assessing disease activity) more frequent scanning is a prerequisite.

These findings suggest that the optimal scanning interval must vary depending on how the optimal treatment outcome is defined (number of active lesions or number of patients with active disease) and whether new or chronic MS patients are being studied.

3.8. Corticospinal tract lesions in multiple sclerosis: relationship between MRI activity and clinical course

3.8.1. Introduction

MS lesions can be seen in any part of the CNS, including the corticospinal tract (CST). Active lesions which differ from stable ones in pathology, distribution, and shape can be identified on serial MRI studies. The CST is a major descending pathway for controlling voluntary movement. We have examined the correlation between active lesions in the CST and clinical findings.

EDSS is a clinical standard for determining neurologic impairment in MS. Poor but significant correlation between MRI lesions and the clinical status of the MS patients was found in previous studies [Issac et al., 1988; Willoughby et al., 1989; Koopmans et al., 1989; Harris et al., 1991; Thompson et al., 1992; Wiebe et al., 1992]. The possible reasons for this lack of correlation between clinical and conventional brain MRI findings may be that there were only small numbers of patients in the studies [Harris et al., 1991; Capra et al., 1992]; the follow up was too short [Issac et al., 1988; Willoughby et al., 1989; Harris et al., 1991; Capra et al., 1992]; on the scan timing was not consistent with the appearance of active lesions so as to miss the time of blood-brain barrier breakdown in the active MS lesions [Kermode et al, 1990; Youl, 1991]. Furthermore, MS lesion location and size are very important factors as well and should be considered in the study analysis [Zhao et al., 1993; Plant, 1992].

This study, as part of the IFNB (Betaseron®) therapeutic trial [The IFNB Multiple Sclerosis Study Group, 1993; Paty et al., 1993], was carried out with 50 MS patients at the University of British Columbia. Patients were scanned every 6 weeks for 2 years. The study was to reveal (1) the dynamics of mostly asymptomatic active lesions; (2)

effectiveness of IFNB on MRI active lesions, and (3) correlation of MRI quantitative measure and clinical measure by EDSS and the correlation between location of active MRI lesions and clinical signs of activity.

3.8.2. Materials and methods

Analysis of Magnetic Resonance Scans

Lesions were identified by experienced and masked observers. Active lesions were identified as (1) new lesions; (2) enlarging lesions; and (3) recurrent lesions.

According to active lesion location and any preexisting, inactive lesions, patients were divided into 3 groups: (1) active lesions involving the CST above the pons only; (2) active lesions involving the CST above the pons with preexisting lesions in the brainstem and CST; (3) active lesions at other sites not in the CST. After all active lesions were identified, clinical data was reviewed.

In this study, lesion size was defined as (1) small, less than 1 cm in diameter; (2) moderate size, 1 - 2 cm; (3) large, greater than 2 cm; (4) confluent (see section 3.5.2).

The active lesions associated with relapse were those which were detected in the scans just before or after 2 weeks of exacerbation.

Clinical Measurement

A relapse was to have occurred with a worsening of an old symptom or an appearance of a new symptom, attributable to MS; accompanied by an appropriate new neurologic abnormality; lasting at least 24 hours in the absence of fever; and preceded by stability or improvement for at least 30 days.

EDSS score was used to measure the disability. The progression of disability was defined as the persistent increase of one or more EDSS points.

3.8.3. Results

Eleven MRI active lesions were identified in group 1, associated with 5 clinical relapses, all with an appropriate motor deficit (table 11). Fourteen MRI active lesions were seen in group 2 with 5 clinical relapses (2 motor, 2 visual, and 1 sensory) (table 12). Thirty active lesions were in group 3 with 8 relapses, none except one of which were appropriately motor in nature (table 13). Both active lesions and relapses were followed up by appropriate serial examinations in this study. The difference was statistically significant when the motor deficit rate in group 1 was compared with the rate in group 3 ($p < 0.05$).

Of 11 active lesions in group 1, 5 (45%) active lesions were small; 6 (55%) active lesions were medium sized, 4 (36%) of the medium lesions were active with relapses. Ten (10) of the total of 14 active lesions in group 2 were medium sized or large and 4/14 were small. 4/10 medium sized and large lesions were active with relapses. Only 1 of the 4 small lesions was correlated with clinical relapses. In group 3, 18/30 active lesions were small and 12/30 were medium sized or large. 4/12 medium or large lesions were active with relapses and 4/18 small lesions were active with relapses, but none of the clinical relapses were appropriate to the CST.

Table 11. Active lesions involving CST above the pons (Group 1)

<u>Active Lesion</u>	<u>CST Lesion Size & Side*</u>	<u>Type of Relapse</u>	<u>MRI Follow-up (weeks)</u>		<u>(Clinical Follow-up (weeks)</u>		<u>Clinical Appropriate to The CST</u>
			<u>Lesion size</u>	<u>Time</u>	<u>Type</u>	<u>Time</u>	
1	Rt. S	Lt. weakness	no change		improved	5	+
2	Lt. M	dysarthria	decrease	6	improved	3	+
3	Lt. M	Rt. leg very weak	disappear	6	improved	6	+
4	Lt. M	Rt. leg weakness	no data (final scan)		gone	15	+
5	Rt. M	Lt. leg weakness	disappear	6	gone	4	+

* S, small lesion in size; M, medium lesion; Rt, right; Lt, left

Table 12. Active lesions involving the CST above the pons with preexisting lesions in the brainstem (Group 2)

Active Lesion	CST Lesion Size & Side*	Type of Relapse	MRI Follow-up (weeks)		(Clinical Follow-up (weeks)		Clinical Appropriate to The CST
			Lesion size	Time	Type	Time	
1	Rt. M	unstable gait	decreased	6	improved	3	+
2	Lt. S	decreased visual acuity	decreased	6	gone	2	-
3	Lt. L	Rt. leg numbness	no data (final scan)		gone	4	-
4	Lt. L	Rt. weakness	decreased	18	improved	13	+
5	Lt. L	decreased visual acuity	decreased	6	gone	2	-

* S, small lesion in size; M, medium lesion; L, large lesion; Rt., right; Lt., left

Table 13. Active lesions not in the CST (Group 3)

Active Lesion	CST Lesion Size & Side*	Type of Relapse	MRI Follow-up (weeks)		(Clinical Follow-up (weeks)		Clinical Appropriate to The CST
			Lesion size	Time	Type	Time	
1	Lt. S	Rt. weakness	gone	6	improved	4	+
2	Rt. M	decreased visual acuity	decreased	6	improved	5	-
3	Lt. M	Rt. arm numbness	decreased	6	gone	7	-
4	Rt. L	Lt. arm numbness	decreased	12	improved	6	-
5	Rt. M	Lt. numbness	decreased	6	improved	3	-
6	Rt. S	Lt. facial numbness	decreased	6	gone	12	-
7	Lt. S	Rt. leg numbness	no change		improved	6	-
8	Lt. S	decreased visual acuity	gone	12	gone	5	-

* S, small lesion in size; M, medium lesion; L, large lesion; Rt., right; Lt., left

3.8.4. Discussion

MS is a disorder characterized by recurrent multifocal inflammation and demyelination within the CNS. CNS dysfunction is usually attributed to conduction block secondary to changes in membrane electrical properties resulting from demyelination [Waxman, 1982]. However, alterations in axons also occur [Barnes, 1991; Matthews, 1991]. The number of axons that traverse a plaque can be reduced to only a small fraction of those originally present and these remaining axons may be thin and exhibit structural abnormalities [Raines et al., 1989]. MRI can detect the natural history of pathology. Our previous MRI studies had found that active lesions can come and go at any time. The new lesions reach their peak in 2 - 4 weeks, either recede in 4 - 8 weeks or remain for relatively long periods. The active lesions are often seen in the deep cerebral white matter and at the gray-white matter junction. Gadolinium enhancing lesions are frequently seen which are considered to represent blood-brain barrier breakdown at the site of inflammation. The inflammatory disturbance may result in demyelination whereas the lesions which totally disappear may be only inflammatory in nature. Our follow-up in active lesions has found a correlation between neurological deficit and location of individual lesions in the corticospinal tract.

The results from this study provide evidence that the active lesions associated with clinical exacerbations were larger than those not associated with exacerbation. These lesions associated with exacerbations also left larger abnormal areas in follow-up scan after 6 months. They, to the most extent, became stable and rarely were re-active. Pathologically, most stable lesions have a rather acellular appearance, consisting of a fibrillary astroglial feltwork that is responsible for the sclerosis, color, and texture of the tissue [Matthews, 1991]. Lesion location and size are important factors in the resulting neurological dysfunction. Large active lesions, if located in the course of corticospinal

tracts and postchiasmal visual pathways, were likely to cause corresponding neurological defects in MS [Zhao et al., 1993; Plant et al., 1992]. This study supports the conclusions and provides further information between lesion activity and clinical status.

In the present study of 50 patients followed for 2 years, a relationship between lesion location and activity in serial MRI and clinical relapses has been seen. The evidence provided in this study is that the many of clinical relapses were associated with medium sized or large lesions. If a lesion was large and/or located in a functional area such as CST, there was a high possibility of neurological symptoms. The same correlation was also observed in previous studies [Filippi et al., 1995] and a recent study [Khouri et al., 1994]. In the study of 281 patients with MS, we had observed two conventional T2-weighted brain MRI scans, separated by an interval of 24 to 36 months. At the time of each scan, clinical disability was rated using the EDSS. There was a significant correlation between the number of new or enlarging lesions and increasing disability in the RR MS [Filippi et al., 1995]. This result supports the present study.

The larger total active area in scans associated with relapses provides evidence that accumulation of destructive pathological changes over time can result in symptoms. Lesion location is very important as well. Symptoms may not occur in patients if an active lesion occurs in a silent area even if the active lesion area is large. However, if a lesion is of considerable size and located in a functional area, symptoms will occur with a higher probability. This study supports the hypothesis that active lesions located in the course of the CST are more likely to be associated with a motor deficit than are other active lesions [Zhao et al., 1993].

In conclusion, the present study has revealed a positive relationship between active lesions seen in the CST on MRI and clinical signs. Large lesions are also more likely than small lesions to be expressed clinically. This result shows that brain serial MRI is a useful supplementary marker of disease activity with clinical correlation in clinical treatment trials in MS.

Chapter 4

Using Lesion Activity as An Outcome Measurement to Monitor Therapeutic Trials in MS

4.1. Introduction

MRI is the most sensitive scanning technique for the demonstration of MS lesions in the CNS and is an objective quantitative outcome measure for assessing the response of MS patients in a therapeutic trial. MS is a common neurologic disease, clinically characterized by the presence of symptomatic lesions in the CNS and by progressive neurologic impairment. Serial MRI has revealed a high rate of lesion activity in MS. Active lesions, which are often silent, occur at a significantly higher frequency than do clinical relapses.

MRI provides the method for monitoring burden of disease as well as lesion activity as outcome measurements. Burden of disease accumulates with active lesion appearance. The hypothesis in a therapeutic trial is that the drug slows or stops the progression of MS if it can slow or stop lesion activity. We previously reported a 2 year interim MRI analysis, using burden of disease measurement, which showed IFNB was effective [The IFNB MS Study Group, 1993; Paty et al., 1993]. In a five year study, using yearly MRI, the positive effects of IFNB on clinical outcome and MRI burden of disease measurement were documented [The IFNB Multiple Sclerosis Study Group and the UBC MS/MRI Analysis Group, submitted, Koopmans, Zhao et al., submitted]

The present study is to examine the efficacy of IFNB treatment by an independent MRI analysis of MS lesion activity based on 6 week MRI scans of our 50 Vancouver patients for a total of 2 years.

4.2. Materials and methods

Patient Population

We studied 50 patients with clinically definite relapsing remitting MS who had been entered into a therapeutic trial using beta-interferon. There were 12 men and 38 women whose ages ranged between 22 and 53 years (mean, 38.6 years). Mean duration of disease was 8.2 years (range, 1.1 to 20.0 years; SD = 5.0 years). Disability of patients was between 0 and 3.5 (mean, 2.0, SD = 1.0) on the EDSS. All patients had an abnormal cerebral MRI characteristic of MS, with scattered areas of increased intensity on the spin-echo (SE) sequence. Patients were randomized into three treatment arms: Placebo (N=17), 1.6 mIU (N=17), 8 mIU (N=16) IFNB self-administered subcutaneously every other day.

Each patient had a cerebral MR scan every 6 weeks for 2 years. Clinical examination was also done on patients every 6 weeks (detailed description on clinical examination and method of patient distribution into three treatment arms seen in our reports [The IFNB Multiple Sclerosis Study Group, 1993; Paty, 1993]).

MR Scan Technique

MR scans were performed using a Picker International Cryogenic MR 2000 scanner with a superconducting magnet operating at a static magnetic field strength of 0.15 Tesla and a 30 cm diameter receiver coil in Vancouver Hospital and Health Science Center, University of British Columbia Site. Twelve contiguous 10 mm thick axial slices were obtained through the brain from the upper cerebral hemisphere to the medulla. The

inplane resolution was 1 mm. A dual echo SE sequence was used with echo delay times (TE) of 60 and 120 msec and a repetition time (TR) of 2133 msec.

The same receiver coil (30-cm diameter) was used for each patient. The repositioning error was minimized through alignment of external and internal landmarks. Initial repositioning was based on two angles from external landmarks (the canthomeatal line and the nasion-tragal line). A midline sagittal slice (pilot) was then obtained and the head position verified using an internal angle (angle between top of the cerebellum and the anterior sphenoid sinus). If this angle differed by more than 2 degrees from the baseline study, the patient was repositioned and the pilot scan repeated. Slices for the actual scans were then programmed so that the middle slice of a simultaneous 12-slice series was centered on the top of the cerebellum. Halfway through an individual scanning session, the pilot scan was repeated to ensure that positioning was still adequate. If movement had occurred, the patient was repositioned and the appropriate sequence repeated.

Quantitative Analysis of Magnetic Resonance Images

An interactive computer program was been designed to display MR images and permit manual tracing of lesions. The lesion borders were outlined on a computer monitor by one technician and checked by a single radiologist in order to reduce interobserver variation. The area of the individual lesions could then be determined and analyzed. The lesion areas were summed slice by slice for a total lesion area and recorded in mm².

Lesions were identified for activity. Changes in lesion size and number between scans were determined by 3 observers by comparative examination of previous scans. Only changes in lesions that were independently agreed on by all observers were recorded. A lesion enlarging continuously over several examinations was counted as active only once. A lesion seen in adjacent slices was also counted as one active lesion only. Active lesions were defined as (1) new when a new lesion seen was identified; (2) enlarging when a previously stable lesion demonstrated increases in size; (3) recurrent

when a lesion reappeared in the same location as one that had previously faded. An active scan was a scan in which active lesions were detected. Active lesions were marked on the films by radiologists and outlined using our computer program on a monitor by a trained technician. Total active area was summed slice by slice. The total active area was defined as the summed area of all active lesions found in all previous scans.

Statistics

As sample averages and medians were similar, descriptive statistics were reported as medians, means, and standard errors (SEs). ANOVA in excel software was reported for testing p values.

4.3. Results

In 50 MS patients, 287 active lesions were detected, in which 63% (181/287) were new, 27% (77/287) enlarging, and 10% (29/287) recurrent. Significant treatment group differences were detected between the placebo and both treatment groups for the active lesion rate in MS patients. The 8 MIU group had a median reduction rate of 83% (Table 14).

The annual rate of new lesions was also significantly lower for both the 8 MIU and 1.6 MIU treatment groups than for the placebo group. The 8 MIU group showed a median reduction of 75% in the rate of new lesion formation (Table 15). We examined the effect of treatment on enlarging and recurrent lesions. No significant differences in the rate of enlarging lesions were observed among the three treatment groups (Table 16). There was no overall significant treatment-group difference in the recurrent lesion rate (Table 17). No significant difference between the placebo and 8 MIU groups was detected.

There were 182 active scans. The percent of scans with activity showed a significant difference between the placebo and the treatment groups (Table 18). The 8 MIU group had a median of 80% fewer active scans than were present in the placebo arm. Mean cumulative percent of active scans per patient per scan week showed a significant difference as well (Figure 9).

Some new lesions come and go totally, but many of them leave a small abnormal area. Of the 139 lesions followed for 36 weeks, 28 out of 139 (20%) were in the 8 MIU group, 82% (23/28) of them disappeared. 32 out of 139 active lesions (23%) were in the 1.6 MIU group and 59% (19/32) of them resolved totally. 79 of the total lesions (57%) were in the placebo group and 59% (47/79) of them disappeared.

It is difficult to determine active lesions on a single conventional unenhanced MRI scan. The first scan in a serial study is used as a baseline to compare with follow-up scans. Therefore, the first active lesion in this study could be detected only as a change in the second scan. The total active area starting with the second scan was accumulated scan by scan for a total of 17 scans. The difference in rate between high dose arm and placebo arm was statistically significant (8 MIU vs. Placebo, $p = 0.0408$; 1.6 MIU vs. Placebo, $p = 0.3702$). The means of the MRI cumulative active area is seen in Figure 10.

Table 14. Active lesion rate in Vancouver patients (N = 50)

Measurement	Statistic	Placebo	1.6 MIU	8 MIU
Active lesion per year	Mean	4.9	1.8	2.0
	Median	3.0	1.0	0.5
	SE	1.3	0.4	0.7
Overall:		$p = 0.0234$		
Placebo vs. 8 MIU		$p = 0.0089$		
Placebo vs. 1.6 MIU		$p = 0.0412$		
1.6 MIU vs. 8 MIU		$p = 0.5070$		

Table 15. New lesion rate in Vancouver patients (N = 50)

Measurement	Statistic	Placebo	IFNB	
			1.6 MIU	8 MIU
New lesion per year	Mean	3.2	1.1	1.2
	Median	2.0	0.5	0.5
	SE	0.9	0.2	0.9
by ANOVA				
	Overall:	$p = 0.0085$		
	Placebo vs. 8 MIU	$p = 0.0026$		
	Placebo vs. 1.6 MIU	$p = 0.0317$		
	1.6 MIU vs. 8 MIU	$p = 0.3207$		

Table 16. Enlarging lesion rate in Vancouver patients (N = 50)

Measurement	Statistic	Placebo	IFNB	
			1.6 MIU	8 MIU
Enlarging lesion per year	Mean	1.2	0.7	0.6
	Median	1.0	0.0	1.0
	SE	0.7	0.5	0.4
by ANOVA				
	Overall:	$p = 0.2484$		
	Placebo vs. 8 MIU	$p = 0.1441$		
	Placebo vs. 1.6 MIU	$p = 0.2501$		
	1.6 MIU vs. 8 MIU	$p = 0.7177$		

Table 17. Recurrent lesion rate in Vancouver patients (N = 50)

Measurement	Statistic	Placebo	IFNB	
			1.6 MIU	8 MIU
Recurrent lesion per year	Mean	0.7	0.3	0.4
	Median	1.0	0.0	1.0
	SE	0.4	0.2	0.2
by ANOVA				
	Overall:	$p = 0.1648$		
	Placebo vs. 8 MIU	$p = 0.2970$		
	Placebo vs. 1.6 MIU	$p = 0.0945$		
	1.6 MIU vs. 8 MIU	$p = 0.3273$		

Fig. 9. Mean Percent of Patients with Active Scans Per Scan Week

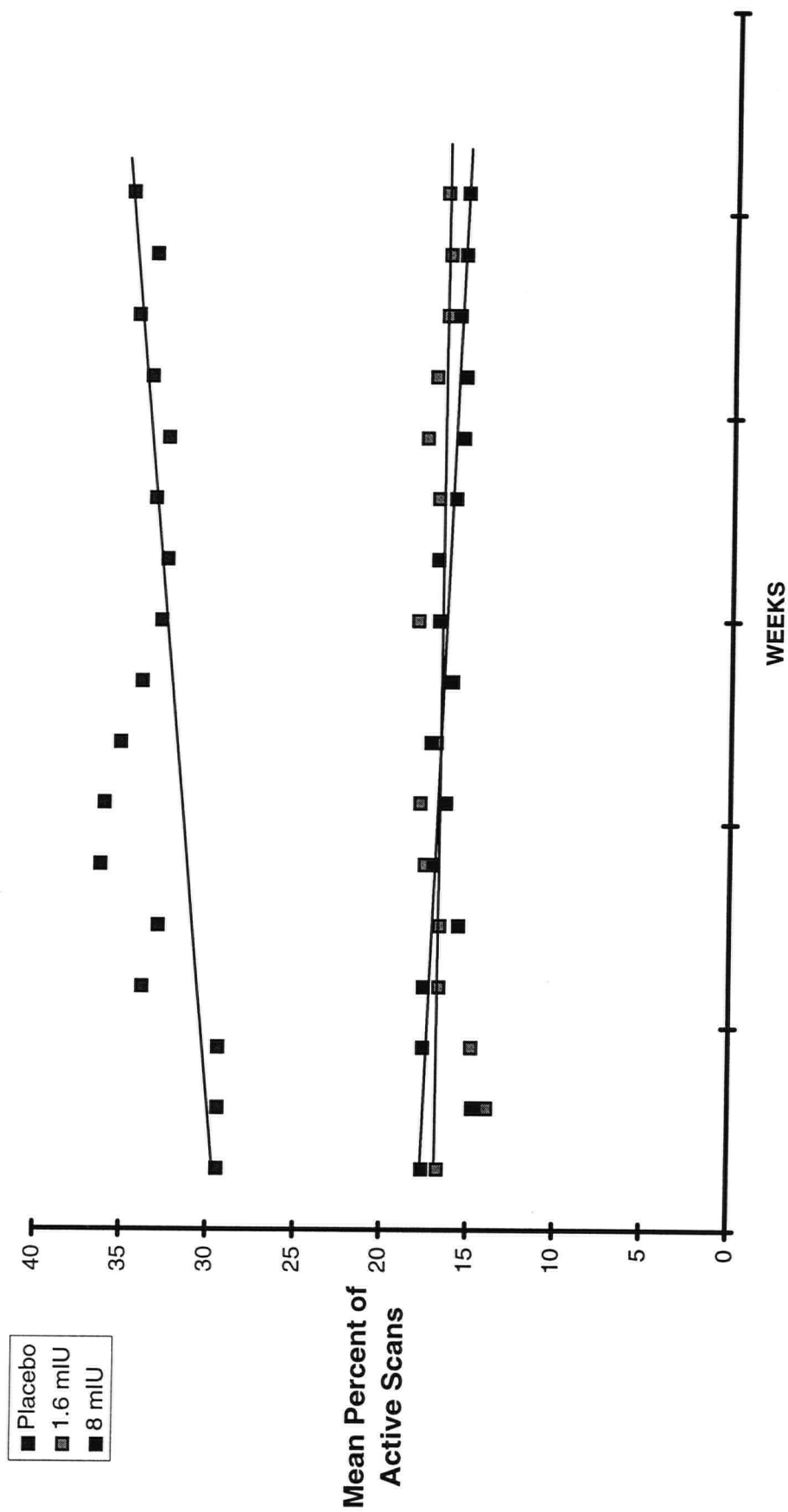


Figure 10. Means of Cumulative MRI Active Area in the Vancouver Cohort over 2 Years

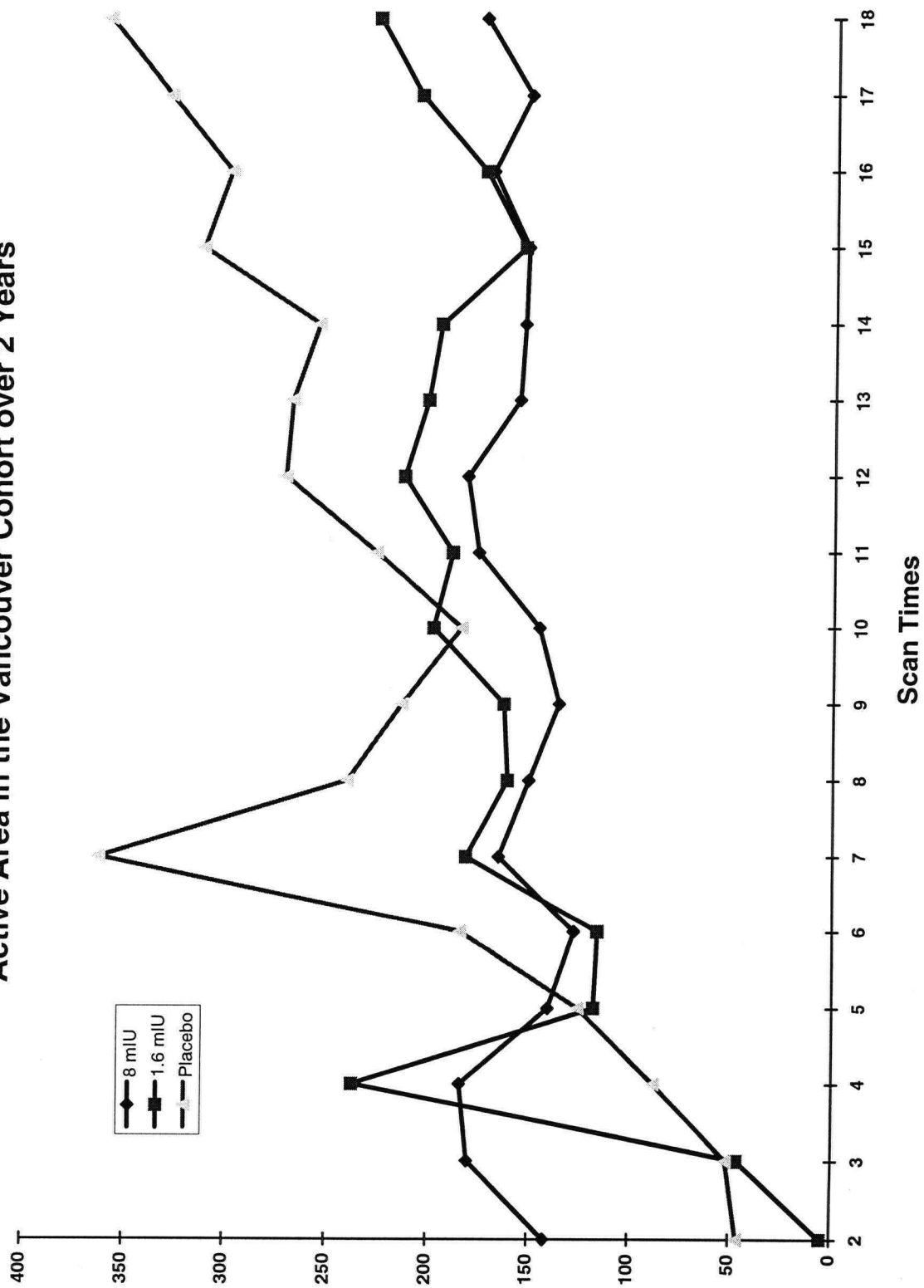


Table 18. Percent of scans with activity in Vancouver patients (N = 50)

Measurement	Statistic	Placebo	IFNB	
			1.6 MIU	8 MIU
Percent of active scans	Mean	34.6	17.0	15.4
	Median	29.4	11.8	5.9
	SE	6.0	3.8	4.5
by ANOVA				
	Overall:	$p = 0.0170$		
	Placebo vs. 8 MIU	$p = 0.0062$		
	Placebo vs. 1.6 MIU	$p = 0.0349$		
	1.6 MIU vs. 8 MIU	$p = 0.4692$		

4.4. Discussion

Natural interferon beta (IFNB) is produced by fibroblasts, and probably by many other cells, if stimulated with synthetic or viral oligonucleotides. Human IFNB consists of a single molecular species that is a glycoprotein. Its gene is encoded on chromosome 9, and it reacts with a cell surface receptor in common with IFN alpha [De Maeyer & De maeyer-Gignard 1988; Panitch, 1992; Clanet M, 1989]. Interferon beta has been reported to be effective in the treatment of RR MS [The IFNB MS Study Group, 1993; Paty et al., 1993]. As part of an interferon beta therapeutic trial, this study provided further evidence

of the drug's effectiveness. The cumulative active area on MRI in the placebo arm increased much faster than those in the treatment arms (fig. 10). There was also a higher proportion of active lesions associated with relapses in the placebo arm than those in the treatment arms. The cumulative active areas in MRI included both new appearing lesions and the lesions which were seen as active ones in all previous scans. Therefore, the cumulative pathology contained the edema, inflammation, demyelination, and/or axon loss.

Measurement of new lesions is important for a therapeutic trial. Increase in lesion load relies mainly on the appearance of new lesions and enlargement of previously stable lesions. In another study [Zhao et al., in preparation] a higher percentage of new lesions was detected in patients with lower initial lesion load. More enlarging lesions were detected in patients with higher initial lesion load. The lower lesion load group had a faster increase in percentage of lesion load and the higher lesion load group had a greater absolute increase in actual lesion load. IFNB decreased both the lesion activity and slowed down the progress of MRI detected pathology.

In addition, using yearly MRI over 5 years [Koopmans, Zhao et al., submitted], we examined the efficacy of IFNB by an independent MRI analysis of yearly MS lesion activity. The data supported the present results, that IFNB decreased MS lesion activity and total lesion load.

The serial MRI data further confirmed the clinical study [The IFNB MS Study Group, 1993] showing a significant reduction in disease activity. The serial MRI data provide a sensitive and objective method to measure lesion activity. By using the frequent MRI method and fewer patients, future therapeutic trials and dosage trials in MS can be shorter in duration and less expensive.

In summary, lesion activity measurement is a sensitive and objective method by which we can monitor a therapeutic trial.

Chapter 5

Future Application of MRI for the Understanding of MS Pathology

5.1. MR Spectroscopy in Multiple Sclerosis

Whereas MRI is an anatomic imaging modality in a long tradition of anatomic imaging techniques in radiology, MR Spectroscopy (MRS) is unique. It is one of few techniques in clinical medicine that provide noninvasive access to living chemistry in situ. In vivo MRS can be used to measure relative concentrations and mobilities of different low-molecular-weight chemicals. Proton MRS can be used to monitor metabolites such as choline, creatine, *N*-acetylaspartate (NAA), mobile lipids, and lactic acid. In vivo MRS can be used to measure relative concentrations and mobilities of different low-molecular-weight chemicals [Gadian et al., 1982].

The lipid-methylene protons resonate at 1.2 ppm in the proton spectrum and contain mainly contributions from triglycerides and other mobile lipids. Lipid metabolism is important in the study of demyelination because the myelin sheath is composed of a lipid-bilayer membrane. Histopathologic techniques have allowed researchers to study lipid changes during demyelination because the lipids stain differently depending on their structural and biochemical state. These studies have shown that abnormal lipid droplets that form during demyelination are a sign of an irreversible lesion. The signal intensity of membrane lipids in MRS is influenced by the fluidity of the membrane structure, which depends on temperature, cholesterol content, lipid composition, lipid-water interactions, and membrane proteins. The proton spectrum of normal brain has little (if any) lipid

signal [Frahm J et al, 1989] because the phospholipids in cell membranes and in myelin have limited mobility. However, it is hypothesized that as myelin degrades and the membrane structure changes, the lipids become less ordered and thus become able to give a detectable MR spectroscopic signal.

The *N*-methyl groups of choline resonate at 3.2 ppm in the proton spectrum in vivo and contain contributions mainly from phosphorylcholine and glycerophosphorylcholine (GPC), which are involved in lipid metabolism. Phosphorylcholine is a precursor molecule that is incorporated into the polar head group of phosphatidylcholine and sphingomyelin [Tunggal B, et al. 1990]. These phospholipids are then incorporated into cell and myelin membranes of the CNS. GPC is a metabolite of the breakdown of phosphatidylcholine. Unfortunately, all the *N*-methyl groups from the choline-containing compounds resonate at the same frequency. The contribution of phosphatidylcholine to this resonance is unknown. Phosphorylcholine and GPC are small molecules with long T₂ relaxation times, whereas phosphatidylcholine is a large molecule with a relatively short T₂ relaxation time that is made even shorter by being incorporated into the highly ordered myelin membrane. However, recent evidence suggests that phosphatidylcholine contributes to the proton MR choline signal [Miller DL, et al. 1991].

The methyl group of *N*-acetylaspartate (NAA) has a sharp resonance at 2.0 ppm in the proton spectrum of the brain. The exact role of NAA in brain tissue is still unclear. Most researchers agree that NAA is a marker for neurons and may have a role in neuronal metabolism [Birken DL, 1989, Gill SS, 1989]. Although some researchers claim that NAA is involved in myelin production, this seems to be contradicted by the fact that NAA is at a much higher concentration in the neurons than in the glial cells [Birken DL, 1989, Gill SS, 1989]. The NAA resonance has been of interest in the study of MS and other brain diseases because of the decrease in NAA:creatine and NAA:choline ratios localized to brain lesions. This decrease in NAA has been attributed to axonal loss [Arnold DL, 1990], active degradation of NAA in injured neurons [Bruhn H, 1989], and

gliosis [Van Hecke P, 1991]. Although MS is primarily a demyelinating disease, injury to neurons becomes more apparent with decade-long progression of the illness, possibly because of the metabolic relationship between axons and myelin-forming oligodendroglia. Along with the injured neurons, the normal-appearing "healthy neurons" may be influenced by the pathologic materials arising from inflammatory foci and the altered BBB.

MS patients have been studied with in-vivo proton spectroscopic techniques in which the spectroscopic localized volume was placed over a region of brain that included a plaque defined by MRI. The main findings have been a decrease in NAA/creatine and an increase in choline/creatine. In some cases, the decrease in NAA did not depend on relative plaque volume inside the spectroscopic localized volume, suggesting that the disease extended into normal-appearing white matter surrounding the active chronic MS plaques. Acute (edema, inflammation) and chronic (demyelination, gliosis) MS plaques appear to have different proton spectra, potentially allowing the number of irreversible chronic plaques in a patient to be determined spectroscopically. The hypothesis is that metabolite ratios are unchanged in hyperacute (edema only) plaque, that demyelination in acute plaques is accompanied by an increased choline/creatine ratio, and that irreversible plaques in the subacute to chronic stage have a decreased NAA/creatine ratio.

Some claims have been made of lipid increases in MS plaques [Koopmans, et al., 1990; Wolinsky, et al., 1990]; however, one of these investigators admits the possibility of lipid contamination from scalp and bone marrow [Koopmans, et al., 1990]. Other proton metabolites that have been reported to change in MS are lactate and inositol.

It is now possible to measure the proton metabolites in patients with MS with spectroscopic imaging techniques. These studies have added insight into the distribution of metabolite concentration within a brain lesion. den Hollander et al. [1987] reported that NAA and choline are not uniform over the whole area of the MS plaques and that certain

plaques have foci of high concentrations of choline or lactate. They hypothesized that these foci may indicate a different stage of the pathology.

The ability to differentiate between early reversible lesions and irreversible lesions would be useful in planning treatment of MS. Some MS lesions are purely edematous or inflammatory and should respond to corticosteroids and other anti-inflammatory drugs. These early lesions should be detectable by an increased intensity on T2-weighted MRI and no changes in proton metabolites on MR spectroscopy. However, as MS progresses, lipids in macrophages and mobile lipids increase months or years later, myelin lipids are lost and are replaced by astrocytic gliosis (decrease in NAA). These older lesions most likely will not respond to anti-inflammatory treatment. Thus, proton MRS along with MR imaging could be helpful in distinguishing those early lesions that might respond to therapy.

5.2. Summary and future directions

MRI has become the most prominent imaging modality for lesions of the CNS. The many tissue and machine parameters involved in the image formation, give MRI a unique capability to separate different healthy tissues and to identify pathological features.

It is clear that MRI has made a major impact in the diagnosis of MS and in monitoring therapeutic trials. It is also possible to compare the site of lesions identified on MRI with the clinical condition of the patient, to establish the "lesion load", the number of events, and to follow the natural history of the different types of lesions as well as different clinical categories of MS. As a consequence, MRI has brought many new insights into the understanding of MS.

Gd-DTPA is a paramagnetic contrast agent, with minimal side-effects, which adds considerable potential to MRI. Due to the variation in Gd-DTPA uptake by various

tissues, it creates the possibility to manipulate tissue contrast on MRI. The increase in contrast is especially effective in the CNS, where the BBB selectively controls tissue perfusion, and normally prevents Gd-DTPA entering the brain. Breakdown of this barrier, for example in inflammation, allows Gd-DTPA to enter the brain, which locally increases MRI contrast considerably. Therefore, in MS, Gd-DTPA is capable of differentiating new, actively inflamed lesions from non-inflammatory, chronic lesions.

This thesis illustrates that active lesions, as a marker of disease activity, reinforces the innovative role of MRI in MS studies. Study in scanning frequency provides a powerful reference in designing new therapeutic trials. The results in monitoring lesion activity as an outcome measure therapeutic trials provide evidence that lesion activity measurement in small group of patients is still a useful and sensitive tool. Furthermore, other important issues in the evolution of MS lesions need to be addressed as well. MRI is capable of providing information on a number of critical issues, such as differentiation of lesions, seen on non-enhanced images, in relation to impairment: partly demyelinated lesions, insufficiently remyelinated lesions, gliotic lesions with preservation of axons, and gliotic lesions with loss of axons.

Future MR research is aimed at identifying specific pathologies and monitoring their response to therapy. Newly developed MR methods might be quite helpful in attaining this goal. Magnetic transfer imaging (MTI) is a new MR technique that can be used to obtain information about large molecules such as phospholipids in myelin membranes. This method offers the possibility to quantitate myelin loss, and possibly also remyelination. Combination of gadolinium-enhancement and MTI may provide synergistic information. Diffusion MRI can identify changes in myelin structure at a very early stage. MR spectroscopy identifies specific biochemical metabolites involved in the formation and breakdown of myelin, neuronal function, and neurotransmission. Proton spectroscopy already has shown differences between active lesions and stable ones. Changes in choline, N-acetyl aspartate, myoinositol, lactate and free lipids have been

demonstrated, but await further confirmation. MR spectroscopy will become especially valuable when combined with imaging techniques.

Each of these new MR techniques could open new insights into the tissue characteristics of MS lesions and their evolution through specific stages, just as lesion activity studies have increased our knowledge about MS. Hopefully, new MR techniques will allow more precise differentiation of tissue alterations and response to therapy. Their use offers a short cut towards detecting and implementing an effective therapy for MS.

References

- Abbott NJ. Glia and the blood-brain barrier. *Nature* 1987; 325:195.
- Adams CWM. The general pathology of multiple sclerosis: Morphological and clinical aspects of the lesions. in *Multiple sclerosis: pathology, diagnosis, and management*. Hallpike JF, Adams CWM, Tourtellotte WW. ed. London, Chapman and Hall. 1983
- Adams CWM. Vascular aspects of multiple sclerosis. In: *A colour Atlas of Multiple Sclerosis and Other Myelin Disorders*. Adams CWM (Ed). London: Wolfe Medical 184-187.
- Adams RD, Victor M. Multiple sclerosis and allied demyelinating diseases. in *Principle of neurology*. McGraw-Hill Info Serv Comp 1989; 755-774
- Alexander JT, et al. The effect of interleukin-2 on the blood-brain barrier in the 9L gliosarcoma rat model. *J Neurosurg* 1989; 70:92-96.
- Andersen O. Restricted dissemination of clinically defined attacks in an MS incidence material. *Acta Neurol Scand* 1980;62(suppl 77):1
- Annunziata P, et al. Frequency of blood-retina and blood-brain barrier changes in multiple sclerosis. *Ital J Neurol Sci* 1988; 9:345-349.
- Arnason BGW. Interferon beta in multiple sclerosis. *Neurology* 1993;43:641-643
- Arnold DL, Matthews PM, Francis G, Antel J. Proton magnetic resonance spectroscopy of human brain in vivo in the evaluation of multiple sclerosis: assessment of the load of disease. *Magn Reson Med* 1990;14:154-159

- Barkhof F. Gadolinium enhanced magnetic resonance imaging in multiple sclerosis. VU University Press, Amsterdam. 1992;29-61
- Barnes D, et al. The characterization of experimental gliosis by quantitative nuclear magnetic resonance imaging. *Brain* 1988; 111:83-94.
- Barnes D, Munro PMG, Youl BD, McDonald WI. The longstanding MS lesion: a quantitative MRI and electron microscopic study. *Brain* 1991;114:1271-1280
- Bastianello S, Pozzilli C, Bernardi S, Bozzao L, Fantozzi LM, Buttinelli C, Fieschi C. Serial study of gadolinium-DTPA MRI enhancement in multiple sclerosis. *Neurology* 1990;40:591-595
- Birken DL, Oldendorf WH. N-acetyl-aspartic acid: a literature review of a compound prominent in ¹H-NMR spectroscopic studies of brain. *Neurosci Biobehav Rev* 1989;13:23-31
- Bloch F, Hansen WW, Packard M. Nuclear induction. *Phys Rev* 1946;70:460-474
- Bobowick AR, Kurtzke JF, Brody JA, et al. Twin study of multiple sclerosis: an epidemiologic inquiry. *Neurology* 1978;28:978-987
- Brainin M, Reisner T, Beuhold A, et al. Topological characteristics of brainstem lesions in clinically definite and clinically probable cases of multiple sclerosis: an MRI-study. *Neuroradiol* 1987;29:530
- Brightman MW, Reese TS. Junctions between intimately apposed cell membranes in the vertebrate brain. *J Cell Biol* 1969; 40:648-677.
- Broman T. Blood-brain barrier damage in multiple sclerosis. sopravital test-observations. *ACTA Neurol Scand* 1964; 40(Suppl 10):21-24.

- Broman T, Andersen O, Bergmann L: Clinical studies on multiple sclerosis. *Acta Neurol Scand* 1981;63:6
- Brown WJ. The capillaries in acute and subacute multiple sclerosis plaques: a morphometric analysis. *Neurol* 1978; 28:84-92.
- Brownell B and Hughes JT. The distribution of plaques in the cerebrum in multiple sclerosis. *J Neurol Neurosurg Psychiat* 1962;25:315-320
- Bruhn H, Frahm J, Gyngell ML, et al. Cerebral metabolism in man after acute stroke: new observations using localized proton NMR spectroscopy. *Magn Reson Med* 1989;9:126-131
- Bundgaard M. Pathways across the vertebrate blood-brain barrier: Morphological viewpoints. In HF Cserr (Ed) *The Neuronal microenvironment*. Annals of the New York Academy of Science 1986; 481:7-19.
- Burnham JA, Wright RR, Dreisbach J, Murray R. The effect of high-dose steroids on MRI gadolinium enhancement in acute demyelinating lesions. *Neurology* 1991; 41:1349-1354.
- Cancilla PA, Berliner JA, Bready JV. Astrocytes and the blood-brain barrier. Kinetics of astrocyte activation after injury and induction effects on endothelium. In Johansson BB, Ovman C, Widner H (Ed). *Pathophysiology of the blood-brain barrier*. Elsevier 1990; 31-39.
- Capra R, Marciano N, Vignolo LA et al. Gadolinium-pentetic acid magnetic resonance imaging in patients with relapsing remitting multiple sclerosis. *Arch Neurol* 1992;49:687-689

- Cendrowski WS. Multiple sclerosis: Discordance in three pairs of dizygotic twins. *J Med Genet* 1968;5:266-268
- Clanet M, Blancher A, Calvas P, Rascol O. Interferon and multiple sclerosis. *Biomed & Pharmacother* 1989;43:355-360
- Claudio L, Kress Y, Norton WT, Brosman CF. Increased vesicular transport and decreased mitochondrial content in blood-brain barrier endothelial cells during experimental autoimmune encephalomyelitis. *Am J Pathol* 1989; 135:1157-1168.
- Claudio L, Kress Y, Factor J, Brosman CF. Mechanisms of edema formation in experimental autoimmune encephalomyelitis. The contribution of inflammatory cell. *Am J Pathol* 1990; 137:1033-1045.
- Cserr HF, Bundgaard M. The neuronal microenvironment : a comparative view. In HF Cserr (Ed) *The neuronal micronment. Annals of the New York Academy of Sciences* 1986; 481:1-7.
- Currier RD, Eldridge R. Possible risk factors in multiple sclerosis as found in a national twin study. *Arch Neurol* 1982;39:140-144
- Damadian R. Tumor detection by nuclear magnetic resonance. *Science* 1971;171:1151-1153
- Damle NK, et al. Interleukin 2-activated human lymphocytes exhibit enhanced adhesion to normal vascular endothelial cells and cause their lysis. *J Immunol* 1987; 138:1779-1785.
- de Graaf J, van der Hoeven JH, Hoogstraten MC, Minderhoud JM. Multiple sclerosis. Utrecht: Bohn, Scheltema & Holkema 1988

- De Maeyer E, De Maeyer-Guignard J. Chapter 2: The interferon gene family. In De Maeyer & De maeyer-Guignard (Eds) Interferons and other regulatory cytokines. John Wiley & Sons, New York 1988:5-38
- den Hollander JA, Luyten PR. Image-guided localized ^1H and ^{31}P NMR spectroscopy of humans. Ann NY Acad Sci 1987; 508:386-398
- Dorovini-Zis K, Bowman PD, Prameya R, et al. Adhesion and migration of human polymorphonuclear leukocytes across cultured bovine microvessel endothelial cells. J Neuropathol Exp Neurol 1992;51:194-205
- Ebers GC, Bulman DE, Sadovnick AD, et al. A population-based study of multiple sclerosis in twins. N Engl J Med 1986;315:1638-1642
- Eickhoff K, et al. Protein profile of cerebrospinal fluid in multiple sclerosis with special reference to the function of the blood-brain barrier. J Neurol 1977; 214-207-215.
- Ellison, et al. Blood-brain barrier dysfunction in cats following recombinant interleukine-2 infusion. Cancer Res 1987; 47:5765-5770.
- Engell T. A clinical patho-anatomical study of clinically silent multiple sclerosis. Acta Neurol. Scand. 1989;79:428-430
- Filippi M, Paty DW, Kappos L, Barkhof F, Compston DAS, Thompson AJ, Zhao GJ, Wiles CM, McDonald WI, Miller DH. Correlations between changes in disability and T_2 -weighted brain MRI activity in multiple sclerosis: a follow up study. Neurology 1995;45:255-260

- Fontana A and Fierz W. The endothelium-astrocyte immune control system of the brain. *Semin Immunopathol* 1985; 8:57-70.
- Frahm J, Bruhn H, Gyngell ML, et al. Localized high-resolution proton NMR spectroscopy using stimulated echoes: initial applications to human brain in vivo. *Magn Reson Med* 1989;9:79-93
- French Research Group on Multiple Sclerosis. Concordance rate is independent of zygotic. *Ann Neurol* 1992;32:724-727
- Frohman EM, Vayuvegula B, van den Noort S, Gupta S. Norepinephrine inhibits gamma-interferon-induced MHC class II (Ia) antigen expression on cultured brain astrocytes. *J Neuroimmunol* 1988; 17:89-101.
- Frohman EM, et al. Astrocytes and intracerebral immune response. *J clin Immunol* 1989; 9:1-9.
- Gadian DG. NMR and its application to living systems. New York, Oxford University Press 1982
- Gay D and Esiri M. Blood-brain barrier damage in acute multiple sclerosis plaques: An immunocytological study. *Brain* 1991;114:557-572
- Gill SS, Small RK, Thomas DGT, et al. Brain metabolites as ^1H NMR markers of neuronal and glial disorders. *NMR Biomed* 1989;2:196-200
- Gross PM, et al. Blood-brain barrier and histamine: quantitative evidence for increased permeability in rat cerebral cortex. *J cereb Blo Flow Metab* 1981; 1:S379-380.
- Grossman RI, et al. Multiple sclerosis: gadolinium enhancement in MR imaging. *Radiology* 1986; 161:721-725.

- Grossman RI, et al. Multiple sclerosis: serial study of gadolinium-enhanced MR imaging. *Radiology* 1988; 169:117-122.
- Gulati A, et al. Evidence for the involvement of histamine in the regulation of blood-brain barrier permeability. *Pharmacol Res Comm* 1985; 17:395.
- Hallpike JF, Adams CWM, Tourtellote WW. Multiple sclerosis: Pathology, diagnosis and management. Baltimore: Williams & Wilkins, 1983.
- Harris JO, Frank JA, Patronas N, McFarlin DE, McFarland HF. Serial gadolinium-enhanced magnetic resonance imaging scans in patients with early, relapsing-remitting multiple sclerosis: implications for clinical trials and natural history. *Ann Neurol* 1991;29:548-555
- Hawkins CP, Munro PMG, Mackenzie F, Kesselring J, Tofts PS, et al. Duration and selectivity of blood-brain barrier breakdown in chronic relapsing experimental allergic encephalomyelitis studied by gadolinium-DTPA and protein markers. *Brain* 1990;113:365-378
- Heurkeas, AHM, et al. Anti-endothelial cell antibodies in patients with rheumatoid arthritis complicated by vasculitis. *Clin Exp Immunol* 1989; 78:7-12.
- Hickey WF, Kimura H. Perivascular microglial cells of the CNS are bone-marrow derived and present antigen in vivo. *Science* 1988; 239:290-193.
- Hreshey LA, et al. Computerized tomography in the diagnosis evaluation of multiple sclerosis. *Ann Neurol* 1979; 5:32-39.
- The IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology* 1993;43:655-661

- The IFNB Multiple Sclerosis Study Group and the UBC MS/MRI Analysis Group. Beta-interferon 1b in the treatment of MS: final outcome of the randomised controlled trial. *Neurology* (in submitted)
- Isaac C, Li DKB, Genton M, et al. Multiple sclerosis: a serial study using MRI in relapsing patients. *Neurology* 1988;38:1511-1515
- Janzer RC, Raff MC. Astrocytes induces blood-brain barrier properties in endothelial cells. *Nature* 1987;325:253-257.
- Johnson MA, Li DKB, Bryant DJ, Payne JA. Magnetic resonance imaging: serial observations in multiple sclerosis. *AJNR* 1984;5:495-499
- Juhler M, et al. Blood-brain and blood-spinal cord barrier permeability during course of experimental allergic encephalomyelitis in the rat. *Brain Res* 1984; 302:347-355.
- Juhler M, et al. A spatial analysis of the blood-brain barrier damage in experimental allergic encephalomyelitis. *J cereb Blo Flow Metab* 1985; 5:545- 553.
- Kajiwara K, Ito H, Fukumoto T. Lymphocyte infiltration into normal rat brain following hyperosmotic blood-brain barrier opening. *J Neuroimmunol*. 1990; 27:133-140.
- Kato S and Nakamura H. Ultrastructural and ultracytochemical studies on the blood-brain barrier in chronic relapsing experimental allergic encephalomyelitis. *ACTA Neuropathol* 1989; 77:455-464.
- Kermode AG, Tofts PS, MacManus DG, Kendall BE, Kingsley DPE, Moseley IF et al. Early lesions of multiple sclerosis. *Lancet* 1988 ii;1203-1204
- Kermode AG, Tofts PS, Thompson AJ, et al. Heterogeneity of blood-brain barrier changes in multiple sclerosis: an MRI study with gadolinium-DTPA enhancement. *Neurology* 1990a;40:229-235

- Kermode AG, Thompson AJ, Tofts PS, et al. Breakdown of the blood-brain barrier precedes symptoms and other MRI signs of new lesions in multiple sclerosis. *Brain* 1990b;113:1477-1489
- Khoury SJ, Guttmann CRG, Orav EJ, et al. Longitudinal MRI in multiple sclerosis: correlation between disability and lesion burden. *Neurology* 1994;44:2120-2124
- Kinrichs DJ, Wegmann KW, Dietsch GN. Transfer of experimental allergic encephalomyelitis to bone-chimeras: endothelial cells are not the restricting element. *J Exp Med* 1987; 166:1906-1911.
- Koopmans RA, Li DKB, Oger JJF, Kastrukoff LF, Jardine C, Costley L, Hall S, Grochowski EW, Paty DW. Chronic progressive multiple sclerosis: serial magnetic resonance brain imaging over six months. *Ann Neurol* 1989a;26:248-256
- Koopmans RA, Li DKB, Oger JJF, Mayo J, Paty DW. The lesion of multiple sclerosis: imaging of acute and chronic stages. *Neurology* 1989b;39:959-963
- Koopmans RA, Zhu G, Li DKB, Allen PA, Javidan M, Paty DW. In-vivo proton MRS of the acute and chronic lesion of multiple sclerosis. In: Book of abstracts. Society of Magnetic Resonance in Medicine 1990;2:1205
- Koopmans, RA, Li DKB, Redekop WK, Zhao GJ, Palmer MR, Kastrukoff LF, Paty DW. The use of magnetic resonance imaging in monitoring interferon therapy of multiple sclerosis. *J Neuroimag* 1993;3:163-168
- Koopmans RA, Li DKB, Zhao GJ, Redekop Wk, Paty DW. MRI assessment of cyclosporine therapy of MS in a multicenter trial. *Neurology* 1992;42(suppl 3):210
- Koopmans RA, Zhao GJ, Li DKB, Paty DW. Interferon b-1b decreases multiple sclerosis lesion activity as detected by yearly MRI. *Neurology* (in submitted)

- Kurtzke JF. Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;33:1444-1452
- Lassmann H, Waksman B, Brosnan C. Mechanism of vascular and tissue damage in demyelinating diseases. *J Neuroimmunol* 1991; 32:83-85.
- Lauterbur PC. Image formation by induced local interaction: examples employing nuclear magnetic resonance. *Nature* 1973;242:190-191
- Li D, Mayo J, Fache S, Robertson WD, Paty, D, Genton M. Early experience in nuclear resonance imaging of multiple sclerosis. *Ann NY Acad Sci* 1984;436:483-486
- Lossinsky AS, et al. Sites of egress of inflammatory cells and horseradish peroxidase transport across the blood-brain barrier in a murine model of chronic relapsing allergic encephalomyelitis. *Acta Neuropathol* 1989; 78:359-371
- Lynch SG, Rose JW, Smoker W, et al. MRI in familial multiple sclerosis. *Neurology* 1990;40:900-903
- MacKay RP, Myrianthopoulos NC. Multiple sclerosis in twins and their relatives. Final report. *Arch Neurol Psychi* 1966;15:449-462
- Mansfield P, Grannell PK. NMR "diffraction" in solids? *J Phys* 1973;[C]6:L422-L426
- Matthews WB, Acheson ED, Batchelor JR, Weller RO. *McAlpine's multiple sclerosis*. New York: Churchill Livingstone, 1985.
- Matthews WB. Pathophysiology. In: Matthews WB, ed. *McAlpine's multiple sclerosis*. 2nd ed. New York: Churchill-Livingstone, 1991:245-246
- Miller BL. A review of chemical issues in ¹H NMR spectroscopy: N-acetyl-L-aspartate, creatine, choline. *NMR Biomed* 1991;4:47-52
- Miller DH, Rudge P, Johnson G, Kendall BE, Macanus DG, Moseley IF, Barnes D, McDonald WI. Serial gadolinium enhanced magnetic resonance imaging in multiple sclerosis. *Brain* 1988;111:927-939
- Miller DH, Rudge P, Johnson G, et al. Serial gadolinium enhanced magnetic resonance imaging in multiple sclerosis. *Brain* 1989;111:927-939

- Miller DH, Barkhof F, Berry L, et al. Magnetic resonance imaging in monitoring the treatment of multiple sclerosis: concerted action guidelines. *J Neurol Neurosurg Psych* 1991;54:683-688
- Miller DH, Barkhof F, Nauta JJP. Gadolinium enhancement increases the sensitivity of MRI in detecting disease activity in multiple sclerosis. *Brain* 1993;116:1077-1094
- Minden SL, Schiffer RB. Affective disorder in multiple sclerosis: review and recommendations for clinical research. *Arch Neurol* 1990;47:98-104
- Mumford CJ, Wood NW, Kellar-Wood H, et al. British isles survey of multiple sclerosis in twins. *Neurology* 1994;44:11-15
- Niendorf HP, Dinger JC, Haustein J, et al. Tolerance data of Gd-DTPA: a review. *Eur J Radiol* 1991;13:15-20
- Ormerod IEC, Miller DH, du Boulay EPGH, McDonald WI. Magnetic resonance imaging in multiple sclerosis. Thieme Med Publisher, New York 1989
- Osborn AG, Hendrick RE. Introduction to MR imaging. part I. basic physics and instrumentation. in *Diagnostic Radiology. MR Imaging*. Ed. Stark DD and Bradley WG. 1988;7-25
- Panitch HS. Interferon in multiple sclerosis: a review of the evidence. *Drugs* 1992;44:946-962
- Pardridge WM, et al. Blood-brain barrier: interface between internal medicine and the brain. *Ann Intern Med* 1986; 105:82-95.
- Paty DW. Multiple sclerosis: assessment of disease progression and effects of treatment. *Can J Neurol Sci* 1987;14:518-520
- Paty DW. Magnetic resonance imaging in the assessment of disease activity in multiple sclerosis. *Can J Neurol Sci* 1988; 15:266-172
- Paty DW, Li DKB, The UBC MS/MRI Study Group, The IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II.

- MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology* 1993a;43:662-667
- Paty DW, Koopmans RA, Zhao GJ, Li DKB, Oger JF, Petkau J. Magnetic resonance imaging (MRI) as an outcome measure in multiple sclerosis. *J Neurol Rehab* 1993b;7:117-129
- Plant GT, Kermode AG, Turano G, et al Symptomatic retrochiasmal lesions in multiple sclerosis: clinical features, visual evoked potentials, and magnetic resonance imaging. *Neurology* 1992;42:68-76
- Pollay M and Robert PA. Blood-brain barrier: a definition of normal and altered function. *Neurosurg* 1980; 6:675-685.
- Poser CM, et al. Neuroimaging and the lesion of multiple sclerosis. *AJNR* 1987; 8:549-552.
- Prineas JW. The neuropathology of multiple sclerosis. In *Handbook of Clinical Neurology*. Koetsier JC. Ed. Elsevier Science Pub. 1985;3(47):213-257
- Prineas JW, Kwon EE, Goldenberg PZ, et al. Multiple sclerosis: oligodendrocyte proliferation and differentiation in fresh lesions. *Lab Invest* 1989;61:489-503
- Prineas JW, Barnard RO, Revesz T, et al. Multiple sclerosis: pathology of recurrent lesions. *Brain* 1993;116:681-693
- Prineas JW. Multiple sclerosis: pathology of the early lesion. In *Multiple Sclerosis: Current Status of Research and Treatment*. Ed. Herndon RM and Seil FJ. Demos. New York 1994:113-130
- Purcell EM, Torrey HC, Pound RV. Resonance absorption by nuclear magnetic moments in a solid. *Phys Rev* 1946;69:37-38
- Raine CS, Cross AH. Axonal dystrophy as a consequence of long-term demyelination. *Lab Invest* 1989;60:714-725
- Raine CS. Demyelinating disease. in *Textbook of Neuropathology*. Ed by Davis RL, Robertson DM. Williams & Wilkins 1991

- Reese TS, Karnovsky MJ. Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J Cell Biol* 1967; 34:207-217.
- Ron MA, Feinstein A. Multiple sclerosis and the mind. *J Neurol Neurosurg Psychia* 1992;55:1-3
- Runge VM, et al. The use of Gd DTPA as a perfusion agent and marker of blood-brain barrier disruption. *Mag Res Imaging* 1985; 3:43-55.
- Sadovnick AD, Armstrong H, Rice GPA, et al. A population-based study of multiple sclerosis in twins: update. *Ann Neurol* 1993;33:281-185
- Sage MR and Wilson AJ. The blood-brain barrier: an important concept in neuroimaging *AJNR* 1994;15:601-622
- Sharma HS and Dey PK. Influence of long-term acute heat exposure on regional blood-brain barrier permeability, cerebral blood flow and 5-HT level in conscious normotensive young rats. *Brain Res* 1987; 424:153-162.
- Schima W, Wimberger D, Stiglbauer R, et al. The importance of magnetic field strength in the MR diagnosis of multiple sclerosis: a comparison of 0.5 and 1.5T. *Rofo. Fortschritte Auf Dem Gebiete Der Rontgenstrahlen Und Der Neuen Bildgebenden Verfahren* 1993;158(4):368-171
- Sibley WA, Paty DW. A comparison of multiple sclerosis in Ontario, Canada and Arizona, USA. *Acta Neurol Scand* 1981;64(suppl 87):60
- Sipe JC, Knobler RL, Braheny SI, et al. A neurologic rating scale (NRS) for use in multiple sclerosis. *Neurology* 1984;34:1368-1372
- Smith ME, Stone LA, Albert PS, et al. Clinical worsening in multiple sclerosis is associated with increased frequency and area of gadopentetate dimeglumine-enhancing magnetic resonance imaging lesions. *Ann Neurol* 1993;33:480-489
- Stenager E, Knudsen L, Jensen K. Psychiatric and cognitive aspects of multiple sclerosis. *Semin Neurol* 1990;10:254-261

- Steward PA, Wiley MJ. Developing nervous tissue induces formation of blood-brain barrier characteristics in invading endothelial cells: A study using quail-chick transplantation chimeras. *Devl Biol* 1981; 84:183-192.
- Stewart WA, Hall LD, Berry K, et al. Magnetic resonance imaging (MRI) in multiple sclerosis (MS): pathological correlation studies in eight cases. *Neurology* 1986;36(suppl 1):320
- Sweeney VP, Sadovnick AD, Brandeys V. Prevaence of multiple sclerosis in British Columbia. *Can J Neurol Sci* 1986;13:47-51
- Tanaka Y, et al. Anti-endothelial cell antibodies and circulating immune complexes in the sera of patients with multiple sclerosis. *J Neuroimmunol* 1987; 17:49-59.
- Thompson AJ, Kermode AG, MacManus DG, et al. Patterns of disease activity in multiple sclerosis: clinical and magnetic resonance imaging study. *Bri Med J* 1990;300:631-634
- Thompson AJ, Miller D, Youl B, MacManus D, Moore S, et al. Serial gadolinium-enhanced MRI in relapsing/remitting multiple sclerosis of varying disease duration. *Neurology* 1992;42:60-63
- Tienari PJ, Salonen O, Wikstrom J, et al. Familial multiple sclerosis: MRI findings in clinically affected and unaffected siblings. *J Neurol Neurosurg Psychiatry* 1992;55:883-886
- Tourtellotte WW and Ma BI. Multiple sclerosis: the blood-brain barrier and the measurement of *de novo* central nervous system IgG synthesis. *Neurology* 1978; 28:76-83.
- Traugott U, and Lebon P. Mulitple sclerosis: involvement of interferons in lesion pathogenesis. *Ann Neurol* 1988; 24:243-251.

- Tsukada N, et al. Autoantibodies to each protein fraction extracted from cerebral endothelial cell membrane in the sera of patients with multiple sclerosis. *J Neuroimmunol* 1989; 24:41-46.
- Tunggal B, Hofmann K, Stoffel W. In vivo ^{13}C nuclear magnetic resonance investigations of choline metabolism in rabbit brain. *Magn Reson Med* 1990;13:90-102
- van Hecke P, Marchal G, Johannik K, et al. Human brain proton localized NMR spectroscopy in multiple sclerosis. *Magn Reson Med* 1991;18:199-206
- Vukmanovic S, et al. Experimental autoimmune encephalomyelitis in "low" and "high" interleukin 2 producer rats. I cellular basis of induction. *Cell Immunol* 1989; 121:237-246.
- Waxman SG. Membranes, myelin, and the pathophysiology of multiple sclerosis. *N Engl J Med* 1982;306:1529-1533
- Weinmann HJ, Brasch RC, Press WR, Wesbey GE. Characteristics of gadolinium-DTPA -complex: a potential NMR contrast agent. *AJR* 1984;142:619-624
- Weinmann HJ, Press WR, Raduchel B, et al. Characteristics of Gd-DTPA and new derivatives. In: Bydder GM, Felix R, Bucheler E, et al. (Eds). *Contrast media in MRI*. Bussum: Medicom Europe 1990:9-14
- Wekerle H. Intercellular interaction in myelin-specific autoimmunity. *J Neuroimmunol* 1988; 20:211-216.
- Westergard E. The effect of serotonin on the blood-brain barrier to protein. *J Neurol Trans* 1978; Suppl14:9-15.

- Wiebe S, Lee DH, Karlik SJ, et al. Serial cranial and spinal cord magnetic resonance imaging in multiple sclerosis. *Ann Neurol* 1992;32:643-650
- Wilcox CE, Ward AMV, Evans A, et al. Endothelial cell expression of the intercellular adhesion molecule-1 (ICAM-1) in the central nervous system of guinea pigs during acute and chronic relapsing experimental allergic encephalomyelitis. *J Neuroimmunol* 1990; 30:43-51.
- Williams A, Eldridge R, MacFarland H, Houff S, et al. Multiple sclerosis in twins. *Neurology* 1980;30:1139-1147
- Willoughby EW, Grochowski E, Li DKB, Oger J, Kastrucoff LF, Paty DW. Serial magnetic resonance scanning in multiple sclerosis: a second prospective study in relapsing patients. *Ann Neurol* 1989;25:43-49
- Wolinsky JS, Narayana PA, Fenstermacher MJ. Proton magnetic resonance spectroscopy in multiple sclerosis. *Neurology* 1990;40:1764-1769
- Wong D, Dorovini-Zis K. Upregulation of intercellular adhesion molecule-1 (ICAM-1) expression in primary cultures of human brain microvessel endothelial cells by cytokines and lipopolysaccharide. *J Neuroimmunol* 1992;39:11-21
- Youl BD, Turano G, Miller DH, et al. The pathophysiology of acute optic neuritis. An association of gadolinium leakage with clinical and electrophysiological deficits. *Brain* 1991;114:2437-2450
- Young IR, Hall AS, Pallis CA, Legg NJ, Bydder GM, Steiner RE. Nuclear magnetic resonance imaging of the brain in multiple sclerosis. *Lancet* 1981;ii:1063-1066

- Zhao GJ, Li DKB, Tanton BL, Paty DW. Assessment of activity of the individual lesions in relapsing-remitting multiple sclerosis on magnetic resonance imaging. *Ann Neurol* 1991;30:270
- Zhao GJ, Li DKB, Tanton BL, Paty DW. Sensitivity of Gd-DTPA MRI and serial MRI in detecting activity of lesion in relapsing-remitting multiple sclerosis. Book of abstracts. Society of MR in Med. 1992; 1:1710
- Zhao GJ, Li DKB, Tanton BL, Paty DW. Features of enhancing and non-enhancing lesions in multiple sclerosis on Gd-DTPA MRI. *J Neuroimaging* 1992;2:52
- Zhao GJ, Li DKB, Koopmans RA, Tanton BL, Paty DW. The growth pattern of enlarging lesions in multiple sclerosis: Observation on serial MRI. *Can J. Neuro Sci* 1993;Suppl.4:S219
- Zhao GJ, Koopmans RA, Li DKB, et al. Corticospinal tract lesions in multiple sclerosis: relationship between MRI activity and clinical course. *Neurology* 1993;43:A246
- Zhao GJ, Li DKB, Koopmans RA, Paty DW, MS/MRI Research Group, MS Study Group. Changes in burden of disease and lesion activity in MS placebo patients: a 5-year study by yearly serial MRI. *Ann Neurol* 1995 (in press)

Appendix A. Expanded Disability Status Scale (EDSS)

- 0 = Normal neurologic exam (all grade 0 in Functional Systems (FS, appendix B); Cerebral grade 1 acceptable).
- 1.0 = No disability, minimal signs in more than one FS (more than one grade 1 excluding Cerebral grade 1).
- 2.0 = Minimal disability in one FS (one FS grade 2, others 0 or 1).
- 2.5 = Minimal disability in two FS (two FS grade 2, others 0 or 1).
- 3.0 = Moderate disability in one FS (one FS grade 3, others 0 or 1), or mild disability in three or four FS (three/four FS grade 2, others 0 or 1) though fully ambulatory.
- 3.5 = Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3; or five FS grade 2 (others 0 or 1).
- 4.0 = Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest some 500 meters.
- 4.5 = Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability, usually consisting of one FS

grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest for some 300 meters.

5.0 = Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (eg, to work full day without special provisions). (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0).

5.5 = Ambulatory without aid or rest for about 100 meters; disability severe enough to preclude full daily activities. (Usual FS equivalents are one grade 5 alone, other 0 or 1; or combinations of lesser grades usually exceeding those for step 4.0).

6.0 = Intermittent or unilateral constant assistance (cane, Crutch, or brace) required to walk about 100 meters with or without resting. (usual FS equivalents are combinations with more than two FS grade 3+).

6.5 = Constant bilateral assistance (canes, crutches, or braces) required to walk about 20 meters without resting. (Usual FS equivalents are combinations with more than two FS grade 3+).

7.0 = Unable to walk beyond about 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in w/c some 12 hours a day. (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely, pyramidal grade 5 alone).

7.5 = Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may

require motorized wheelchair. (Usual FS equivalents are combinations with more than one FS grade 4+).

8.0 = Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms. (Usual FS equivalents are combinations generally grade 4+ in several systems).

8.5 = Essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions. (Usual FS equivalents are combinations generally 4+ in several systems).

9.0 = Helpless bed patient; can communicate and eat. (Usual FS equivalents are combinations, mostly grade 4+).

9.5 = Totally helpless bed patient; unable to communicate effectively or eat/swallow. (Usual FS equivalents are combinations, almost all grade 4+).

10. = Death due to MS.

Appendix B. Functional Systems

Pyramidal Functions

- 0. Normal.
- 1. Abnormal signs without disability.
- 2. Minimal disability.
- 3. Mild or moderate peraparesis or hemiparesis; severe monoparesis.
- 4. Marked paraparesis or hemiparesis; moderate quadriparesis; or monoplegia.
- 5. Paraplegia, hemiplegia, or marked quadriparesis.
- 6. Quadriplegia.
- V. Unknown.

Cerebellar Functions

- 0. Normal.
- 1. Abnormal signs without disability.
- 2. Mild ataxia.
- 3. Moderate truncal or limb ataxia.
- 4. Severe ataxia, all limbs.
- 5. Unable to perform coordinated movements due to ataxia.
- V. Unknown.
- X. Is used throughout after each number when weakness (grade 3 or more on pyramidal) interferes with testing.

Brain Stem Functions

- 0. Normal.
- 1. Signs only.

2. Moderate nystagmus or other mild disability.
3. Severe nystagmus, marked extraocular weakness, or moderate disability of other cranial nerves.
4. Marked dysarthria or marked disability.
5. Inability to swallow or speak.
- V. Unknown.

Sensory Functions

0. Normal.
1. Vibration or figure-writing decrease only, in one or two limbs.
2. Mild decrease in touch or pain or position sense, and/or moderate decrease in vibration in one or two limbs; or vibratory (c/s figure writing) decrease alone in three or four limbs.
3. Moderate decrease in touch or pain or position sense, and/or essentially lost vibration in one or two limbs; or mild decrease in touch or pain and/or moderate decrease in all proprioceptive tests in three or four limbs.
4. Marked decrease in touch or pain or loss of proprioception, alone or combined, in one or two limbs; or moderate decrease in touch or pain and/or severe proprioceptive decrease in more than two limbs.
5. Loss (essentially) of sensation in one or two limbs; or moderate decrease in touch or pain and/or loss of proprioception for most of the body below the head.
6. Sensation essentially lost below the head.
- V. Unknown.

Bowel and Bladder Function

0. Normal.
1. Mild urinary hesitancy, urgency, or retention.

2. Moderate hesitancy, urgency, retention of bowel or bladder, or rare urinary incontinence.
3. Frequent urinary incontinence.
4. In need of almost constant catheterization.
5. Loss of bladder function.
6. Loss of bowel and bladder function.
- V. Unknown.

Visual (or Optic) Functions

0. Normal.
1. Scotoma with visual acuity (corrected) better than 20/30.
2. Worse eye with scotoma with maximal visual acuity (corrected) of 20/30 to 20/59.
3. Worse eye with large scotoma, or moderate decrease in fields, but with maximal visual acuity (corrected) of 20/60 to 20/99.
4. Worse eye with marked decrease of fields and maximal visual acuity (corrected) of 20/100 to 20/200; grade 3 plus maximal acuity of better eye of 20/60 or less.
5. Worse eye with maximal visual acuity (corrected) less than 20/200; grade 4 plus maximal acuity of better eye of 20/60 or less.
6. Grade 5 plus maximal visual acuity of better eye of 20/60 or less.
- V. Unknown.
- X. Is added to grades 0 to 6 for presence of temporal pallor.

Cerebral (or Mental) Functions

0. Normal.
1. Mood alteration only (Does not affect DSS score).
2. Mild decrease in mentation.
3. Moderate decrease in mentation.

4. Marked decrease in mentation (chronic brain syndrome - moderate).

5. Dementia or chronic brain syndrome-severe or incompetent.

V. Unknown.

Other Functions

0. None.

1. Any other neurologic findings attributed to MS (specify).

V. Unknown.