Published in final edited form as:

J Neurol Sci. 2009 July 15; 282(1-2): 28–33. doi:10.1016/j.jns.2008.12.036.

White Matter Hemodynamic Abnormalities precede Sub-cortical Gray Matter Changes in Multiple Sclerosis

Andrew W. Varga, M.D., Ph.D.¹, Glyn Johnson, Ph.D.¹, James S. Babb, Ph.D.¹, Joseph Herbert, M.D.², Robert I. Grossman, M.D.³, and Matilde Inglese, M.D., Ph.D.^{1,2}

¹ Department of Radiology, New York University, New York, NY, USA

² Department of Neurology, New York University, New York, NY, USA

³ Dean's Office at New York University, NY USA

Abstract

Background—Hypoperfusion has been reported in lesions, normal-appearing white (NAWM) and gray matter (NAGM) of patients with clinically definite multiple sclerosis (MS) by using perfusion MRI. However, it is still unknown how early such changes in perfusion occur. The aim of our study was to assess the presence of hemodynamic changes in the NAWM and subcortical NAGM of patients with clinically isolated syndrome (CIS) in comparison to healthy controls and to patients with early relapsing-remitting (RR) MS.

Methods—Absolute cerebral blood flow (CBF), blood volume (CBV) and mean transit time (MTT) were measured in the periventricular and frontal NAWM, thalamus and putamen nuclei of 12 patients with CIS, 12 with early RR-MS and 12 healthy controls using dynamic susceptibility contrast enhanced (DSC) T2*-weighted MRI.

Results—Compared to controls, CBF was significantly decreased in the periventricular NAWM of CIS patients and in the periventricular NAWM and putamen of RR-MS patients. Compared to CIS, RR-MS patients showed a significant CBF decrease in the putamen.

Conclusions—CBF was decreased in the NAWM of both CIS and RR-MS patients and in the subcortical NAGM of RR-MS patients suggesting a continuum of tissue perfusion decreases beginning in white matter and spreading to gray matter, as the disease progresses.

Keywords

clinically isolated syndrome; relapsing-remitting MS; normal-appearing white matter; normal-appearing gray matter; Dynamic Susceptibility Contrast Perfusion MRI; conventional MRI

Introduction

Multiple sclerosis (MS) is the leading cause of non traumatic disability in young adults and affects 2 million people worldwide [1]. Neuro-axonal loss is considered the major contributor to permanent disability and there is increasing evidence that it occurs since the earliest stages

Correspondence to: Matilde Inglese, MD, PhD, New York University Langone Medical Center, Center for Biomedical Imaging, 660 First Avenue, 4th Floor, New York, NY 10016, USA. Phone: 1 212 263 3322. Fax: 1 212 263 7541. E-mail: matilde.inglese@med.nyu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

of the disease [2,3]. Although neuro-axonal injury is a pathological hallmark of the disease in addition to inflammation and demyelination, the molecular and cellular mechanisms leading to neurodegeneration are still poorly understood.

Several immunopathological studies suggest that vascular factors may contribute to the pathogenesis of MS [4-12]. The typical “Dawson's fingers” [4] are in fact, perivenular areas of demyelination, and histopathological examination of acute plaques demonstrates perivenular lymphocytic cuffing [6,7]. Perivascular and intravascular fibrin deposition [8,9] and venous occlusive changes have been demonstrated in active MS lesions [10]. In chronic lesions, perivenular inflammation is limited, yet there are indications of vascular damage in the form of vein wall hyalinization [9]. Furthermore, in both MS plaques and the serum of MS patients, vascular endothelial growth factor is upregulated [11,12]. Endothelial cell activation, confirmed by class II antigen expression and focal endothelial cell associated fibrin deposition, has also been suggested as an early event in the vascular injury in MS [10].

Until the recent advancement in quantitative magnetic resonance imaging (MRI), the extent of microvascular abnormalities and their role in lesion development has been difficult to assess *in vivo*. Indeed, several studies employing either bolus-tracking or arterial spin labeling (ASL) MRI techniques have documented the presence of microvascular abnormalities in lesions, normal appearing white matter (NAWM) and gray matter (NAGM) in various subtypes of MS [13-16]. Although there are some discrepancies, most of the studies have shown that WM and cortical and sub-cortical GM perfusion is decreased in patients with clinically definite MS.

In WM, inflammatory-related ischemia is exacerbated by the marginal blood supply due to: (a) the venous watershed existing in the paraventricular white matter and subcortical white matter [17]; (b) very slow flow seen in the veins draining white matter [18]; (c) white matter having 4-5 times less blood flow than gray matter [19,20]; (d) poor collateral in the white matter compared with gray matter. Unlike WM, which is more vulnerable to vascular compromise, GM hypoperfusion in MS is likely to be secondary to white matter injury and tissue loss. However, the question of how early such WM and GM changes in perfusion occur remains open.

The aim of this study was to use MRI to assess the presence and extent of changes in the perfusion of NAWM and sub-cortical NAGM in patients with clinically isolated syndrome (CIS) in comparison with patients with early RR-MS and healthy controls. Since WM is more vulnerable to changes in tissue perfusion compared to GM, we sought to specifically test the hypothesis that cerebral NAWM perfusion is decreased since the earliest stages of the disease whereas sub-cortical NAGM perfusion develops as the disease progresses.

Materials and Methods

Subjects

The MRI data were acquired from a cohort of CIS patients recruited prospectively in our MS clinical center and from a cohort of newly diagnosed RR-MS patients prospectively recruited in an ongoing MRI research project. There were twelve (9 female) patients with CIS (six had optic neuritis, four had a brainstem-cerebellar syndrome and two had a spinal cord syndrome) and 12 (7 female) patients with RR-MS meeting the McDonald criteria [21]. All alternative neurological diseases were excluded by appropriate investigations [21]. To be included, both CIS and RR-MS patients had to be within 5 years of first symptoms. Patients were not included if they had corticosteroid use or relapses within 3 months prior to MRI. Neurological disability was assessed within 1 week of MRI scan by a single experienced neurologist blind to the MRI findings using the Expanded disability status scale (EDSS) score [22]. The patients with CIS had a mean age of 41.4 (range, 28-56) yrs, mean disease duration of 18.6 (range, 3-58 months

and median EDSS score of 1.0 (range, 0-2). The patients with RR-MS had a mean age of 47.5 (range, 32-62) yrs, mean disease duration of 47.7 (range, 12-62) months and median EDSS score of 1.5 (range, 0-2.5). Subsets of both CIS and RR-MS patients were under immunomodulatory treatment with Interferon beta-1a (Avonex, Biogen, Cambridge Mass), Interferon beta-1a (Rebif, Serono, Rockland, MA) or glatiramer acetate (Copaxone, Teva, Petah Tiqvah, Israel). For comparison, twelve age-matched healthy controls (6 female) were recruited. Their mean age was 42.0 (range, 24-54) yrs. Approval for this study was obtained from the Institutional Board of Research Associates of New York University Medical Center and informed consent was obtained from all subjects. Demographic, clinical and conventional MRI characteristics of the three subject groups are given in Table 1.

MR Imaging Acquisition

MRI was performed using a 3.0-T scanner (Trio, Siemens Medical Systems, Erlangen, Germany) with an 8-channel phased-array head coil. The following sequences were collected in all subjects during a single MR session: *a*) dual-echo turbo spin-echo (repetition time [TR] = 5,500, echo time [TE] = 12/99, 96 contiguous, 3 mm-thick, axial slices with a 256×205 matrix and a 220×190 mm field of view [FOV]; in-plane voxel-size $0.85 \text{ mm} \times 0.92 \text{ mm}$; parallel imaging acceleration factor of 2); *b*) gradient-echo echo-planar imaging (TR = 1000, TE = 32; 10 contiguous, 3 mm-thick axial slices with a 128×128 matrix; 220×220 FOV; flip angle, 30° ; and signal bandwidth, 1396 Hz/pixel; in-plane voxel size, $1.7 \times 1.7 \text{ mm}$). DSC MR images were acquired during the first pass of a standard-dose (0.1 mmol/kg) bolus of gadopentetate dimeglumine (Magnevist; Berlex Laboratories, Wayne, NJ). Contrast was injected at a rate of 5 mL/sec, followed by a 20-mL bolus of saline also at a rate of 5 mL/sec. A total of 60 images were acquired at 1-second intervals, with the injection occurring at the fifth image, so that the bolus would typically arrive at the 15th to 20th image. *c*) Post-Gd T1-weighted spin-echo (TR = 471, TE = 12, 50 contiguous, 3 mm-thick, axial slices with a 256×205 matrix and a $220 \times 220 \text{ mm}$ FOV, in-plane voxel size $1.7 \text{ mm} \times 0.85 \text{ mm}$).

Image Processing and Evaluation

Data were transferred to a Linux workstation for offline perfusion analysis using programs developed in-house using the IDL programming languages. In all cases contrast agent concentration, C , is first found using the simple relationship [23]

$$C \propto -\ln\left(\frac{S}{S_0}\right)$$

where S is the signal intensity and S_0 is the pre-bolus signal intensity averaged between acquisition 2 and 11. This equation assumes that T1 shortening effects are negligible which is true in practice since we use a relatively low flip angle to minimize saturation.

Absolute cerebral blood volume (CBV), cerebral blood flow (CBF) and mean transit time (MTT) were calculated using the method of Rempp *et al.* [24]. These parameters can be calculated from the following equations:

$$\begin{aligned}
 MTT &= \frac{\int C dt}{C_{\max}} \\
 CBV &= \frac{\int C dt}{\int AIF dt} \\
 CBF &= \frac{CBV}{MTT}
 \end{aligned}$$

where C is the tissue concentration following an ideal, instantaneous bolus and $C_{\max}$ is the maximum value of C . The bolus is not instantaneous, of course, but an approximation to the idealized response can be found by deconvolving the measured tissue concentration with the arterial input function (AIF). Deconvolution of the measured value of C by the AIF was performed by standard Fourier methods. Before deconvolution, gamma variate functions were fitted to both measured curves (C and AIF) in order to reduce noise and to correct for any leakage due to blood brain barrier (BBB) disruption (although in this study we expected little or no contrast extravasation). The AIF was found using an automated method similar to that described by Rempp *et al.* [24] and Carroll *et al.* [25].

MTT, CBV, and CBF were calculated in regions of interest (ROIs) in the following brain regions: periventricular and frontal NAWM, the thalamus, and the putament nuclei bilaterally. To optimize reproducibility, CBF, CBV, and MTT measurements were taken in ROIs from three consecutive slices in each of the eight locations. ROIs were fixed in size (radius 1 image pixel, 1.7 mm) and were placed to avoid arterial and venous structures in the NAWM, particularly in the subcortical and periventricular areas. When placing the reference ROI it is important to avoid blood vessels. The ROI is therefore positioned using images acquired at the bolus peak when vessels appear black and are quite distinct, rather than the initial images where vessels are isointense with brain tissue. ROIs were placed in the same section positions in patients and controls. Within these section positions, ROIs were placed in the same locations within the eight brain regions described above. Perfusion measurements were obtained by two authors (A.W.V. and M.I.) with more than five years of experience with this type of measurement in clinical and research settings. To exclude inter-observer and minimize intra-observer variability as to the location of ROI placement between patients, each patient data set was reviewed by both authors at the same time [26]. Furthermore, since all MRI sequences were centered to the same position, regardless of the number of slices, the ROI were placed on the axial gradient-echo echo planar image using the corresponding transverse T2-W image as reference to avoid the inclusion of lesions.

Lesion Volume Measurements

T2-hyperintense and T1-hypointense lesion volume (LV) measurements were performed by a single trained technician, blinded to subject identity, using a semiautomated segmentation technique based on local thresholding (Jim version 3, Xinapse Systems, UK, <http://www.xinapse.com>), using the marked hardcopies as a reference [27]. Out of 24 patients, only one affected by RR-MS presented 2 gadolinium-enhancing lesion on post-contrast T1 scans.

Statistical Analysis

The subject groups were compared with respect to each perfusion measure (CBF, CBV, MTT) in each brain region using mixed model regression based on ranks. Thus, the comparison with respect to a given measure in a specific region used the ranks of the measure as the dependent variable and the correlation structure was modeled by assuming observations to be correlated only when derived for the same subject and by allowing the error variance to differ across groups. The regression model included subject gender and subject group as fixed classification

factors and subject age as a numeric covariate. Regression was also used to compare subject groups in terms of T2-W and T1-W LVs. Since type 3 p values assess the effect of one factor adjusted for the effects of all other factors in the same model, they were used to assess differences between groups adjusted for the effects of age and gender. Spearman rank correlations were used to evaluate the association of each perfusion measure with T2-W and T1-W lesion volumes, EDSS and disease duration. Spearman rank correlations were also used to evaluate the association of each perfusion measure in the white matter with those in the gray matter. The perfusion measures were variously represented for each subject as an average over the two white matter regions or an average over the two gray matter regions. The correlations were assessed for each subject group separately. All reported p values are two-sided and were declared statistically significant when less than 0.05. SAS version 9.0 (SAS Institute, Cary, NC, 2002) was used for all statistical computations.

Results

None of the healthy controls showed lesions on T2- and T1-W scans. T2 and T1 LV for the CIS and RR-MS patients are given in Table 1. Since there were no significant differences between corresponding regions from the two hemispheres in terms of perfusion values (data not shown), CBF, CBV and MTT from periventricular and frontal NAWM, the thalamus, and the putamen nuclei averaged over the two hemispheres entered the statistical analysis. In addition, CBF, CBV and MTT values averaged over the two regions of NAWM and the two regions of NAGM were used to assess the associations with LVs, disease duration and EDSS. There were no significant differences among the three groups of subjects in terms of CBV and MTT in any of the examined brain areas ($p > 0.3$ for all comparisons). Results pertaining to group comparisons with respect to CBF, CBV, and MTT are given in Table 2.

Comparison of Perfusion metrics between Patients with CIS, RR-MS and Controls

After adjustment for age and sex, significant decreases were found between the CIS patients and the controls with respect to the CBF in periventricular ($p = 0.03$), but not in the frontal NAWM, or thalamus and putamen ($p > 0.4$) (Fig. 2). Also, significant decreases were found between the early RR-MS patients and the controls with respect to CBF in the periventricular NAWM ($p = 0.01$) and in the putamen ($p = 0.03$) but not in the frontal NAWM and in the thalamus ($p > 0.08$) (Fig. 2). Finally, compared to CIS, RR-MS patients showed a significant CBF decrease in the putamen ($p = 0.04$) and a trend towards statistical significance in the thalamus ($p = 0.06$) but not in the periventricular and frontal NAWM ($p > 0.4$) (Fig 2).

Neither T2 and T1 LVs, nor disease duration and EDSS scores correlated with the perfusion metrics from the four brain regions studied in both CIS and RR-MS patients. No association was found between CBF, CBV and MTT in NAWM and those in NAGM in any of the three groups of subjects. However, when CIS and RR-MS patients were considered as a whole group, a significant positive association was found between CBF in NAWM and in NAGM ($r = 0.6$; $p = 0.002$) and between CBV in NAWM and NAGM ($r = 0.5$; $p = 0.03$).

Discussion

This study used DSC perfusion imaging to measure CBF, CBV, and MTT in NAWM and sub-cortical NAGM of patients with CIS in comparison to those with early RR-MS and to healthy controls. While CBF was decreased in the periventricular NAWM in both CIS and RR-MS patients in comparison to controls, sub-cortical GM CBF was decreased in patients with early RR-MS but not in those with CIS.

Our findings in the early RR-MS group of patients are quite consistent with those of prior MR perfusion studies in patients with the same clinical course [13-16,28]. One of the early studies

using DSC MRI demonstrated that acute gadolinium-enhancing lesions in MS patients have higher relative CBV than contra-lateral NAWM [29]. However, since NAWM may be inappropriate as reference due to pathology of NAWM in its own right, more recent studies employed absolute measurements of CBF, CBV and MTT. The overall perfusion of MS patients in chronic lesions, and several regions of NAWM and NAGM is decreased compared to healthy control subjects [13,15,16,30].

A novel finding of our study is the presence of reduced perfusion in the NAWM of patients with CIS, albeit at a lesser degree than in patients with RR-MS. This is not surprising since patients with CIS already show evidence of “hidden” WM damage detected by non-conventional MRI techniques [31]. For example, magnetization transfer ratios in NAWM of CIS patients have been shown to be decreased [32,33] suggesting demyelination and axonal injury. Likewise, mean diffusivity was increased and fractional anisotropy was decreased in the NAWM of CIS patients [34]. Furthermore, spectroscopy studies in patients with CIS have shown decreases in whole-brain N-acetylaspartate reflecting axonal pathology [35] and increased myo-inositol in the NAWM, reflecting glial activity [36].

The interpretation of NAWM hypoperfusion in MS is not only based on the close vicinity of evolving plaques to deep WM venules, and on the intrinsic vulnerability of WM to hypoxia, but also on several histological and pathophysiological observations. Microvascular damage such as small venous thrombosis, vein wall hyalinization and intravascular fibrin deposition has been observed in MS lesions at histological examination [9,10]. Toxic inflammatory mediators might contribute to tissue hypoxia through mitochondrial injury [37,38]. Recent studies provide evidence for mitochondrial damage in MS lesions, which could be mediated by reactive oxygen and nitric oxide (NO) species [37,39]. In addition to NO and cytokine mediated changes in cerebral perfusion, altered glial cell homeostasis may contribute to vascular changes in MS [40]. Recently, it was reported that a rise of calcium levels within astrocytes induces constriction of blood vessels and consequently reduces CBF [41]. Finally, recent studies on gene expression in the NAWM of patients with MS showed that mRNAs of multiple genes involved in hypoxic preconditioning are significantly unregulated in comparison with control WM [42]. This notion is also supported by the observation that one of the four patterns of pathology described in MS lesions, distal dying-back oligodendroglialopathy, is the same pattern found in acute WM stroke, likely mediated in part by hypoxia inducible factor (HIF 1 alpha) [43,44]. Interestingly, this particular type of lesion has also been described in a study on early stages of MS lesions [45].

Unlike in patients with RR-MS, tissue perfusion was not decreased in the sub-cortical GM of patients with CIS suggesting a continuum of brain perfusion decreases in MS beginning in periventricular white matter, and spreading to other WM as well as GM regions during disease progression. Several direct and indirect mechanisms might have contributed to the deep GM hemodynamic impairment in RR-MS patients. First of all, MS lesions are frequently found in the thalamus and basal ganglia at post-mortem examination. However, lesions are often missed on T2-W images due to their small size and poor contrast with the surrounding GM. In addition, diminished blood supply has been described in MS plaques [29,30]. Although macroscopic deep GM lesions were not detected in our MS patients, theoretically, small deep GM lesions, not detected on MRI, could have contributed to the decrease of perfusion. Second, axonal transection in active WM lesions could lead indirectly to anterograde and retrograde degeneration of axons running within the thalamus and basal ganglia. Finally, axonal loss itself secondary to hypoperfusion, demyelination or Wallerian degeneration might contribute to CBF decrease via reduction in local metabolic activity. All these mechanisms, already active since the earliest stages of the disease, may lead to GM hypoperfusion as the disease progresses. Furthermore, the secondary nature of GM hypoperfusion seems to be supported by the association between WM and GM hypoperfusion in our patient's group.

To our knowledge, this is the first study to suggest heretofore that perfusion in the NAWM of CIS patients is decreased compared to healthy controls and that sub-cortical GM might be affected as the disease progresses secondarily to WM injury. There are several caveats to our study. First of all our sample size was small and, although we suggest that GM hypoperfusion develops as disease progresses, caution must be exercised before drawing firm conclusions since the patients with RR-MS were not the same ones who had CIS some years earlier. Second, our study is cross-sectional and we are essentially examining snapshots in time, which may have overlooked the dynamics of tissue perfusion changes. For instance, a previous longitudinal perfusion MRI study examining RR-MS patients found that increases in NAWM perfusion could be detected as much as 3 weeks before the development of a subsequent lesion [14], however these increases were over the background of overall decreased perfusion. Finally, the relative low resolution of DSC MRI and the high density of vessels in the cortical GM precluded us from measuring tissue perfusion in the cortex by means of region of interest analysis.

Examining perfusion patterns in patients with CIS and following them over time will help confirm our findings regarding the spread of perfusion decreases if and when those patients convert to clinically definite MS.

Acknowledgments

This study was supported by the National Institute of Health (NIH) grants RO1 NS051623 and RO1 NS 29029.

References

1. Hauser, SL. Multiple sclerosis and other demyelinating diseases. In: Isselbacher, KJ.; W, J.; Martin, JB.; Fauci, AS.; Kasper, DL., editors. *Harrison's Principles of Internal Medicine*. New York: McGraw-Hill; 1994. p. 2287-2295.
2. Bjartmar C, Trapp BD. Axonal degeneration and progressive neurologic disability in multiple sclerosis. *Neurotox Res* 2003;5(12):157–164. [PubMed: 12832230]
3. Dalton CM, Chard DT, Davies GR, Miszkil KA, Altmann DR, Fernando K, Plant GT, Thompson AJ, Miller DH. Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes. *Brain* 2004;127(Pt 5):1101–1107. [PubMed: 14998914]Epub 2004 Mar 1103
4. Dawson J. The histology of disseminated sclerosis. *Trans R Soc Edinb* 1916;108:397–399.
5. Putnam T. The pathogenesis of multiple sclerosis: a possible vascular factor. *New England Journal of Medicine* 1933;209:786–790.
6. Adams CW, Poston RN, Buk SJ. Pathology, histochemistry and immunocytochemistry of lesions in acute multiple sclerosis. *Journal of the Neurological Sciences* 1989;92(23):291–306. [PubMed: 2809622]
7. Tanaka R, Iwasaki Y, Koprowski H. Ultrastructural studies of perivascular cuffing cells in multiple sclerosis brain. *American Journal of Pathology* 1975;81(3):467–478. [PubMed: 1211421]
8. Putnam T. Evidences of vascular occlusion in multiple sclerosis and encephalomyelitis. *Arch Neurol Neuropsychol* 1935;32:1298–1321.
9. Adams CW, Poston RN, Buk SJ, Sidhu YS, Vipond H. Inflammatory vasculitis in multiple sclerosis. *J Neurol Sci* 1985;69(3):269–283. [PubMed: 4031947]
10. Wakefield AJ, More LJ, Difford J, McLaughlin JE. Immunohistochemical study of vascular injury in acute multiple sclerosis. *J Clin Pathol* 1994;47(2):129–133. [PubMed: 8132826]
11. Proescholdt MA, Jacobson S, Tresser N, Oldfield EH, Merrill MJ. Vascular endothelial growth factor is expressed in multiple sclerosis plaques and can induce inflammatory lesions in experimental allergic encephalomyelitis rats. *Journal of Neuropathology & Experimental Neurology* 2002;61(10): 914–925. [PubMed: 12387457]

12. Su JJ, Osoegawa M, Matsuoka T, Minohara M, Tanaka M, Ishizu T, Mihara F, Taniwaki T, Kira J. Upregulation of vascular growth factors in multiple sclerosis: correlation with MRI findings. *Journal of the Neurological Sciences* 2006;243(12):21–30. [PubMed: 16376944]
13. Rashid W, Parkes LM, Ingle GT, Chard DT, Toosy AT, Altmann DR, Symms MR, Tofts PS, Thompson AJ, Miller DH. Abnormalities of cerebral perfusion in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2004;75(9):1288–1293. [PubMed: 15314117]
14. Wuerfel J, Bellmann-Strobl J, Brunecker P, Aktas O, McFarland H, Villringer A, Zipp F. Changes in cerebral perfusion precede plaque formation in multiple sclerosis: a longitudinal perfusion MRI study. *Brain* 2004;127(Pt 1):111–119. [PubMed: 14570816]
15. Adhya S, Johnson G, Herbert J, Jaggi H, Babb JS, Grossman RI, Inglese M. Pattern of hemodynamic impairment in multiple sclerosis: dynamic susceptibility contrast perfusion MR imaging at 3.0 T. *Neuroimage* 2006;33(4):1029–1035. [PubMed: 16996280]Epub 2006 Sep 1022
16. Inglese M, Park SJ, Johnson G, Babb JS, Miles L, Jaggi H, Herbert J, Grossman RI. Deep gray matter perfusion in multiple sclerosis: dynamic susceptibility contrast perfusion magnetic resonance imaging at 3 T. *Arch Neurol* 2007;64(2):196–202. [PubMed: 17296835]
17. Andeweg J. The anatomy of collateral venous flow from the brain and its value in aetiological interpretation of intracranial pathology. *Neuroradiology* 1996;38(7):621–628. [PubMed: 8912316]
18. White E, Greitz T. Subependymal venous filling sequence at cerebral angiography. Influence of grey and white matter distribution. *Acta Radiol Diagn (Stockh)* 1972;13(1):272–285. [PubMed: 4660131]
19. Herzog H, Seitz RJ, Tellmann L, Rota Kops E, Julicher F, Schlaug G, Kleinschmidt A, Muller-Gartner HW. Quantitation of regional cerebral blood flow with 15O-butanol and positron emission tomography in humans. *J Cereb Blood Flow Metab* 1996;16(4):645–649. [PubMed: 8964804]
20. Helenius J, Perkio J, Soinne L, Ostergaard L, Carano RA, Salonen O, Savolainen S, Kaste M, Aronen HJ, Tatlisumak T. Cerebral hemodynamics in a healthy population measured by dynamic susceptibility contrast MR imaging. *Acta Radiol* 2003;44(5):538–546. [PubMed: 14510762]
21. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van den Noort S, Weinshenker BY, Wolinsky JS. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001;50(1):121–127. [PubMed: 11456302]
22. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;33(11):1444–1452. [PubMed: 6685237]
23. Rosen BR, Belliveau JW, Buchbinder BR, McKinsty RC, Porkka LM, Kennedy DN, Neuder MS, Fisel CR, Aronen HJ, Kwong KK, et al. Contrast agents and cerebral hemodynamics. *Magn Reson Med* 1991;19(2):285–292. [PubMed: 1881317]
24. Rempp KA, Brix G, Wenz F, Becker CR, Guckel F, Lorenz WJ. Quantification of regional cerebral blood flow and volume with dynamic susceptibility contrast-enhanced MR imaging. *Radiology* 1994;193(3):637–641. [PubMed: 7972800]
25. Carroll TJ, Rowley HA, Haughton VM. Automatic calculation of the arterial input function for cerebral perfusion imaging with MR imaging. *Radiology* 2003;227(2):593–600. [PubMed: 12663823]
26. Wetzel SG, Cha S, Johnson G, Lee P, Law M, Kasow DL, Pierce SD, Xue X. Relative cerebral blood volume measurements in intracranial mass lesions: interobserver and intraobserver reproducibility study. *Radiology* 2002;224(3):797–803. [PubMed: 12202717]
27. Bermel RA, Sharma J, Tjoa CW, Puli SR, Bakshi R. A semiautomated measure of whole-brain atrophy in multiple sclerosis. *J Neurol Sci* 2003;208(12):57–65. [PubMed: 12639726]
28. Law M, Saindane AM, Ge Y, Babb JS, Johnson G, Mannon LJ, Herbert J, Grossman RI. Microvascular abnormality in relapsing-remitting multiple sclerosis: perfusion MR imaging findings in normal-appearing white matter. *Radiology* 2004;231(3):645–652. [PubMed: 15163806]
29. Haselhorst R, Kappos L, Bilecen D, Scheffler K, Mori D, Radu EW, Seelig J. Dynamic susceptibility contrast MR imaging of plaque development in multiple sclerosis: application of an extended blood-brain barrier leakage correction. *J Magn Reson Imaging* 2000;11(5):495–505. [PubMed: 10813859]
30. Ge Y, Law M, Johnson G, Herbert J, Babb JS, Mannon LJ, Grossman RI. Dynamic susceptibility contrast perfusion MR imaging of multiple sclerosis lesions: characterizing hemodynamic

- impairment and inflammatory activity. *AJNR Am J Neuroradiol* 2005;26(6):1539–1547. [PubMed: 15956527]
31. Miller D, Barkhof F, Montalban X, Thompson A, Filippi M. Clinically isolated syndromes suggestive of multiple sclerosis, part 2: non-conventional MRI, recovery processes, and management. *Lancet Neurology* 2005;4(6):341–348. [PubMed: 15907738]
 32. Iannucci G, Tortorella C, Rovaris M, Sormani MP, Comi G, Filippi M. Prognostic value of MR and magnetization transfer imaging findings in patients with clinically isolated syndromes suggestive of multiple sclerosis at presentation. *Ajnr: American Journal of Neuroradiology* 2000;21(6):1034–1038. [PubMed: 10871009]
 33. Brex PA, Leary SM, Plant GT, Thompson AJ, Miller DH. Magnetization transfer imaging in patients with clinically isolated syndromes suggestive of multiple sclerosis. *Ajnr: American Journal of Neuroradiology* 2001;22(5):947–951. [PubMed: 11337341]
 34. Gallo A, Rovaris M, Riva R, Ghezzi A, Benedetti B, Martinelli V, Falini A, Comi G, Filippi M. Diffusion-tensor magnetic resonance imaging detects normal-appearing white matter damage unrelated to short-term disease activity in patients at the earliest clinical stage of multiple sclerosis. *Archives of Neurology* 2005;62(5):803–808. [PubMed: 15883269]
 35. Filippi M, Bozzali M, Rovaris M, Gonen O, Kesavadas C, Ghezzi A, Martinelli V, Grossman RI, Scotti G, Comi G, Falini A. Evidence for widespread axonal damage at the earliest clinical stage of multiple sclerosis. *Brain* 2003;126(Pt 2):433–437. [PubMed: 12538409]
 36. Fernando KT, McLean MA, Chard DT, MacManus DG, Dalton CM, Miskiel KA, Gordon RM, Plant GT, Thompson AJ, Miller DH. Elevated white matter myo-inositol in clinically isolated syndromes suggestive of multiple sclerosis. *Brain* 2004;127(Pt 6):1361–1369. [PubMed: 15128615]
 37. Lu F, Selak M, O'Connor J, Croul S, Lorenzana C, Butunoi C, Kalman B. Oxidative damage to mitochondrial DNA and activity of mitochondrial enzymes in chronic active lesions of multiple sclerosis. *J Neurol Sci* 2000;177(2):95–103. [PubMed: 10980305]
 38. Kalman B, Leist TP. A mitochondrial component of neurodegeneration in multiple sclerosis. *Neuromolecular Med* 2003;3(3):147–158. [PubMed: 12835510]
 39. Heales SJ, Bolanos JP, Stewart VC, Brookes PS, Land JM, Clark JB. Nitric oxide, mitochondria and neurological disease. *Biochim Biophys Acta* 1999;1410(2):215–228. [PubMed: 10076028]
 40. De Keyser J, Steen C, Mostert JP, Koch MW. Hypoperfusion of the cerebral white matter in multiple sclerosis: possible mechanisms and pathophysiological significance. *J Cereb Blood Flow Metab* 2008;28(10):1645–1651. [PubMed: 18594554] Epub 2008 Jul 1642
 41. Mulligan SJ, MacVicar BA. Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. *Nature* 2004;431(7005):195–199. [PubMed: 15356633]
 42. Graumann U, Reynolds R, Steck AJ, Schaeren-Wiemers N. Molecular changes in normal appearing white matter in multiple sclerosis are characteristic of neuroprotective mechanisms against hypoxic insult. *Brain Pathol* 2003;13(4):554–573. [PubMed: 14655760]
 43. Lassmann H. Hypoxia-like tissue injury as a component of multiple sclerosis lesions. *J Neurol Sci* 2003;206(2):187–191. [PubMed: 12559509]
 44. Semenza GL. Surviving ischemia: adaptive responses mediated by hypoxia-inducible factor 1. *Journal of Clinical Investigation* 2000;106(7):809–812. [PubMed: 11018065]
 45. Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann Neurol* 2004;55(4):458–468. [PubMed: 15048884]

Figure 1. Selected axial gradient-echo echo-planar magnetic resonance images (A, C), and color-coded cerebral blood flow (CBF) map (B, D) from a patient with clinically isolated syndrome. Circular regions of interest were placed on the frontal and periventricular NAWM and on the thalamus and putamen nuclei bilaterally. The color bars indicate the CBF values (in milliliters per 100 grams per minute).

Figure 2.

Box plots display the 25% to 75% values (boxes) $\pm$ 95% values (whiskers), median values (horizontal lines within boxes) of mean absolute cerebral blood flow (CBF) value distribution in the putamen nucleus (A) and in the periventricular NAWM (B) among healthy controls (CTRL) (empty box), patients with clinically isolated syndrome (CIS) (hatched box) and patients with relapsing-remitting multiple sclerosis (RR-MS) (crosshatched box). Only significant P values are reported. Note significantly lower CBF values in the periventricular NAWM and putamen of patients with RR-MS and in the periventricular NAWM of patients with CIS.

Table 1

Demographic, clinical and conventional MRI characteristics of CIS, MS patients and healthy controls.

	CIS Patients (12)	RR-MS Patients (12)	Healthy Controls (12)
Mean Age, (range) y	41.4 (28-56)	47.5 (32-62)	42.0 (24-54)
Gender, F/M	9/3	7/5	6/6
Median EDSS Score, (range)	1.0 (0.0-2.0)	1.5 (0.0-2.5)	-
Mean Disease Duration, (range) months	18.6 (3.0-58)	47.7 (12-62)	-
Mean T2 LV (ml) (range)	2.0 (0.2-11.8)	3.8 (0.6-27.8)	-
Mean T1 LV (ml) (range)	0.05 (0.0-0.12)	0.85 (0.01-3.2)	-

CIS = clinically isolated syndrome; RR-MS = relapsing-remitting multiple sclerosis; EDSS = Expanded Disability Status Scale; LV = lesion volume

Table 2

CBF, CBV, and MTT values from NAWM and subcortical NAGM in healthy controls and patients with CIS and RR-MS.

	Healthy Controls	CIS Patients	RR-MS Patients
CBF (ml/100g/min)			
Periventricular NAWM	39.73 ± 5.0	36.24 ± 9.1 *	33.53 ± 6.1 *
Frontal NAWM	39.03 ± 6.6	37.97 ± 12.5	34.53 ± 7.2
Thalamus	68.45 ± 13.3	68.74 ± 8.0	61.82 ± 5.4
Putamen	71.91 ± 8.9	72.52 ± 12.0 **	63.92 ± 6.4 ***
CBV (ml/100g)			
Periventricular NAWM	2.62 ± 0.6	2.50 ± 0.8	2.40 ± 0.4
Frontal NAWM	2.63 ± 0.6	2.89 ± 1.3	2.43 ± 0.6
Thalamus	4.90 ± 0.9	4.27 ± 0.9	4.54 ± 1.2
Putamen	4.94 ± 0.6	4.46 ± 0.9	4.55 ± 0.8
MTT (Seconds)			
Periventricular NAWM	3.94 ± 0.7	4.14 ± 0.7	4.33 ± 0.5
Frontal NAWM	4.03 ± 0.6	4.39 ± 0.6	4.20 ± 0.6
Thalamus	4.36 ± 0.6	3.79 ± 0.9	4.27 ± 0.7
Putamen	4.15 ± 0.6	3.80 ± 0.8	4.31 ± 0.7

Values are given as means ± SD.

CBF = cerebral brain flow; CBV = cerebral brain volume; MTT = mean transit time; NAWM = normal-appearing white matter; NAGM = normal-appearing gray matter

* p<0.05 versus healthy controls,

** p<0.05 comparison between CIS and RR patients