Journal of Clinical Neuroscience (2005) **12**(8), 895–901
0967-5868/$ - see front matter
doi:10.1016/j.jocn.2004.11.010

Clinical study

Asymmetries in the spatial distributions of enhancing lesions and black holes in relapsing-remitting MS

James A Koziol[1] PhD, Simone Wagner[1,2] MD, David F Sobel[3] MD, Anne C Feng[1] MS, Hans-Peter Adams[1] DIM

[1]Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, [2]Department of Neurology, Ruprecht - Karls - University, Heidelberg, Germany [3]Department of Radiology, Scripps Clinic, La Jolla, CA

Summary Magnetic resonance imaging (MRI) is the most important paraclinical test in the diagnosis of multiple sclerosis (MS) and for delineating its natural history. We investigate MRIs from a longitudinal study of 24 relapsing-remitting MS patients who had monthly MRI examinations for one year, and were not receiving active MS therapy during this period. We hypothesized that lesions occur randomly throughout the brain, and that patients are homogeneous with regard to spatial patterns of lesion presentation. We recorded the numbers and locations of enhancing lesions and hypointense lesions (black holes) in all scans, and found asymmetrical patterns of lesions about the mid-transaxial, mid-coronal, and mid-sagittal planes. Furthermore, in distinct subsets of patients, enhancing lesions and black holes tend to occur in the same locations. Clustering in lesion locations may be of functional significance, with consequent therapeutic implications.

Keywords: enhancing lesions, black holes, relapsing-remitting MS, spatial distributions

BACKGROUND

Multiple sclerosis (MS) is a common demyelinating disease in humans, with a prevalence in excess of 250,000 cases in the United States. The disease usually begins in adults between 15 and 45 years of age and typically has a relapsing-remitting course. The pathologic hallmark of MS is the plaque, an area of demyelination in the white matter often accompanied by inflammation. Cranial magnetic resonance imaging (MRI) has become the most important paraclinical test for diagnosing MS, for delineating its natural history, and potentially for use as an objective quantitative outcome measure in assessing the response of MS patients to experimental therapy. The images reveal the multiple lesions that grow and shrink at different rates in various regions of the brain.

We investigate the MRI lesion findings in a longitudinal study of twenty-four relapsing-remitting MS patients who had monthly MRI examinations for one year, and were not receiving active therapy for MS over this period. In particular, we examine the spatial distributions of enhancing lesions and hypointense lesions (black holes) in these patients. Our aims are to assess the hypotheses that lesions occur randomly throughout the brain, and that patients are homogeneous with regard to spatial patterns of lesion presentation.

METHODS

Study design

A randomized, double-blind, placebo-controlled clinical trial of Cladribine for treatment of relapsing-remitting multiple sclerosis (MS) was conducted in the General Clinical Research Center of Scripps Clinic.[1] Fifty-two patients, all of whom had clinically definite relapsing-remitting MS of at least two years' duration, were enrolled in the trial, after having provided informed consent. Patients were stratified on the basis of age and severity (baseline Scripps Neurologic Rating Scale, SNRS), and then randomized to either a placebo arm ($n = 25$) or a Cladribine arm ($n = 27$). The two groups did not differ significantly at baseline with regard to sex, race, age, years with symptoms, SNRS, or Expanded Disability Status Score (EDSS) values. Two patients, one in each arm, dropped out prior to the planned one year study duration. The primary results of the trial are reported elsewhere.[1] The analyses reported here are based on the 24 placebo patients who were evaluable at 12 months, as Cladribine has a profound effect on the incidence of enhancing lesions (which in turn would confound our findings).

Received 30 September 2004
Accepted 19 November 2004

Correspondence to: James A. Koziol, PhD, The Scripps Research Institute, Department of Molecular and Experimental Medicine, MEM216, 10550 N. Torrey Pines Rd, La Jolla, CA, 92037. Tel.: +858 784 2703;
Fax: +858 784 2664;
E-mail: koziol@scripps.edu

Magnetic resonance imaging

Patients received MRI examinations at baseline (time of entry into the trial), and then at one-month intervals thereafter over the one year duration of the trial. There were no missing scans among the 24 patients considered herein. All MRI examinations were performed on a 1.5 T General Electric Signa scanner, at the MRI facility of Scripps Clinic. T2- and proton density-weighted images were obtained using a conventional spin-echo sequence with repetition times of 2,500 ms and echo delay times of 30 and 90 ms. Sections were 4 mm thick with a 1 mm interslice gap. The image matrix was 256×256 fort all images, and the field of view was set to 22×22 cm. T1-weighted images (repetition time 600 ms, echo delay time 20 ms) of 3 mm thickness and 0 mm interslice gap were taken approximately 10 minutes after gadopentotate dimeglumine (Magnevist, Berlex Laboratories, Wayne, NJ, USA) injection (0.1 mmol/kg).

Two observers (SW, DFS) agreed on the definition of hypointense lesions as described by Truyen et al.[2] and, in a preliminary study, evaluated a number of images of patients with MS who were not included in the clinical trial. Subsequently, one observer (SW) undertook quantification of numbers of all enhancing lesions, new enhancing lesions, all black holes, and new black holes on a monthly basis for each patient; clarification and review were provided by the other observer (DFS). Both observers were blinded to the clinical data, including treatment assignment. For both enhancing lesions and black holes, each lesion was further classified as occurring on the left side or the right side of: the brain stem, cerebellum, frontal, temporal, parietal, or occipital

periventricular regions (that is, adjacent to the periventricular system), or, other frontal, temporal, parietal, or occipital white matter nonadjacent to the periventricular system (that is, a total of 20 spatial locations).

Reproducibility of counts was assessed by the random selection of 56 images, and reevaluation was performed by the primary observer (SW) approximately two months after completion of all counting. The kappa statistic was calculated to assess the intrarater level of agreement with replicate counts. Reproducibility was reasonably good with regard to counts of both enhancing lesions (kappa = 0.67, standard error = 0.07) and black holes (kappa = 0.70, standard error = 0.08). In addition, counts of enhancing lesions were found to be strongly correlated with corresponding lesion volumes, $r = 0.92$.[3] See Wagner et al. for further considerations relating to lesion counts, and MR methodology.[3]

Statistical methods

Within each patient, we investigated symmetry in lesion locations about three planes: a midtransaxial plane, dividing the brain into infratentorial versus supratentorial regions; a mid-coronal plane, dividing the brain into anterior versus posterior regions; and, a mid-sagittal plane, dividing the brain into left versus right regions. Since our methodology for examining bilateral symmetry about each of these planes is similar, we therefore describe solely our methods for investigating bilateral symmetry in lesion locations about the midsagittal plane. We first amalgamated lesion counts over all of the monthly MRIs, with the dichotomization of locations as either on the left or on the right side. Exact binomial tests were then used to assess the null hypotheses that enhancing lesions and black holes would occur equally frequently on the left side and the right side of the brain, against the two-sided alternative that lesions would occur preferentially on one or the other side [as a priori we would not expect a particular side to be favored or disfavored]. In addition, exact 95% confidence intervals were constructed about the observed proportions of lesions occurring on the predominant side [Fig. 2]. An omnibus test of the null hypothesis that lesion locations were bilaterally symmetric was obtained by first computing the two-sided p-value of the binomial test within each patient,[4] adjusting for discreteness of the binomial distribution,[5] and then combining the p-values across patients with Fisher's combination procedure with attendant test statistic F_C:[6] under the null hypothesis, F_C would be distributed as a (central) chi-square random variable, and large values of F_C would lead to rejection. Summary estimates of sidedness of lesion locations [Fig. 2] were calculated from a random effects model as applied to the individual binomial proportions;[7] in particular, the random effects model assumes that the patients are a random sample from a larger population, and explicitly incorporates a measure of heterogeneity between the subjects into the summary point and interval estimates.

Between patient comparisons of lesion locations were first undertaken with Fisher's exact tests (denoted F_E) for $R \times C$ contingency tables, after amalgamating individual lesion counts over all of the monthly MRIs, and over both sides of the brain. [This resulted in the categorization of enhancing lesions and black holes into 10 locations: brain stem, cerebellum, periventricular region (frontal, temporal, parietal, or occipital), or other white matter (frontal, temporal, parietal, or occipital).] In this setting, Fisher's exact test assesses the null hypothesis that the distributions of lesion locations are homogeneous across patients. Because many of the cell counts in the classification of lesion counts by locations were small, the large sample distribution theory attending Fisher's exact test is likely invalid, and exact p-values were estimated from Monte Carlo simulation of the underlying permutation distributions using StatXact 5 (Cytel Software Corp., Cambridge, MA, USA, 2001). After these overall tests of homogeneity were rejected in each case, hierarchical cluster analyses[8] were used to identify subsets of patients with relatively homogeneous patterns of lesion presentation in terms of locations. In this regard, we first normalized individual patient data relating to total enhancing lesions and total black holes to proportions occurring in each of the 10 locations. Distances between patients were computed with a standard Euclidean metric; and, Ward's method[9] was used for cluster identification.

The Mantel-Haenszel method[7] was used to compute the summary odds ratio of location vs. type of lesion across all patients, after tabulating the frequencies of enhancing lesions and black holes occurring in the periventricular region or other white matter for each patient.

RESULTS

Baseline demographic and clinical characteristics

Demographics relating to the 24 patients with relapsing-remitting multiple sclerosis randomized onto the placebo arm of the clinical trial and evaluable at 12 months are presented in Table 1. As might be expected, the female:male ratio is about 2:1, with all patients being Caucasian. None of the 24 patients were prescribed corticosteroids for treatment of exacerbations during the course of the trial [although such treatment was allowable under the protocol].

Lesion locations: within patient comparisons

We first investigated whether enhancing lesions and black holes demonstrate bilateral symmetry about the mid-transaxial plane, the mid-coronal plane, and the mid-sagittal plane within individual patients. All lesions in each patient had initially been classified from the monthly MRIs as occurring in the brain stem, cerebellum, periventricular region (frontal, temporal, parietal, or occipital), and other white matter (frontal, temporal, parietal, or occipital), with all lesion locations further identified as occurring on the left side or the right side of the brain. Within each patient, we amalgamated counts over all 12 months and specific locations, resulting in dichotomous counts, as follows: for transaxial symmetry, we compared supratentorial (frontal, temporal, parietal, or occipital) versus infratentorial (cerebellum, brain stem) lesions; for midcoronal symmetry, we compared frontal and parietal versus temporal, occipital, cerebellum, and brain stem lesions; and for midsagittal symmetry, we compared left-side versus right-side lesions.

Table 1 Baseline demographic and clinical characteristics of 24 relapsing-remitting multiple sclerosis patients with monthly MRIs over one year

Sex	Male	7
	Female	17
Race	White	24
	Other	0
Number of Exacerbations	1	12
	2	5
	3 or 4	7

		Percentile			
	Mean	25th	50th	75th	Range
Age (yrs)	40.1	36.5	41.0	44.0	31–52
Symptoms (yrs)	9.1	3.5	9.0	12.5	1–25
Baseline EDSS	3.8	2.5	3.5	5.3	2–6.5