

Seasonal Variations in Exacerbations and MRI Parameters in Relapsing-Remitting Multiple Sclerosis

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Key Words

Seasonality · Cyclic variation · Periodic regression · Relapsing-remitting multiple sclerosis

Abstract

Environmental factors may be involved in the etiology of multiple sclerosis (MS). We investigate prevalence of exacerbations and MRI findings in a cohort of relapsing-remitting multiple sclerosis patients, for evidence of seasonal variation or cyclic trends. We find only weak evidence of seasonality in our data. Differences in reports of seasonal variation in multiple sclerosis disease activity may be due to regional climatic differences or other geographic variables that change with latitude as well as genetic predisposition.

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Introduction

Multiple sclerosis is an inflammatory demyelinating disease, one of the most common disabling neurologic diseases among adults in North America and northern Europe. Epidemiological studies have suggested that environmental factors are involved in the etiology of the disease [1]. In this report, we investigate the hypothesis that various events associated with relapsing-remitting multiple sclerosis, including not only clinical exacerbations but

also various documented events on magnetic resonance images (MRIs), evince seasonal variation or annual cyclic trends. The study was conducted on a cohort of 24 individuals with relapsing-remitting multiple sclerosis, who were seen monthly over the course of one year as part of a randomized clinical trial [2]. In particular, documentation of clinical exacerbations was complete and each individual received monthly MRIs. We here examine the exacerbation and MRI data for evidence of seasonal variation or cyclic trends, supportive of the notion that environmental factors contribute to the etiology of multiple sclerosis.

Methods

Study Design

A randomized, double-blind, placebo-controlled clinical trial of cladribine for treatment of relapsing-remitting multiple sclerosis was conducted in the General Clinical Research Center of Scripps Clinic [2]. Fifty-two patients, all of whom had clinically definite relapsing-remitting multiple sclerosis of at least two years' duration [3], were enrolled in the trial, after having given informed consent. All patients had a history of two or more relapses in the previous two years and Extended Disability Status Scores (EDSS) of 6.5 or less at time of study entry. 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 only other treatment allowed during the study period was corticosteroids for acute relapses (exacerbations), at the discretion of the attending neurologists. The two groups did not differ significantly at baseline with regard to sex, race, age, years with symptoms, SNRS, or

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 [2]. The analyses summarized here are based on the 24 placebo patients who were evaluable at 12 months, as cladribine has a profound impact on the incidence of enhancing lesions and the occurrence of exacerbations (which in turn would confound our findings). All of these 24 placebo patients were enrolled in a 13-month window from June 1994 to July 1995, with 17 of the 24 entering in the initial 6 months of the trial.

Clinical neurologic examinations of all patients were performed at study entry and were repeated every month for one calendar year, as well as within 48 h of report by a patient of a relapse (exacerbation). A clinical relapse was defined as the appearance of new symptoms or worsening of an existing symptom attributable to multiple sclerosis and accompanied by objective worsening of neurologic findings. To be scored as a relapse, the alteration must have been preceded by disease stability or improvement lasting for at least 30 days and the worsening must have lasted at least 24 h and have occurred in the absence of fever. All relapses were identified by the attending neurologists who were blinded to treatment assignment, as described previously [2].

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. 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 for 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 min after gadopentotate dimeglumine (Magnevist, Berlex Laboratories, Wayne, NJ, USA) injection (0.1 mmol/kg).

Quantification of enhancing lesions and hypointense lesions (black holes) on the monthly MRI scans was undertaken in a blinded fashion by one experienced neurologist; details are reported elsewhere [4].

Statistical Methods

The statistical problem inherent in these data is to compare the null hypothesis of a uniform distribution of events throughout the year (i.e., no seasonal variation) against the alternative of a seasonal fluctuation of frequency of events over the year. Edwards [5] proposed a method that has been widely used in epidemiological investigations for assessing this hypothesis, with the alternative that the frequencies follow a sinusoidal curve of period 12 months. Note that such a curve has just one peak and one trough during the year, precisely the pattern reported by Auer et al. [6] and others. Hence we tallied the numbers of exacerbations observed per month from the clinical records of the placebo patients. Since each patient was observed for exactly one year, each patient contributed 12 months of observation to the total. Similarly, we tallied the numbers of months in which the monthly MRIs had new T₁-enhancing lesions, any T₁-enhancing lesions, new black holes, or any black holes. We chose to use a variant of Edwards' test, the score test of Roger [7], to examine evidence of cyclicity in these exacerbation and MRI data. (Roger's test should be more accurate than Edwards' test with moderate num-

bers of events, that is, small to medium-sized samples.) For illustrative purposes, we chose 3-month moving averages so as to emphasize any potential seasonal effect [8].

We also examined whether cyclicity exists in the actual counts of both enhancing lesions and black holes from the monthly MRIs. In this regard, there exist well-established regression models for cyclical variation [9, 10], which are immediately applicable to our setting. In particular, the periodic regression model may here be written

$$Y_{ij} = m_i + a_i \cos \{(2 \pi t_{ij}/12) - \phi_i\} + e_{ij},$$

where Y_{ij} is the j th observation (count) from the i th individual at month t_{ij} , $i = t_{ij} = 12$. The unknown parameters m_i , a_i and ϕ_i represent the mean level, amplitude, and phase respectively for the i th individual, $1 = i = 24$. The errors e_{ij} are assumed to be normally distributed, independent across patients. The phase angle ϕ_i can be determined from the regression equation, as explained by Bliss [9]. Also, a useful index of variability about the periodic regression line is the ratio $B_i = a_i/m_i$; for example, $B_i = 0.1$ denotes a swing in counts of 10% from the mean in both directions during the year.

We used a random effects formulation [11] to determine the overall fit of the data to the periodic model, while allowing the individual patient fits to vary appropriately. We fit this model using the LME routine in the statistical software package S-Plus 5 (MathSoft Inc., Seattle, 1998).

Results

Our investigation is based on the monthly clinical and MRI findings of 24 patients with relapsing-remitting multiple sclerosis, each of whom was observed over a one-year period during the course of a clinical trial during which he or she received a placebo [2]. All 24 patients were Caucasian; the female:male ratio was 17:7; their median age was 41 years (range 31–52 years); median years with symptoms was 9.0 (range 1–25 years); the median EDSS at start of the trial was 3.5 (range 2–6.5); the median SNRS at start of the trial was 75.5 (range 54–98).

In figure 1 we plot the monthly numbers of exacerbations cumulatively experienced by the 24 patients over the one-year period of observation for each patient. We also smooth the data slightly, using a 3-month moving average. There is some suggestion of a cyclic trend, with exacerbations peaking in the months of May, July, August and September, dipping in the months October through March, though the late spring-summer peak is counteracted by the singular lack of any exacerbations in June. Formally, Roger's statistic $R = 0.47$, $p = 0.79$, provides no evidence in support of a cyclic trend.

In figure 2 we plot the proportions of monthly MRIs in the 24 patients in whom new enhancing lesions, any enhancing lesions, new black holes, or any black holes were detected. Again, we smooth the data with 3-month moving averages. New enhancing lesions exhibit peaks in

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Fig. 1. Number of exacerbations (left Y-axis) and proportion of patients with exacerbations (right Y-axis) per month, in a cohort of 24 relapsing-remitting multiple sclerosis patients, over the course of one year. Dots denote the actual numbers and proportions, which have been smoothed with a three-month moving average (solid line).

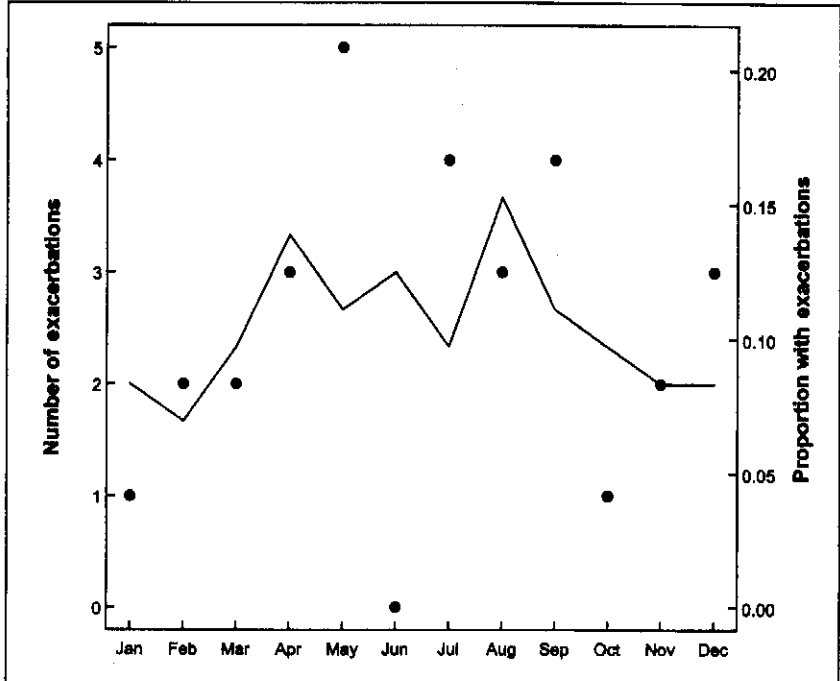
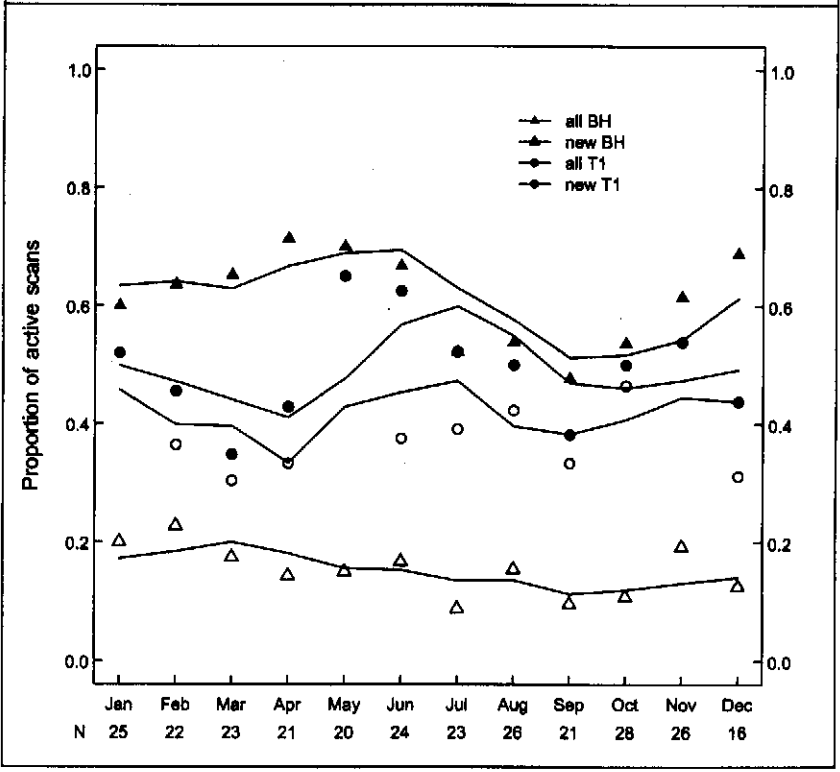


Fig. 2. Proportions of active MRI scans in a cohort of 24 relapsing-remitting multiple sclerosis patients, each of whom received a monthly MRI scan over the course of one year. Four parameters reflecting MRI activity are depicted: presence of any enhancing lesions on the MRI scans (denoted all T1); presence of new enhancing lesions on the MRI scans (new T1); presence of any black holes on the MRI scans (all BH); and, presence of new black holes on the MRI scans (new BH). Symbols denote the actual proportions; the solid lines are three-month moving averages. Numbers of scans evaluated per month are indicated beneath the X-axis, denoted N.



both summer and winter, compared to all enhancing lesions, which are maximal from June through August. There is less variability in the frequency of new black holes, which appear to peak in January and February; in comparison, all black holes are maximal roughly from

March through June. The Roger statistics again afford little support for cyclicity with these data: new enhancing lesions, $R = 0.09$, $p = 0.95$; any enhancing lesions, $R = 0.93$, $p = 0.63$; new black holes, $R = 2.78$, $p = 0.25$; any black holes, $R = 1.84$, $p = 0.40$.

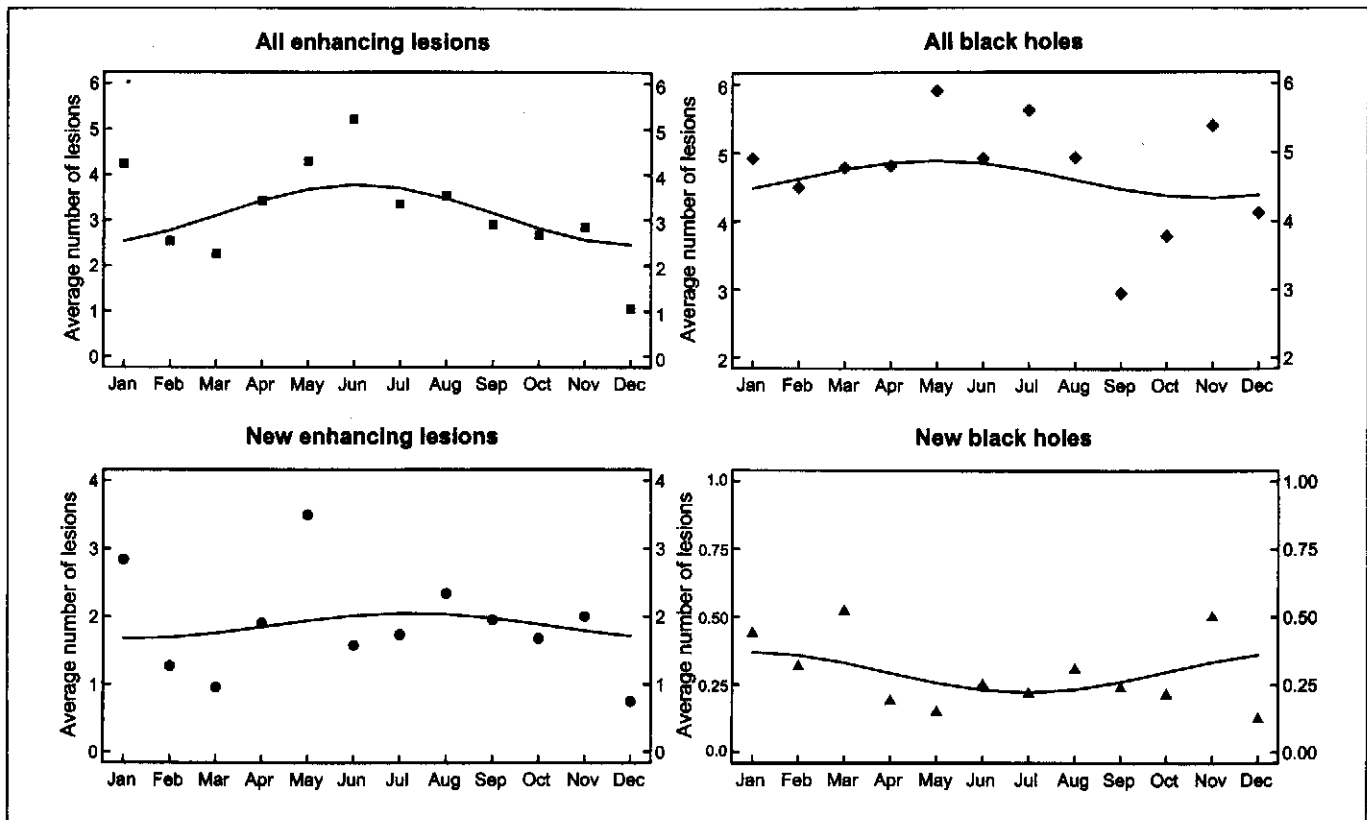


Fig. 3. Periodic regression fits to numbers of lesions observed on the MRI scans in a cohort of 24 relapsing-remitting multiple sclerosis patients, each of whom received a monthly MRI scan over the course

of one year. See Methods for description of the periodic regression model. Symbols denote the average number of lesions; the solid lines are the summary fits.

We used a random effects formulation of a standard periodic regression model to examine the extent of periodicity in the actual counts of enhancing lesions and black holes on a monthly basis. The summary fits are presented in figure 3, along with the raw data. The summary fits are in conformity with the prevalence plots in figure 2. For all enhancing lesions, the summary fit peaks at day 182, with index of variability $B = 0.21$; for new enhancing lesions, the summary fit peaks at day 220, with $B = 0.10$. In comparison, the summary fit for all black holes peaks at day 147, with $B = 0.06$; and, the summary fit for new black holes peaks at day 29, with $B = 0.25$.

Lastly, a reviewer has asked us to comment on whether stratification by duration of illness showed any seasonality. We therefore divided our cohort into two subsets, the first with duration of illness 6 years or less ($n = 11$) and the second with duration of illness exceeding 6 years ($n = 13$). We found no evidence of seasonality in either of the subsets, but this may well be attributable to the relatively small sample sizes per subset and the relatively modest

overall indications of seasonality noted above. More fundamentally, we investigate whether clinical exacerbations and MRI parameters are at all related to duration of illness, disregarding seasonality. In figure 4 we present scatter plots of exacerbations and MRI parameters for the two subsets of patients stratified by duration of illness. Wilcoxon tests comparing the various observations between the two groups were all insignificant: exacerbations, $p = 0.81$; total enhancing lesions, $p = 0.77$; total black holes, $p = 0.75$; new enhancing lesions, $p = 0.75$; new black holes, $p = 0.81$.

Discussion

An investigation of seasonality, or cyclic variation throughout the year, is a common component of the basic etiological description of diseases. Here we have examined monthly patterns of exacerbations and MRI parameters in a cohort of relapsing-remitting multiple sclerosis

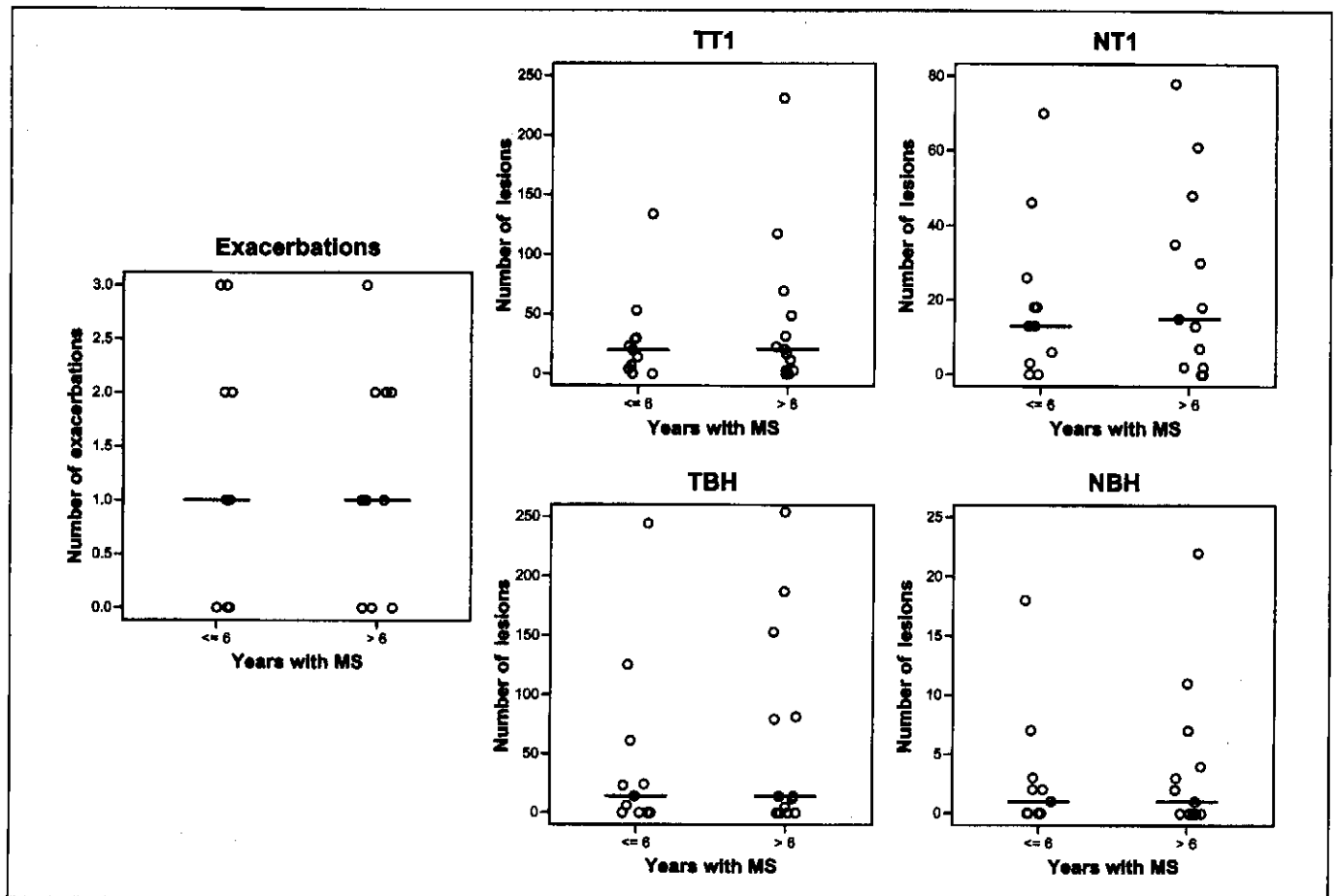


Fig. 4. Scatter plots of exacerbations, total numbers of enhancing lesions (TT1), total numbers of black holes (TBH), numbers of new enhancing lesions (NT1), and numbers of new black holes (NBH) observed per patient over the one-year period of the study. Patients

were stratified into two groups: patients with multiple sclerosis symptoms for 6 or fewer years ($n = 11$) and patients with multiple sclerosis symptoms for greater than 6 years ($n = 13$). Vertical lines are drawn at the medians.

patients, but found no statistically supported evidence for cyclicity, that is, seasonal dependence. Exacerbations were less frequent in October and November, and January through March, than in the spring and summer months (fig. 1), but this pattern failed to achieve statistical significance with a statistical procedure especially designed to be sensitive to seasonality [5, 7]. Similarly, patterns in the proportions of active monthly MRI scans (activity connoting the presence of any enhancing lesions, or new enhancing lesions, or any black holes, or new black holes) were somewhat cyclical (fig. 2), but none was found to be statistically significant. Quantification of actual numbers of lesions also led to some indication of seasonality (fig. 3), though again evidence is not conclusive.

We remark that the data we have presented here were accumulated during a randomized clinical trial, in which

the 24 patients were observed over the course of a one-year period. All exacerbations were carefully documented; and, each patient underwent a monthly neurological examination, as well as an MRI. The trial was double-blinded, so that neither patients nor evaluators were aware of treatment assignment. We believe, therefore, that our assessments of the prevalence of exacerbations as well as quantitation of MRI parameters in our cohort are quite accurate. Nevertheless, as a reviewer has pointed out, ascertainment bias may yet accrue from the fact that any cohort of patients enrolled in clinical trials is likely a biased representation of the disease as a whole given entry criteria meant to improve chances of showing a therapeutic effect. In this regard, we acknowledge that entry criteria attempted to identify patients with more active disease. Furthermore, we may well have an overcount of exacerbations

tions relative to relapsing-remitting multiple sclerosis patients not enrolled in clinical trials, as strenuous efforts were made during the course of the trial to identify and verify even mild exacerbations. Patients not enrolled in clinical trials might conceivably overlook such periods if disability is not pronounced. In the present study, we would expect MRI quantitation to be rather objective, and note that all exacerbations were verified and ranked in severity using prespecified criteria by the attending neurologists. In addition, these 24 patients constituted the placebo arm of the trial, hence clinical and MRI findings are not confounded by any potential treatment (cladribine) effect.

A potential limitation of the present study is possible confounding of results by other therapeutic options, such as concurrent interferon treatment or use of corticosteroids for treatment of exacerbations. Although other therapeutic interventions such as interferon were expressly disallowed per the protocol, corticosteroid usage for exacerbations was permitted, per the attending neurologist. Interestingly, chart review of the 24 placebo patients revealed that none was prescribed corticosteroids for alleviation of exacerbations during the period of the clinical trial. Hence corticosteroid usage was not considered a covariate in any of our analyses. Similarly, chart review revealed fewer than 10 serious viral infections among these patients, and we could find no strong correlation between viral infection and either exacerbations or MRI events.

Previous studies have reported periodicities in the monthly occurrences of exacerbations and MRI parameters in various cohorts of multiple sclerosis patients, but their findings are not altogether congruent. Wuthrich and Rieder [12] present a cyclic representation (period one year) in multiple sclerosis bouts (exacerbations) from a cohort of patients in Basel, with peak incidence occurring in March. In comparison, Bamford et al. [13] present a monthly pattern in the frequency of exacerbations in Arizona which peaks in the warmer months (with the curious exception of a dip in May, similar to our observed dip in June). One might speculate that climatic or environmental factors (e.g., sunshine exposure, temperature, humidity, rainfall) could contribute to these variations in phase. Note, however, that O'Reilly and O'Reilly [17] found no significant correlation between the monthly frequency of relapses (exacerbations) and various climatic variables (mean total rainfall, relative humidity, temperature variables, sunshine or solar radiation) in a retrospective study of multiple sclerosis patients in Ireland (although, these climatic variables were found to be correlated with the duration of relapses). In an analysis of placebo patients

from a multicenter clinical trial, Rovaris et al. [18] found no evidence of significant seasonal fluctuations in T₁-enhancing lesions. Similarly, using patients from a single-center clinical trial, Killestein et al. [19] could find no significant seasonal variation in active MRI lesions or in the numbers of patients experiencing clinical relapses.

On the other hand, we found a rather wide range in phases of the MRI summary findings. Enhancing lesions tend to peak in July and August, whereas new black holes tend to peak in January, and all black holes, in May. Our indices of variability were all quite small, ranging from 0.06 to 0.25. In contrast, Auer et al. [6] found a much more prominent annual variation in the average number of enhancing lesions from MRI scans of a mixed cohort of relapsing-remitting and secondary progressive multiple sclerosis patients in Munich: the peak occurred about April, and, we estimate their index of variability to be about 0.6. (Subsequently, Embry et al. [15] proposed that vitamin D levels, which vary with seasonal UV light exposure, are the main environmental factor involved in this seasonal fluctuation.) From simulation studies, we estimate that the Roger tests would have detected cyclic patterns of incidence of exacerbations or proportions of active scans with powers exceeding 0.8 in our cohort of 24 patients, had there been underlying cyclicality with indices of variability 0.6. Similar powers would have been attained with our periodic regression models, again had there been more pronounced cyclicality with indices of variability 0.6. Nevertheless, we caution that our sample size is relatively small and that the average number of new lesions is rather low for detecting smaller seasonal patterns with reasonable power.

One can speculate on reasons for the disparity in findings. (1) Multiple sclerosis has a unique geographic distribution: temperate zones have a low prevalence and more northerly regions have a prevalence more than ten times that in warmer climates [14]. Variations in multiple sclerosis exacerbations and MRI manifestations may well be more pronounced in more northerly (mid to high latitude) temperate climatic areas with distinctive seasonal weather pattern changes (as with the aforementioned continental Europe studies), compared to more southerly regions such as San Diego or Arizona, which experience more gradual changes in climate from one part of the year to another [16]. (2) Many of the earlier studies of cyclicality had pooled data over a number of years of observation. If seasonal patterns are reproducible from year to year, then this strategy should lead to enhanced sensitivity to cyclicality. But if seasonal patterns evince substantial annual variations, as with rainfall and sunlight, temperature and

humidity, then pooling across years may obscure any cyclicality. One might therefore argue that studies such as those by Rovaris et al. [18], Killestein et al. [19], and ours, in which patients are enrolled over a limited 12- to 18-month window, might better focus on seasonal variations. Even here, however, we may have chosen a window in which a particular seasonal pattern by chance is either missing or exaggerated. [In our trial, 17 of the 24 patients studied here were enrolled in the initial 6 months of the study. Seasonality (or lack thereof) in the clinical course of disease in these patients necessarily predominates in our findings.] (3) Most of the studies cited previously had pooled data over different geographic areas, which would tend to weaken the prominence of environmental components unique to one region. And, the observational studies may well have been confounded by various treatment modalities, and by corticosteroid usage.

At the suggestion of a reviewer, we also investigated whether stratification by duration of illness showed any seasonality. We dichotomized duration of illness with a demarcation of 6 years or less versus greater than 6 years, yielding subset sample sizes of 11 and 13, respectively. We found no indication of seasonality in either subset, but this may well be attributable to the reduced sample sizes in the subsets. More fundamentally, we investigated whether duration of illness was at all related to clinical exacerbations and MRI findings (fig. 4). Somewhat surprisingly, we found no indication whatsoever that any of these manifestations of disease was related to duration of disease. We remark that the natural history of multiple

sclerosis transitions from a relapsing-remitting course to a secondary progressive course in the majority of patients starting with relapsing-remitting disease, but with widely varying transition times to secondary progressive status. One might therefore expect that individuals in our cohort [all of whom were diagnosed with relapsing-remitting disease] with longer times of duration of disease might have somewhat fewer manifestations of 'acute' disease, in particular, clinical exacerbations and active enhancing (T₁) lesions, compared with individuals with more recent onset of disease. On the other hand, all patients had a history of two or more relapses in the two years preceding trial entry, indicative of an active relapsing-remitting disease process. (As noted previously, entry criteria for the trial attempted to identify patients with more active disease.)

In summary, although it is difficult to dismiss the belief that the presence of environmental factors on a background of genetic susceptibility is involved in the etiology of multiple sclerosis, the complexity of interpreting seasonal trends with a model sensitive to cyclicality remains challenging.

Acknowledgments

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