

## Effects of Seasons on Magnetic Resonance Imaging–Measured Disease Activity in Patients with Multiple Sclerosis

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The number of gadolinium (Gd)-enhancing lesions on monthly magnetic resonance imaging (MRI) scans of the brain is widely used to monitor multiple sclerosis (MS) clinical trials.<sup>1,2</sup> In relapsing-remitting (RR) MS, serial monthly Gd-enhanced MRI scans are five to ten times more sensitive than clinical measures in detecting disease activity<sup>3</sup> and, therefore, allow treatment effects to be investigated by studying fewer patients for shorter follow-up periods than when using clinically-based end points.<sup>4</sup> In addition, counting the numbers of enhancing lesions is a very reproducible process in experienced hands,<sup>5</sup> and it also allows the observers to be unaware of the treatment regimen of individual patients, thus avoiding bias due to unblinding.

A retrospective study by Auer et al<sup>6</sup> recently reported that the frequency and extent of Gd-enhancing lesions on serial MRI scans from MS patients is influenced by seasonal fluctuations, being significantly higher in the first than in the second half of the year. This study was, however, based on relatively small samples of patients and scans (202 scans were obtained from 53 patients, ie, an average number of less than four scans per patient) and the frequency of scanning was highly variable. Therefore, its results might have been heavily influenced by MRI findings from individual patients. Because the MRI activity at a given timepoint is significantly correlated with that seen during the previous and the subsequent months,<sup>3</sup> a cluster of few scans with very high numbers of Gd-enhancing lesions could have artificially increased the average activity during certain periods. The month-to-month fluctuations of the number of enhancing lesions might, therefore, be a chance effect depending on the variable timing of patient sampling and on the high interpatient variability of MRI activity.

Because Auer et al<sup>6</sup> concluded that such a marked seasonal variation of Gd-enhancing lesions in MS patients may prevent us from reaching reliable conclusions from MRI-monitored trials, we re-addressed this issue by studying the seasonal fluctuations of the number of Gd-enhancing lesions as seen on 11 consecutive brain MRI scans obtained every 4 weeks over a 10-month period from 120 RRMS patients, who were part of the untreated arm of a previous multi-center, randomized, double-blind, placebo-controlled trial.<sup>7</sup> All the scans were collected between February 1997 and August 1998, using a standardized imaging protocol across the participating centers. The only treatment allowed during the study period was steroid for acute relapses. Enhancing lesions were counted by two experienced observers by consensus.

The average number of enhancing lesions per scan was 4.1 (SD = 7.1). The Figure shows the month-to-month variation of the mean number of enhancing lesions, which is

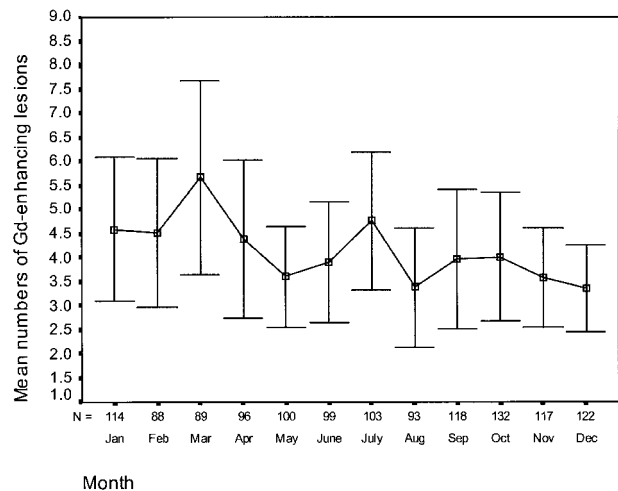


Fig. Mean numbers of gadolinium-enhancing lesions per scans obtained during the different months of the year from 120 relapsing-remitting multiple sclerosis patients ( $N$  = number of available scans; vertical bars represent 95% confidence interval limits). All magnetic resonance imaging (MRI) scans were obtained every 4 weeks using a standardized brain imaging protocol. The seasonal fluctuations of MRI activity were not statistically significant (see text for details and statistical analysis).

higher in March [mean number of enhancing lesions (SD) = 5.7 (9.5)] and lower in December [mean number of enhancing lesions (SD) = 3.3 (5.0)]. To avoid interpretation bias due to the interpatient variability of MRI activity, we made a statistical analysis of the pairwise comparisons between the numbers of enhancing lesions during the four seasons in 92 patients who had at least one scan for each of the seasons (Friedman test for nonparametric data). The mean numbers of enhancing lesions (SD) were 4.4 (6.6), 5.1 (8.5), 4.5 (6.7), and 4.5 (7.6) for winter, spring, summer, and autumn, respectively. No significant difference of MRI activity between seasons was found.

These results demonstrate that, although MRI activity in RRMS patients varies in the different seasons, this fluctuation is not significant. Our data were obtained from a large sample of patients with heterogeneous geographical origins, who were recruited from different European and Canadian centers.<sup>7</sup> In addition, baseline MRI scans were collected over a period covering all the four seasons. For these reasons, our results should not be biased by other factors that are known to influence MS activity. In conclusion, the seasonal fluctuations of subclinical activity in patients with RRMS should not affect the interpretation of the results from MRI-monitored clinical trials a great deal.

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## Central Acetylcholinesterase Inhibition in Alzheimer Patients

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Kuhl and colleagues<sup>1</sup> measured with direct positron emission tomography acetylcholinesterase activity in the brain of living Alzheimer's disease patients treated chronically with donepezil, a widely used cholinesterase inhibitor. After 10 mg/day donepezil, they measured an average inhibition in cerebral cortex of 27%. The cholinesterase inhibition in control patients receiving physostigmine (1.5 mg/hour intravenously) was found to be considerably higher (52%). They judged that the extent of central inhibition with donepezil is much less than that measured in red blood cells during large double-blind clinical trials and concluded that peripheral and central pharmacodynamic activity of cholinesterase inhibitors correlate poorly. They also suggest that the limited efficacy of cholinesterase inhibitors may be due to limited central cholinesterase inhibition reached with currently available drugs.

The report is extremely interesting because it proposes a relatively noninvasive technique for measuring central acetylcholinesterase activity with potential implications for the optimization of Alzheimer's disease therapy with cholinesterase inhibitors. However, in our opinion there are a number of pitfalls that could limit their conclusions.

Unfortunately, Kuhl and colleagues did not simultaneously measure acetylcholinesterase activity in red blood

cells, thus rendering impossible a direct comparison of central and peripheral cholinesterase inhibition. Central acetylcholinesterase inhibition after donepezil was measured in only 6 patients and displayed high variability, with individual inhibitions in cerebral cortex ranging from 2 to 50%. Variability of cortical cholinesterase inhibition was similar to that measured in red blood cells (standard deviations of 11–12 vs 11%).<sup>2</sup> With this high variability, many more patients need to be studied to get a reliable estimate of average cholinesterase inhibition. In a study involving 139 Alzheimer's disease patients, an average of 75% inhibition was measured peripherally after 10 mg/day donepezil,<sup>2</sup> a value not far from that found by Kuhl and colleagues in other brain regions (47–61%). However, the extent of acetylcholinesterase inhibition measured by Kuhl and colleagues in cerebral cortex ( $52 \pm 9\%$ ) after physostigmine infusion (1.5 mg/hour) was comparable to that reported in plasma ( $47 \pm 4\%$ ).<sup>3</sup>

Although Kuhl and colleagues found that acetylcholinesterase inhibition with physostigmine in cerebral cortex was considerably higher than after donepezil (52 vs 27%), large double-blind clinical trials have not indicated that cognitive effects of physostigmine are superior to those of donepezil.<sup>4</sup> This suggests that a clear correlation between the extent of central cholinesterase inhibition and clinical efficacy of cholinesterase inhibitors cannot be easily established.

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## Reply

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We reported on direct positron emission tomography (PET) studies<sup>1</sup> in Alzheimer's disease (AD) patients after chronic treatment with donepezil (5 and 10 mg/day). We found less inhibition of cerebral cortical acetylcholinesterase (AChE) activity than has been inferred by others<sup>2</sup> based on surrogate assays of peripheral red cells from similar patients treated by identical dose schedules. At 5 mg/day dosage, the 95% confidence interval ranged from 15 to 36% inhibition for our PET group (mean 25%, standard deviation 12%,  $n = 9$ ). In contrast, the 95% confidence interval ranged from 62 to 66% for the cited red cell assay group<sup>2</sup> (mean 64%, standard

deviation 12%, n = 142). Clearly, these ranges do not overlap. Therefore, our data refute the hypothesis that cerebral cortical cholinesterase inhibition is the same as that found in peripheral red cells.

We commented that, unless current drug schedules already provide maximum AChE inhibition in AD cerebral cortex, there is promise that alternative drugs or dose schedules might do better and be more effective. The issue is unsettled whether there is a clear correlation between extent of cerebral cortical inhibition and clinical efficacy of cholinesterase inhibitors. Direct *in vivo* measures using radiotracers should help address the question. We advocate direct examination of brain pharmacodynamic effects of established and investigational pharmaceuticals. This is necessary, because our findings emphasize that extrapolations from preclinical

studies or from surveys of surrogate peripheral sites may not reflect accurately the state of the living human brain.

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