Development of Hypointense Lesions on T1-Weighted Spin-Echo Magnetic Resonance Images in Multiple Sclerosis

Relation to Inflammatory Activity

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Objective: To evaluate whether degree of inflammatory activity in multiple sclerosis, expressed by frequency of gadolinium enhancement, has prognostic value for development of hypointense lesions on T1-weighted spin-echo magnetic resonance images, a putative marker of tissue destruction.

Design: Cohort design with long-term follow-up. Thirty-eight patients with multiple sclerosis who in the past had been monitored with monthly gadolinium-enhanced magnetic resonance imaging for a median period of 10 months (range, 6-12 months) were reexamined after a median period of 40.5 months (range, 33-80 months).

Setting: Magnetic Resonance Center for Multiple Sclerosis Research, Amsterdam, the Netherlands, referral center.

Main Outcome Measures: The new enhancing lesion rate (median number of gadolinium-enhancing lesions per monthly scan) during initial monthly follow-up; hypointense T1 and hyperintense T2 lesion load at first and last visit.

Results: The number of enhancing lesions on entry scan correlated with the new enhancing lesions rate ($r = 0.64$; $P < .001$, Spearman rank correlation coefficient). The new enhancing lesion rate correlated with yearly increase in T1 ($r = 0.42$; $P < .01$, Spearman rank correlation coefficient) and T2 ($r = 0.47$; $P < .01$, Spearman rank correlation coefficient) lesion load. Initial T1 lesion load correlated more strongly with yearly increase in T1 lesion load ($r = 0.68$; $P < .01$, Spearman rank correlation coefficient).

Conclusions: Degree of inflammatory activity only partially predicted increase in T1 (and T2) lesion load at long-term follow-up. Initial T1 lesion load strongly contributed to subsequent increase in hypointense T1 lesion load, suggesting that there is a subpopulation of patients with multiple sclerosis who are prone to develop destructive lesions.

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THE INABILITY to predict the clinical course of multiple sclerosis (MS) in individual patients has made it difficult to decide which patients are suitable for early treatment, since some will not develop disability for many years. Some therapies (interferon beta and copolymer 1) have been shown to suppress both relapse rate and the rate of gadolinium (Gd)-enhancing lesions, although a robust effect on disability is more difficult to establish.1-4

Recent studies have focused on the relationship between accumulation of hypointense lesions (“black holes”) on T1-weighted spin-echo magnetic resonance (MR) images and development of disability5-8 in patients with MS. At autopsy, these lesions correlate with axonal loss and matrix destruction,2 supporting the hypothesis that hypointense lesions may be used as a putative marker of severe tissue destruction in MS. These autopsy findings are in line with recent biopsy findings: hypointensity on T1-weighted scans was shown to be affected mainly by 2 factors, the extent of axonal reduction and extracellular edema, capable of expanding the extracellular space.8 Further, recent recommendations already include change in T1 lesion load (in addition to change in T2 lesion load) as an MR imaging outcome measure in definite (phase 3) clinical trials in patients with established MS.9

Factors influencing development of hypointense lesions have been obscure. Truyen et al6 hypothesized that hypointense lesions may develop as a result of exhaustion of repair mechanisms (eg, remyelination), associated with the secondary progressive phase of the disease. Lassmann et al10 suggested that exhaustion of repair mechanisms is the consequence of a high degree of inflammatory activity or of repeated inflammation of the same lesion.

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PATIENTS AND METHODS

PATIENTS

Thirty-eight patients (20 women and 18 men) with clinically definite MS who had been monitored between February 1, 1990, and November 30, 1993, with monthly Gd-enhanced MR imaging for at least 6 months were re-examined. Informed consent was obtained after the nature of the procedure(s) had been fully explained. These patients previously had been involved in follow-up studies and natural course studies, and some of the patients (n = 24) had been monitored with monthly MR imaging to investigate treatment efficacy of the monoclonal anti-CD4 cM-T412 antibody. Nine of these 24 patients were also involved in a recent follow-up study investigating monthly enhanced MR imaging lesion rate and changes in T1 lesion volume.

Regarding monthly Gd-enhanced MR imaging examinations, 3 patients had been followed up for 6 months, 1 patient for 8 months, 23 patients for 10 months, and 9 patients for 12 months. At the time of the initial examination, 26 patients had relapsing-remitting MS (RRMS) and 12 patients had secondary progressive MS (SPMS); median age at first visit was 33.0 years (range, 23-52 years), and median disease duration was 3.5 years (range, 0-26 years). Full neurologic history and examination at study entry and exit were performed in all cases, with disability assessed by means of the Kurtzke Expanded Disability Status Scale (EDSS). Because of the nature of this study, different neurologists performed these examinations at study entry and exit. Patients using immunosuppressive drugs other than infrequent short courses of intravenous methylprednisolone during relapses were excluded. Clinical and MR imaging examinations were not performed within 1 month after treatment with intravenous corticosteroids. Patients who were treated with the monoclonal anti-CD4 antibody cM-T412 (13 patients were receiving active treatment for 6 consecutive months) were not excluded because of lack of treatment effect on degree of MS activity as measured by monthly Gd-enhanced MR imaging.

MR IMAGING

The first series of scans were all performed at 0.6 T (Technicare, Solon, Ohio) and the last scan was performed at 1.5 T (Vision; Siemens, Erlangen, Germany) with the use of standard head coils. During the initial phase, median monthly follow-up was 10 months (range, 6-12 months). Median interval between first and last visit was 40.5 months (range, 33-80 months). A comparable MR imaging protocol was used for the first series and for the last scan, including an equal number of slices and equal dose of Gd on both occasions. Identical slice positioning was achieved by means of unenhanced images in 2 or 3 consecutive planes, correcting for positioning differences according to internal landmarks. After administration of gadopentetate dimeglumine (Magnevist; Schering AG, Berlin, Germany) (0.1 mmol/kg), axial T2-weighted spin-echo images (0.6 T; repetition time, 2755 ms; echo time, 95 ms) were obtained. Gd-enhanced T1-weighted spin-echo images (0.6 T; repetition time, 800 ms; echo time, 14 ms) were obtained at follow-up visits. Gd-enhanced images in 2 or 3 consecutive planes, correcting for slice positioning differences according to internal landmarks, were recorded. The number of slices and equal dose of Gd on both occasions. Iden-

CLINICAL CHARACTERISTICS

Significant differences were present between patients with RRMS and SPMS regarding age, disease duration, and EDSS score at entry and exit of the study (Table 1). Regarding the total study group, median change in EDSS score per year was 0.16 (range, −1.3 to +1.1). During the study, an increase in EDSS was present in 21 patients; a decrease, in 6 patients; and no change, in 10 patients. In 1 patient EDSS score was not available for 1 data point. Significant increase in disability (increase in EDSS score, >0.5) occurred in 16 patients (42%) during the study (Figure 1).

MR IMAGING CHARACTERISTICS

Thirty-four patients showed enhancing lesions during initial monthly follow-up; in 4 patients no enhancing lesions were observed. For the total study group, median new enhancing lesion rate was 0.86 (range, 0-9.25). The median number of enhancing lesions on the first scan was 0 (range, 0-9). Median new enhancing lesion rate was 1.1 (range, 0-9.25) for patients with RRMS and 0.67 (range, 0-1.7) for patients with SPMS (Mann-Whitney test, P <.001). Hypointense T1 lesion load increased in 37 of 38 patients, with a median increase of 1.6 cm3 (range, 0-12.9 cm3); final hypointense lesion load was 3.4 cm3. In 1 patient with RRMS, no hypointense lesions were present on the initial scan and no hypointense lesions developed during follow-up. Median T2 lesion load at entry was 10.8 cm3 (range, 1.3-51.8 cm3) and was significantly higher in patients with SPMS than in those with RRMS (Mann-Whitney test, P <.001). Hypointense T1 lesion load increased in 28 patients (21 with RRMS and 7 with SPMS) and decreased in 9 patients (5 with RRMS and 4 with SPMS). For 1 patient, the initial T2 lesion load could not be analyzed because of data storage problems. For the whole study group, median change in T1 lesion load per year was 0.74 cm3 (range, 0-6.7 cm3) and median change in T2 lesion load per year was 0.93 cm3 (range, −2.6 to +7.8 cm3); no differences were present between the subgroups. For the total study group, median initial T1/T2 ratio was 0.13
millimeters; echo time, 60 and 120 milliseconds; number of excitations, 2; 1.5 T: 2800, 60 and 120, and 1, respectively) and T1-weighted images (0.6 T: 450, 28, and 4, respectively; 1.5 T: 550, 15, and 2, respectively) were obtained. Nineteen slices with an in-plane resolution of 1.0 × 1.3 mm (0.6 T) or 0.98 × 0.98 (1.5 T) and a slice thickness of 5 mm were obtained with an interslice gap of 1.25 mm.

ANALYSIS

Monthly postcontrast T1-weighted images were analyzed for number of new enhancing lesions by 2 readers (L.T. and F.B.) by consensus. The new enhancing lesion rate was calculated per patient and defined as total number of new enhancing lesions on monthly MR imaging divided by number of monthly follow-up scans. The entry and exit scans were analyzed by 1 observer (M.A.A.v.W.) who was blinded to clinical data. Hypointense lesions on postcontrast T1-weighted images obtained at both first and last visit were analyzed and marked on hard copies. Cutoff levels for hypointense lesions was based on visual analysis and defined as regions with low signal intensity compared with surrounding normal-appearing white matter, corresponding to a hypointense region on T1-weighted images. Hypointense lesions on T1-weighted images assessed at first and last visit were analyzed and marked on hard copies; they were defined as sharply demarcated regions of high signal intensity (separated from surrounding normal-appearing white matter by a distinct border). Both T2- and T1-weighted images of the first and last visit were transferred to a computer workstation (Sparc 5; SUN, Palo Alto, Calif) for quantitative analysis. Hyperintense T1 and hypointense T1 lesion loads were quantified by a single observer, blinded to clinical data, by means of a seed-growing technique with home-developed software. T1 and T2 lesion volumes were calculated by adding the area of all lesions multiplied by 0.625 (interslice distance + slice thickness + interslice gap). To evaluate intraobserver variation, scans of 5 patients with varying lesion loads were re-assessed. The intraobserver coefficient of variance for this method was 2.8% for T1-weighted images and 3.0% for T1-weighted images.

STATISTICAL ANALYSIS

Change in T1 and T2 lesion load and change in EDSS during the study period for each patient was adjusted for the length of study. Adjusted annual values were entered into the analysis. The new enhancing lesion rate was adjusted for the duration of the initial monthly follow-up to provide monthly enhancing lesion rates for patients (lesions per month per patient). Since most data were not normally distributed, medians were used to describe the data. Spearman rank correlation coefficient (r) was calculated for correlations. All comparisons underwent 2-sided testing with nonparametric tests, with a significance level of .01 to correct for multiple comparisons. Multiple regression analysis (forward stepwise, F to enter = 0.05) was used to estimate the main predictive variables for development of hypointense lesions and for progression of clinical disability.

CORRELATIONS FOR BASELINE AND FOLLOW-UP DATA

Exclusion of patients who were treated with the monoclonal anti-CD4 antibody cM-T412 (n = 13) yielded the following MR imaging characteristics: new enhancing lesion rate of 0.9 (range, 0.9-25); absolute difference in T1 lesion load, 2.8 cm3 (range, −7.4 to +16.7 cm3); absolute difference in T1 lesion load, 1.6 cm3 (range, 0.12-9.9 cm3); median change in T1 lesion load per year, 0.53 cm3 (range, 0.6-7.0 cm3); median change in T1 lesion load per year, 0.90 cm3 (range, −2.6 to +7.8 cm3); initial T1/T2 ratio, 0.12 (range, 0.05-56); and T1/T2 ratio at exit, 0.22 (range, 0.05-53). In comparison with the MR imaging characteristics of all patients, no significant difference was present for any of these MR imaging variables (Mann-Whitney test, P>.10).

Exclusion of patients treated with anti-CD4 antibody cM-T412 did not change the correlations for baseline and follow-up data significantly. Therefore, correlations referring to all patients are described. Number of enhancing lesions on entry scan correlated with the new enhancing lesion rate at monthly follow-up (r = 0.64; P<.001). Similarly, the number of enhancing lesions at entry scan correlated with the number of new enhancing lesions in the first 3 months of the study (r = 0.87; P<.01). The number of enhancing lesions on entry scan did not correlate with yearly increase in hypointense T1 or T2 lesion load during long-term follow-up. For the whole group, new enhancing lesion rate during monthly follow-up correlated (r = 0.42; P<.01) with yearly increase in hypointense T1 lesion load and correlated more with yearly increase in T2 lesion load (r = 0.47; P<.01) (Figure 3). In patients with SPMS, correlations between new enhancing lesion rate and yearly increase in hypointense lesion load differed (r = 0.54; P = .07) from those in patients with RRMS (r = 0.48; P = .01). For the total group of patients, new enhancing lesion rate did not correlate with yearly change in T1/T2 ratio. The new enhancing lesion rate was higher for patients who showed an increase in T2 lesion load (median, 1.1; range, 0–9.3) than for patients with a decrease in T2 lesion load (median, 0.3; range, 0–1.9). New enhancing lesion rate was only slightly higher for patients who showed a significant increase in EDSS score (median, 1.4; range, 0–3.7) than for patients with an increase in EDSS score of less than 1 full point during long-term follow-up (median, 1.2; range, 0–9.3). No correlation was present between new enhancing lesion rate and yearly change in EDSS score (Figure 4), although a positive correlation was present for patients with SPMS (r = 0.50; P = .09). Baseline T1 lesion load correlated significantly with subsequent increase in hypointense lesion load (r = 0.68; P<.01) (Figure 5). The correlation between baseline T1 lesion load and new enhancing lesion rate showed a trend for patients with SPMS only (r = 0.67; P = .02).

Initial EDSS score correlated with initial hypointense T1 lesion load (r = 0.58; P<.01) and to a lesser ex-
Multiple regression analysis (forward stepwise) was performed to determine the main factors predicting hypointense $T_1$ lesion load at study exit (dependent variable). Independent variables investigated were age, disease duration, EDSS score at entry, initial hypointense $T_1$ and $T_2$ lesion loads, absolute and relative changes in hypointense $T_1$ and $T_2$ lesion load, the initial $T_1/T_2$ ratio, and the new enhancing lesion rate. This yielded a model (multiple $R = 0.63$; $R^2 = 0.39$) that included the EDSS score at entry ($\beta = 0.63$; $P < .001$) as the only variable.

In this study we found correlations between clinical disability and hypointense lesion load at study entry and exit. These correlations were stronger than for $T_2$ lesion load. Disability correlated with the ratio of hypointense $T_1$ lesion load over $T_2$ lesion load, this ratio being higher in patients with SPMS than those with RRMS. These results are in line with previous results from preliminary studies and further support the hypothesis that hypointense lesions may be used as a putative marker to monitor fixed deficit in MS.

Since the ultimate goal of treatment in MS is to prevent accumulation of fixed deficit, it is important to evaluate which factors influence development of hypointense lesions. Our results show that degree of inflammatory activity, as depicted by Gd-enhancing lesions on monthly MR imaging, predicts only to some degree the increase in hypointense $T_1$ and hyperintense $T_2$ lesion load at long-term follow-up. Our long-term observations extend the correlation found between enhancing lesion rate and change in $T_2$ lesion load in recent studies and further support the hypothesis that hypointense lesions may be used as a putative marker to monitor fixed deficit in MS.
be different for acute and chronic hypointense lesions, they should be considered separately in follow-up studies. In our follow-up study, only postcontrast T1-weighted images were available; therefore, a distinction between acute and chronic hypointense lesions was not possible. However, the contribution of acute hypointense lesions to our T1 lesion load measurement has probably been small. First, most acute hypointense lesions return to isointensity within 3 months after enhancement; their impact in a long-term follow-up study will therefore be minimal. Second, our T1-weighted images were performed after the administration of Gd. Since most acute hypointense lesions will show enhancement, they subsequently were not included in our T1 lesion load measurements.

Our data suggest that inflammatory activity as expressed by Gd enhancement is only one of several factors related to development of destructive lesions, and that so far unknown factors may independently have a prominent role. This observation is in line with a number of clinical observations that relapse rate only shows moderate correlations with development of future disability. Further research should focus on the identification of these factors that, in addition to inflammatory activity, influence development of destructive and disabling lesions. Heterogeneity, with regard to pathological char-

**Table 2. Magnetic Resonance Imaging Characteristics of Patients**

<table>
<thead>
<tr>
<th></th>
<th>All Patients (N = 38)</th>
<th>RRMS (n = 26)</th>
<th>SPMS (n = 12)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 LL at entry, cm³</td>
<td>1.5 (0-22.8)</td>
<td>0.9 (0-10.2)</td>
<td>5.8 (0-22.8)</td>
<td>.001</td>
</tr>
<tr>
<td>T2 LL at entry, cm³</td>
<td>10.8 (1.3-51.8)</td>
<td>9.1 (1.3-43.9)</td>
<td>20.6 (2.3-51.8)</td>
<td>.01</td>
</tr>
<tr>
<td>T1 LL at exit, cm³</td>
<td>3.4 (0-26.9)</td>
<td>2.0 (0-16.0)</td>
<td>9.2 (0-26.9)</td>
<td>.02</td>
</tr>
<tr>
<td>T2 LL at exit, cm³</td>
<td>17.0 (1.2-51.2)</td>
<td>10.2 (1.2-46.7)</td>
<td>26.2 (1.9-51.2)</td>
<td>.02</td>
</tr>
<tr>
<td>T1/T2 ratio at entry</td>
<td>0.1 (0-0.6)</td>
<td>0.1 (0-0.6)</td>
<td>0.3 (0-0.4)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>T1/T2 ratio at exit</td>
<td>0.2 (0-0.6)</td>
<td>0.2 (0-0.4)</td>
<td>0.4 (0-1.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>New enhancing lesion rate‡</td>
<td>0.9 (0-9.2)</td>
<td>1.1 (0-9.2)</td>
<td>0.7 (0-1.7)</td>
<td>.30</td>
</tr>
</tbody>
</table>

*RRMS indicates relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; and LL, lesion load.
†Mann-Whitney test.
‡Number of new enhancing lesions per month.
acteristics as well as to genetics of MS, may explain part of the observed variability. This hypothesis is in agreement with our observation that patients most likely to show an increase in hypointense $T_1$ lesion load are those who already have high hypointense $T_1$ lesion loads, thereby indicating that there is a subpopulation of patients with MS who are prone to develop destructive lesions. Hypointense “burden of disease” therefore could be used for stratification of patients at baseline in treatment trials where changes in $T_1$ lesion load will be analyzed as a secondary outcome measure. This shows strong similarity to the use of Gd-enhanced scanning to monitor new inflammatory activity, for which it was also shown that MR imaging activity at a single time point can predict future MR activity, suggesting that activity at baseline can be used for the stratification of patients for treatment trials. In agreement with previous publications, the influence of inflammatory activity on changes in clinical disability is difficult to establish, since only a positive correlation could be observed for patients with SPMS. The absence of a stronger correlation could be related to our relatively short follow-up period (approximately 40 months) and to the small number of patients with SPMS included (12 patients). Of these 12 patients with SPMS, only 6 showed an increase in EDSS score greater than 0.5 during follow-up. Most importantly, interobserver variation for EDSS assessment, which in our study was scored by 2 neurologists, may have resulted in loss of sensitivity.

Of technical concern in our follow-up study is the difference in field strength at study entry and study exit. Apart from increase in signal to noise, the relative sensitivity for detection of lesions probably varies between 0.6 T (first series of scans) and 1.5 T (last scan), since measurement of $T_1$ lesion load—needs to be standardized between MR imaging centers. Further research is needed to develop guidelines for a clear definition of acute and chronic hypointense lesions, to optimize and standardize the pulse sequences used to identify hypointense lesions, and to improve intrarater and interrater variability in hypointense lesion load measurements.

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the amount of T₁ and T₂ weighting differs. The MR images at study entry and study exit therefore were analyzed comparatively for each patient. Further, because this difference in field strength may have influenced calculation of lesion volumes and with that our descriptive data, rank-order and cross-sectional correlations have been used to evaluate our data.

Despite limitations in clinical evaluations, (changes in) MR imaging variables, and duration of follow-up, it is clear that progressive disability only correlates weakly with presently analyzed MR variables. Therefore, a search for other laboratory markers is still needed. In terms of MR imaging variables, for example, cerebral atrophy was shown to be related to clinical worsening, even in the absence of new (enhancing) lesions. Most likely a more general disease process occurs “in between” focal lesions. Further research is warranted to characterize this diffuse process in brain and spinal cord.

In conclusion, we have demonstrated that the degree of inflammatory activity as depicted by Gd-enhancing lesions is correlated to some degree with increase in hypointense lesion load at long-term follow-up, but other, so far unidentified, factors may have a prominent role. Initial hypointense T₁ lesion load strongly contributes to subsequent increase in T₁ lesion load, which indicates that there is a subpopulation of patients with MS who are prone to develop more destructive lesions.

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