

## Original Investigation

# Association of Vitamin D Levels With Multiple Sclerosis Activity and Progression in Patients Receiving Interferon Beta-1b

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**IMPORTANCE** Low serum 25-hydroxyvitamin D (25[OH]D) levels are associated with an increased risk of multiple sclerosis (MS) as well as with increased disease activity and rate of progression in clinically isolated syndromes and early MS.

**OBJECTIVE** To assess the association between 25(OH)D and disease course and prognosis in patients with relapsing-remitting MS treated with interferon beta-1b.

**DESIGN, SETTING, AND PARTICIPANTS** We conducted a prospective cohort study assessing 25(OH)D levels and subsequent MS disease course and progression characterized by magnetic resonance imaging (MRI) and clinical end points. The study took place between November 2003 and June 2005; data analysis was performed between June 2013 and December 2014. The study was conducted among participants in the Betaferon Efficacy Yielding Outcomes of a New Dose (BEYOND) study, a large, phase 3, prospective, multicenter, blinded, randomized clinical trial. Patients were monitored for at least 2 years. Clinic visits were scheduled every 3 months, and MRI was performed at baseline and annually thereafter. Eligible patients included 1482 participants randomized to receive 250 µg or 500 µg of interferon-1b with at least 2 measurements of 25(OH)D obtained 6 months apart.

**EXPOSURES** Serum 25(OH)D measurements were performed at baseline, 6 months, and 12 months.

**MAIN OUTCOMES AND MEASURES** Main outcomes included cumulative number of new active lesions (T2 lesions and gadolinium acetate-enhancing lesions), change in normalized brain volume, relapse rate, and progression determined by the Expanded Disability Status Scale (EDSS). Statistical analyses were adjusted for age, sex, randomized treatment, region, disease duration, and baseline EDSS score.

**RESULTS** Overall, average 25(OH)D levels in 1482 patients were significantly inversely correlated with the cumulative number of new active lesions between baseline and the last MRI, with a 50.0-nmol/L increase in serum 25(OH)D levels associated with a 31% lower rate of new lesions (relative rate [RR], 0.69; 95% CI, 0.55-0.86;  $P = .001$ ). The lowest rate of new lesions was observed among patients with 25(OH)D levels greater than 100.0 nmol/L (RR, 0.53; 95% CI, 0.37-0.78;  $P = .002$ ). No significant associations were found between 25(OH)D levels and change in brain volume, relapse rates, or EDSS scores. Results were consistent following adjustment for HLA-DRB1\*15 or vitamin D-binding protein status.

**CONCLUSIONS AND RELEVANCE** Among patients with MS treated with interferon beta-1b, higher 25(OH)D levels were associated with lower rates of MS activity observed on MRI. Results for brain atrophy and clinical progression were more equivocal.

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**M**ultiple sclerosis (MS) is a debilitating disease of the central nervous system characterized by episodic periods of inflammatory demyelination and progressive neurologic deficits.<sup>1,2</sup> The higher MS risk among individuals with low vitamin D intake<sup>3</sup> or low circulating 25-hydroxyvitamin D (25[OH]D)<sup>4-6</sup> levels and the worse prognosis among patients with MS who have low 25(OH)D levels<sup>7-12</sup> suggest that these low levels may contribute to the disease process. Most recently, a previous study indicated that individuals with average serum 25(OH)D levels less than <50.0 nmol/L (to convert to nanograms per milliliter, divide by 2.496) in the 12 months following a first demyelinating event had worse clinical and imaging outcomes after 5 years than did those with higher levels.<sup>7</sup> However, since average 25(OH)D levels in that study were low and there was no clear ceiling effect, the optimal serum level of 25(OH)D could not be identified. In addition, it is important to determine whether this association between 25(OH)D and MS outcomes differs among subgroups of patients and particularly whether it applies to patients with high disease activity who may benefit from other potential therapeutic alternatives.<sup>13</sup> To address these issues, we conducted a prospective study among a large group of patients with relapsing-remitting MS treated with interferon beta-1b with a wide distribution of serum 25(OH)D levels and disease severity.

## Methods

### Study Population

Eligible patients included 1796 individuals with MS randomized to receive either 250 µg or 500 µg of interferon beta-1b (Betaferon) in the Betaferon Efficacy Yielding Outcomes of a New Dose (BEYOND) trial.<sup>14</sup> The present study was approved by the Harvard T. H. Chan School of Public Health. Participants in the BEYOND trial provided written informed consent. The present study used de-identified data and biological samples and had no direct contact with BEYOND participants. Full descriptions of the study population and results have been reported.<sup>14,15</sup> Briefly, BEYOND was a large, phase 3, prospective, multicenter, blinded, randomized clinical trial with interferon beta-1b, 250 µg (n = 897); interferon beta-1b, 500 µg (n = 899); and glatiramer acetate, 20 mg (n = 448), treatment arms. Following enrollment from November 2003 to June 2005, patients were monitored for at least 2 years. Clinic visits were scheduled every 3 months for assessment of relapse, Expanded Disability Status Scale (EDSS) score, safety, and tolerability, and magnetic resonance imaging (MRI) was performed at baseline and annually thereafter. Both T2- and T1-weighted images (with and without 0.1 mmol/kg of gadolinium-diethylenetriamine pentaacetic acid) of the whole brain were obtained and evaluated in a blinded manner by the Neuroimaging Research Unit in Milan, Italy. Observers identified T1- and T2-weighted lesions, and a semiautomated local threshold technique was used to determine lesion volume.<sup>16</sup> Normalized brain volume at baseline was assessed using Siena and longitudinal brain volume percent change was evaluated with Siena.<sup>16</sup>

Levels of 25(OH)D were measured in serum samples collected at baseline, 6 months, and 12 months from patients randomized to receive 250 µg or 500 µg of interferon beta-1b; patients randomized to glatiramer acetate were not included. Because the primary purpose of the study was to estimate the effects of yearly average 25(OH)D levels, we included in the analyses only the 1482 (from the 1796 eligible) patients with at least two 25(OH)D measurements performed 6 months apart; all of these patients had 25(OH)D levels determined at 6 months, 1465 patients (98.9%) had the baseline measurement, and 1426 individuals (96.2%) had 25(OH)D levels obtained at 12 months. Of these 1482 participants, 1456 (98.2%) had follow-up information on MRI measurements after the baseline assessment. Patients from 26 countries participated in the BEYOND trial<sup>14</sup> and were initially grouped into 9 countries/regions as follows: Western Europe (Austria, Belgium, Switzerland, Germany, and the Netherlands), the Mediterranean (Spain, France, Italy, Greece, and Israel), Scandinavia (Denmark, Sweden, Finland, and Norway), Eastern Europe (Ukraine, Latvia, Hungary, Slovenia, and Poland), North America (United States and Canada), Russia, Argentina, Brazil, and Australia. For the purpose of adjusting the primary analyses of 25(OH)D levels and MS outcomes, these regions were further collapsed into 4 broad areas based both on geography and patient characteristics: Eastern Europe (including Russia), Western Europe (including Israel), North America, and the southern hemisphere (Australia, Argentina, and Brazil). Although these categories are arbitrary and not entirely homogeneous (eg, 25[OH]D levels were lower in Russia than Eastern Europe), sensitivity analyses, in which confounding by geography was controlled using the original 9 regions, gave virtually identical results.

### Measurement of 25(OH)D

Samples were shipped on dry ice to the central laboratory in Schwentinal, Germany, within 3 days of being drawn and then maintained at -20°C until further analysis. Serum 25(OH)D was measured separately in each treatment group (250 or 500 µg of interferon beta-1b) using a chemiluminescence assay (Roche Diagnostics) in June 2013. The average intra-assay coefficients of variation derived from blind quality control samples were 2.6% for the samples from patients randomized to 250 µg of interferon beta-1b and 4.0% for patients randomized to 500 µg of interferon beta-1b. The corresponding between-assay coefficients of variation were 5.0% and 7.1%, and the intraclass correlation for quality control samples run with both the 250- and the 500-µg doses of interferon beta-1b was 0.95. Baseline level of 25(OH)D was strongly correlated with levels at both 6 months (Spearman correlation coefficient,  $r = 0.75$ ) and 12 months ( $r = 0.89$ ); 25(OH)D levels displayed the expected seasonal variations. Because we were interested in the effects of long-term average 25(OH)D levels and as a result of large between-region variation, we performed season adjustment as previously described<sup>4</sup> within each region (using the 4-region categorization).

### Genotype Assessment

All samples were genotyped (GeneChip, version 6.0 platform; Affymetrix). Both HLA-DRB1\*15 and vitamin D-bind-

ing protein were imputed with MaCH<sup>17</sup> using the 120 phased chromosomes from the CEU (Utah residents with ancestry from northern and western Europe) or samples in HapMap phase II (<http://hapmap.ncbi.nlm.nih.gov/>) as the reference.

### Statistical Analysis

Our data analysis was performed from June 2013 to December 2014. We treated the season-adjusted serum 25(OH)D level as a time-dependent variable and considered the value at each time point to be an average of all previous values. The association between season-adjusted 25(OH)D levels and MS outcomes was examined in both cross-sectional and longitudinal analyses. In the cross-sectional analysis, we assessed the association between baseline season-adjusted serum 25(OH)D levels with baseline MRI measurements (including volume of T2 hyperintense lesions, number of T1-enhancing lesions, volume of T1-nonenhancing hypointense lesions, and normalized brain volume) and clinical measurements (including baseline EDSS score and number of relapses in the 2 years before study baseline). We also assessed the cross-sectional association between the cumulative average mean 25(OH)D level at 12 months and MRI measurements performed at year 1. In longitudinal analyses, we considered the association of time-dependent, season-adjusted average 25(OH)D levels with the cumulative number of new active lesions (the sum of new T2 lesions and new gadolinium-enhancing lesions), change in normalized brain volume, relapse rate, and confirmed EDSS score progression (defined as an increase of 1 point compared with baseline and sustained for  $\geq 3$  months).

We modeled lesion counts using negative binomial regression and continuous outcomes (T2 lesion volume and normalized brain volume) using generalized linear regression (applying log transformations to skewed data), allowing for patient-specific random intercepts in longitudinal analyses.<sup>18</sup> We assessed relapse rate using an Andersen-Gill model<sup>19</sup> for recurrent events and assessed time to confirmed EDSS score progression using Cox regression analysis.

Because of large differences in 25(OH)D levels across regions, categorization using either quintiles or prespecified cut points of 25(OH)D levels resulted in groups that were largely region dependent. Given these differences and the varied baseline disease characteristics across regions, in primary analyses, we modeled baseline and cumulative averaged 25(OH)D using linear terms. We assessed the robustness of this modeling strategy using restricted cubic splines and found no evidence of thresholds or departure from linearity in any model for either MRI or clinical outcome. Further, we conducted analyses restricted to individuals with average 25(OH)D levels of at least 50.0 nmol/L and, separately, categorical analyses using the following a priori categories of average 25(OH)D values: less than 37.5, 37.5-49.9, 50.0-74.9, 75.0-99.9, and 100.0 nmol/L or higher (both overall and stratified by region).

All analyses were adjusted for age, sex, randomized treatment (interferon beta-1b, 250 or 500  $\mu$ g), disease duration ( $\leq 1$  year, 2-5 years, 6-10 years, and  $>10$  years), and region (North America, Western Europe, Eastern Europe, and the southern hemisphere, with the exception of analyses stratified by region). Longitudinal analyses were additionally adjusted for

baseline disease characteristics, including EDSS score, and, in sensitivity analyses, for baseline T2 lesion volume and race/ethnicity. We also assessed the effects of 25(OH)D following prespecified subgroups based on baseline characteristics: age (median or less vs greater than the median), sex, relapses in the previous 2 years ( $<2$  vs  $\geq 2$ ), disease duration ( $\leq 1$  year, 2-5 years, 6-10 years, and  $>10$  years), T2-lesion volume (median or less vs greater than the median), EDSS score ( $<3$  vs  $\geq 3$ ), and disease activity (high vs low). *High disease activity* was defined as having at least 2 relapses in the previous 2 years (to study baseline) and at least 1 T1-enhancing lesion noted on baseline MRI.

We also conducted analyses assessing the association between MS disease activity and serum 25(OH)D levels adjusting for HLA-DRB1 (using rs3135388 as a marker for DRB1\*15; imputation  $r^2 > 0.99$ ) or vitamin D-binding protein/GC (using rs4588 as a marker for GC-1s/f vs GC-2; imputation  $r^2 = 0.96$ ) and assessed whether either single-nucleotide polymorphism modified such association.

## Results

Among the 1796 individuals with MS included in our study, average 25(OH)D levels varied markedly by region: the lowest levels were observed in Russia (median, 41.9 nmol/L), and the highest levels were documented in Australia (median, 66.4 nmol/L), Brazil (68.9 nmol/L), and North America (59.7 nmol/L) (eTable 1 in the Supplement). Significant differences were also observed in several clinical parameters. The most salient differences were the higher volume of T2 hyperintense lesions, younger age at onset, and longer disease duration among patients from Eastern Europe and Russia compared with those from Western Europe and North America (eTable 1 in the Supplement). Following standardization by age and region, at baseline patients with higher 25(OH)D levels tended to have lower body mass index, a lower EDSS score and Multiple Sclerosis Severity Score, fewer relapses in the previous 2 years, lower T1 and T2 lesion volumes, and higher normalized brain volume (Table 1).

### MRI Evidence of Disease Activity

In cross-sectional analyses adjusted for age, sex, randomization treatment group (interferon beta-1b, 250 vs 500  $\mu$ g), baseline EDSS score, disease duration, and region of residence, baseline 25(OH)D levels, reported as estimates (95% CIs), were inversely associated with T2 hyperintense lesion volume (a 50-nmol/L higher level corresponding to a difference of  $-0.11$  cm<sup>3</sup> in log[T2 lesion volume];  $-0.20$  to  $-0.02$ ;  $P = .02$ ) and the total number of T1-enhancing lesions ( $-0.24$ ;  $-0.45$  to  $-0.02$ ;  $P = .03$ ) at baseline (Table 2). Similar results were obtained for the association between 25(OH)D and MRI parameters at 12 months, with a difference in log(T2 lesion volume) of  $-0.19$  cm<sup>3</sup> ( $-0.31$  to  $0.07$ ) (Table 2). Participant 25(OH)D levels were also inversely associated with the cumulative number of new active lesions (defined as the sum of new T2- and T1-enhancing lesions) between baseline and 12 months ( $0.76$ ;  $0.60$  to  $0.98$ ;  $P = .03$ ) (Table 2).

Table 1. Selected Age- and Region-Adjusted Characteristics of BEYOND Participants<sup>a</sup>

Characteristic	Category of Season-Adjusted 25(OH)D Level				
	<37.5 nmol/L	37.5-49.9 nmol/L	50.0-74.9 nmol/L	75.0-99.9 nmol/L	≥100.0 nmol/L
No. of patients	299	331	534	194	98
Season-adjusted 25(OH)D level					
Mean (SD), nmol/L	30.0 (5.0)	43.8 (3.3)	60.8 (7.0)	86.5 (5.9)	117.1 (12.9)
Median (IQR), nmol/L	31.2 (7.8)	43.6 (6.0)	59.5 (11.8)	86.9 (11.8)	114.0 (18.2)
Age, mean (SD), y <sup>b</sup>	36.1 (8.4)	36.0 (8.5)	35.9 (9.0)	35.8 (7.7)	35.0 (5.7)
Residing in North America, No. (%) <sup>b</sup>	25	23	29	38	51
Male sex, No. (%)	27	31	35	39	15
White race, No. (%)	85	91	92	96	96
Age at onset, mean (SD), y	31.5 (8.2)	31.5 (8.2)	31.4 (9.0)	30.8 (8.2)	30.5 (5.5)
BMI, mean (SD)	27.2 (7.0)	25.3 (4.9)	25.2 (5.0)	25.0 (4.1)	23.3 (2.9)
Baseline disease characteristics					
EDSS score, mean (SD)	2.5 (1.2)	2.5 (1.1)	2.3 (1.1)	1.9 (0.8)	1.9 (0.8)
No. of relapses in previous 2 years (at baseline), mean (SD)	2.0 (0.8)	2.1 (0.9)	2.1 (1.0)	1.9 (0.7)	1.9 (0.6)
Disease duration, median (IQR)	3.0 (7.0)	5.2 (5.0)	5.1 (5.8)	5.5 (4.8)	5.0 (3.9)
MSSS, mean (SD)	4.9 (2.2)	4.8 (2.3)	4.5 (2.3)	4.2 (1.9)	3.8 (1.7)
Normalized brain volume, mean (SD), cm <sup>3</sup>	1490 (112)	1486 (103)	1488 (103)	1481 (95)	1501 (78)
No. of T1 hyperintense lesions, median (IQR)	2.5 (3)	2.6 (2)	1.8 (2)	1.9 (2)	1.8 (1)
Volume of T2 hyperintense lesions, mean (SD), cm <sup>3</sup>	9.6 (10.1)	9.5 (10.9)	8.4 (8.9)	8.8 (7.8)	6.4 (5.9)
Volume of T1-nonenhancing hypointense lesions, mean (SD), cm <sup>3</sup>	1.9 (3.3)	1.8 (3.5)	1.6 (2.8)	1.8 (4.7)	1.1 (1.6)

Abbreviations: BEYOND, Betaferon Efficacy Yielding Outcomes of a New Dose; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); EDSS, Expanded Disability Status Scale; IQR, interquartile range; MSSS, Multiple Sclerosis Severity Score; 25(OH)D, 25-hydroxyvitamin D.

SI conversion factor: To convert 25(OH)D to nanograms per milliliter, divide by 2.496.

<sup>a</sup> Participants received interferon beta-1b, 250 µg or 500 µg.

<sup>b</sup> Value was not age or region adjusted.

In longitudinal analyses, 25(OH)D was significantly inversely correlated with the cumulative number of active lesions between baseline and the last MRI (average follow-up time, 2 years), with a 50-nmol/L higher level in serum 25(OH)D levels associated with a 31% lower rate of new lesions (relative rate [RR], 0.69; 95% CI, 0.55-0.86;  $P = .001$ ). This inverse association was also strong and significant in analyses restricted to patients with 25(OH)D levels greater than 50.0 nmol/L (RR, 0.62; 95% CI, 0.46-0.84;  $P = .002$ ), and was consistently observed in each of the 4 regions (Figure 1). The dose-response association between 25(OH)D and the cumulative number of active lesions was further explored in categorical analyses stratified by region (Table 3). Patients with serum 25(OH)D levels greater than >100.0 nmol/L had a 47% lower rate of new active lesions compared with patients who had serum levels of 50.0 to 74.9 nmol/L (RR, 0.53; 95% CI, 0.37-0.78;  $P = .002$ ) and were relatively consistent within the region (Table 3). Following the potential threshold effect we observed (Table 3), we fit an additional regression model to test for a threshold at 100.0 nmol/L. Results of this analysis suggested no significant differences in the effect of 25(OH)D on the rate of cumulative new active lesions for individuals with levels less than 100.0 nmol/L and those with levels of 100.0 nmol/L or more ( $P = .39$ ). In analyses further adjusted for HLA-DRB1\*15 or vitamin D-binding protein status, results were slightly stronger (adjustment for HLA-DRB1\*15: RR per 50.0 nmol/L, 0.65; 95% CI, 0.46-0.93;  $P = .02$ ; adjustment for vitamin D-binding protein: RR per 50.0 nmol/L, 0.63; 95% CI, 0.45-0.90;  $P = .01$ ). We did not observe evidence of an inter-

Table 2. MRI Evidence of Disease Activity per 50.0-nmol/L Increase in 25(OH)D in Cross-Sectional Analyses

Characteristic	MRI Outcome, Estimate (95% CI) <sup>a</sup>	P Value
Baseline		
Difference in log(T2 lesion volume), cm <sup>3</sup>	-0.11 (-0.20 to -0.02)	.02
No. of T1-enhancing lesions	-0.24 (-0.45 to -0.02)	.03
Difference in log(T1 hypointense lesion volume), cm <sup>3</sup>	-0.05 (-0.12 to 0.02)	.17
12 mo <sup>b</sup>		
Difference in log(T2 lesion volume), cm <sup>3</sup>	-0.19 (-0.31 to 0.07)	.003
Difference in log(T1 hypointense lesion volume), cm <sup>3</sup>	-0.07 (-0.14 to 0.00)	.055
Rate of T2 lesion count during year 1	0.78 (0.62 to 0.99)	.04
Rate of T1-enhancing lesions during year 1	0.74 (0.50 to 1.08)	.12
Rate of new active lesions during year 1	0.76 (0.60 to 0.98)	.03

Abbreviations: EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; 25(OH)D, 25-hydroxyvitamin D.

<sup>a</sup> Adjusted for age, sex, randomization status, baseline EDSS, disease duration (≤1, 2-5, 6-10, and >10), and region of residence (North America, Western Europe, Eastern Europe and southern hemisphere).

<sup>b</sup> Magnetic resonance imaging performed at month 12.

action between single-nucleotide polymorphism or 25(OH)D levels and MS.

In additional analyses, the association between serum 25(OH)D and MS activity was slightly attenuated following

adjustment for race/ethnicity and baseline T2 lesion volume but remained statistically significant (RR of cumulative new active lesions for 50.0-nmol/L increase, 0.74; 95% CI, 0.60-0.92;  $P = .009$ ). Results were also consistent in analyses using the 12-month MRI measurements as the baseline; in these analyses, a 50.0-nmol/L higher level in serum 25(OH)D levels was associated with a 25% lower rate of new lesions (RR, 0.75; 95% CI, 0.60-0.94;  $P = .01$ ). Similarly, additional analyses adjusting for body mass index did not materially influence the results (for cumulative number of active lesions: RR for 50.0-nmol/L increase, 0.69; 95% CI, 0.55-0.86;  $P = .001$ ). The inverse association between average 25(OH)D levels and the cu-

mulative number of new active lesions was similar across strata in exploratory analyses defined by age, sex, disease activity and severity at baseline, disease duration, T2 lesion volume, and EDSS score (Figure 2).

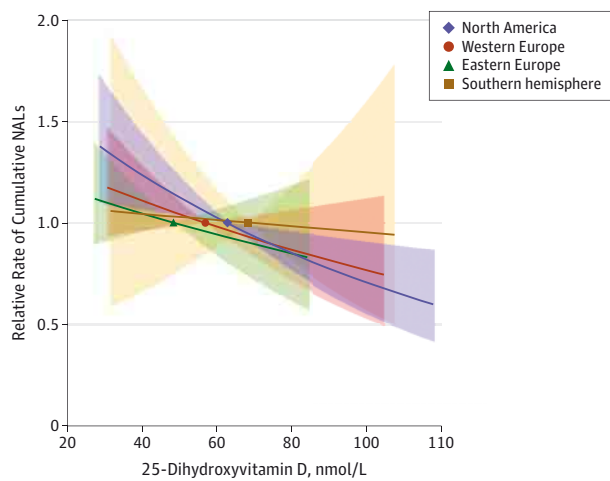
**Relapses and EDSS**

No significant association was found in a cross-sectional analysis between baseline 25(OH)D levels and the number of relapses in the previous 2 years (relapse rate, 1.01; 95% CI, 0.94 to 1.09;  $P = .77$ ) or between 25(OH)D level and the number of relapses from baseline to the last visit (relapse rate, 1.01; 95% CI, 0.84 to 1.21;  $P = .92$ ). Baseline 25(OH)D level was inversely associated with EDSS scores at baseline: a 50.0-nmol/L higher level of 25(OH)D corresponded to a mean difference of 0.28 in EDSS scores (mean difference, -0.28; 95% CI, -0.40 to -0.16;  $P < .001$ ). However, analyses were attenuated in longitudinal analyses adjusted for age, sex, randomization status, region of residence, and baseline disease duration, with 25(OH)D no longer significantly associated with confirmed EDSS progression (defined as a 1-point increase from baseline that was sustained for  $\geq 3$  months; RR per 50.0-nmol/L increase: 0.84; 95% CI, 0.86 to 1.15;  $P = .14$ ). Results were slightly stronger using month 12 as the baseline but remained insignificant (confirmed EDSS progression: RR per 50.0-nmol/L increase, 0.77; 95% CI, 0.56 to 1.06;  $P = .11$ ).

**Brain Volume**

No associations were found between 25(OH)D levels and brain volume at baseline or at 12 months. Similarly, we did not observe an association between 25(OH)D or relative change in brain volume from baseline or 12 months to 24 months (eTable 2 in the Supplement).

**Figure 1. Relative Rate of Cumulative New Active Lesions (NALs) vs Average of Baseline, 6-Month, and 12-Month 25-Hydroxyvitamin D (25[OH]D) Level Stratified by Geographic Region**



The solid lines and shaded regions represent the relative rate ratios of cumulative NALs for changes in 25(OH)D relative to the median level and the corresponding 95% CIs, respectively. Analyses were adjusted for age, sex, randomization status, baseline Expanded Disability Status Scale score, and disease duration ( $\leq 1$ , 2-5, 6-10, and  $>10$  years). Models assume a linear association between the logarithm of the rate of cumulative NALs and serum 25(OH)D. Analyses using cubic splines revealed no significant deviation from linearity. To convert 25(OH)D values to nanograms per milliliter, divide by 2.496.

**Discussion**

In this large prospective study among patients with relapsing-remitting MS treated with interferon beta-1b and with median disease duration of 3 years, we found that, in cross-sectional analyses, those with higher serum 25(OH)D levels had

**Table 3. Categorical Season-Adjusted 25(OH)D and Rate of New Active Lesions**

Characteristic	Category of Season-Adjusted 25(OH)D, nmol/L				
	<37.5	37.5-49.9	50.0-74.9	75.0-99.9	$\geq 100.0$
Overall <sup>a</sup>	0.95 (0.73-1.24)	0.97 (0.81-1.17)	1 [Reference]	0.93 (0.75-1.14)	0.53 (0.37-0.78)
Region <sup>b</sup>					
North America	1.19 (0.70-2.00)	1.17 (0.82-1.68)	1 [Reference]	1.10 (0.77-1.55)	0.61 (0.34-1.12)
Western Europe	0.78 (0.47-1.30)	1.02 (0.74-1.40)	1 [Reference]	0.95 (0.65-1.39)	0.36 (0.16-0.81)
Eastern Europe	0.97 (0.65-1.43)	0.85 (0.64-1.11)	1 [Reference]	0.82 (0.52-1.30) <sup>c</sup>	NA
Southern hemisphere	0.82 (0.30-2.21) <sup>c</sup>	NA	1 [Reference]	0.64 (0.35-1.18) <sup>c</sup>	NA

Abbreviations: NA, not applicable; 25(OH)D, 25-hydroxyvitamin D.

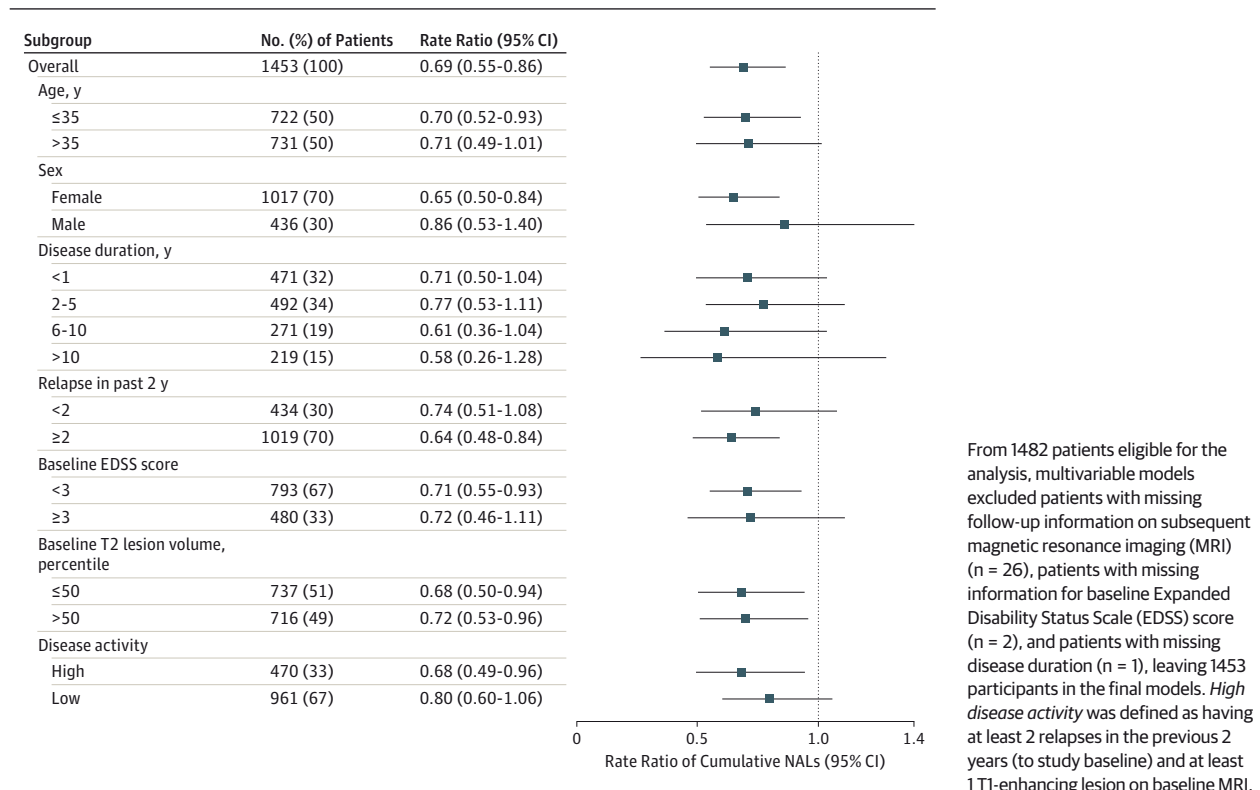
SI conversion factor: To convert 25(OH)D to nanograms per milliliter, divide by 2.496.

<sup>a</sup> Adjusted for age, sex, randomization status, baseline Expanded Disability Status Scale score, disease duration ( $\leq 1$ , 2-5, 6-10, and  $>10$  years), and region of residence (North America, Western Europe, Eastern Europe, and southern hemisphere).

<sup>b</sup> Adjusted for all of the above factors except region.

<sup>c</sup> Categories were collapsed when the number of patients was less than 20. The categories for less than 37.5 nmol/L and 37.5 to 49.9 nmol/L and for 75.0 to 99.9 nmol/L and greater than or equal to 100.0 nmol/L were collapsed; data shown represent the effect estimates for the combined ranges.

**Figure 2. Relative Rate of Cumulative New Active Lesions (NALs) vs Average of Baseline, 6-Month, and 12-Month 25-Hydroxyvitamin D Level Stratified by Baseline Disease Characteristics**



a lower disease burden noted on MRI and a lower baseline EDSS score. Furthermore, patients with higher serum 25(OH)D levels during the first 12 months of the study developed fewer new active lesions observed on MRI during a median follow-up time of 2 years. This inverse longitudinal association between 25(OH)D levels and MS activity was consistent with and independent of HLA-DRB1\*15 or vitamin D-binding protein status and was present across various subgroups of patients with different baseline characteristics. A decrease in the number of new active lesions with increasing serum 25(OH)D levels was observed even after excluding patients with vitamin D levels less than 50.0 nmol/L, a result suggesting that 50.0 nmol/L may be too low a target in patients with MS. However, despite the uniform results for MRI measures of disease activity, serum 25(OH)D levels were not significantly correlated with brain atrophy or clinical outcomes.

Strengths of this study include its large number of participants, the regionally diverse population with varying baseline characteristics, and the repeated measurements of 25(OH)D, which helped to characterize patients' long-term vitamin D status. Furthermore, the results could be adjusted for known confounders, such as baseline disease severity, which affects both serum 25(OH)D levels and MS progression, and the wide distribution of serum 25(OH)D levels allowed us to assess whether optimal levels for preventing MS progression exceed the moderate levels observed in most previous studies.<sup>7,9,10</sup> Our observation of the lowest level of MS activity among patients with serum 25(OH)D levels above 100.0 nmol/L is consistent with the results of a previous

investigation in the United States<sup>20</sup> and suggests that the 25(OH)D levels in most patients with MS who are not receiving supplemental vitamin D may be suboptimal. Although circulating levels of 25(OH)D of 100.0 nmol/L are achievable in most patients with supplementation of cholecalciferol, 1000 to 4000 IU/d, which is considered a tolerable dose, there is no convincing evidence that increasing 25(OH)D levels above the physiologic limit (approximately 150.0 nmol/L) is beneficial, and it could potentially be detrimental. Considering the results of a randomized trial<sup>21</sup> in which higher (14 000 IU/d) doses of cholecalciferol were administered to patients with MS, intake of more than 4000 IU/d should be discouraged.

Our study also has important limitations. Length of follow-up was relatively short (median, 2 years). This limited follow-up may explain the lack of association between serum 25(OH)D levels and measures of brain atrophy or clinical endpoints, both of which have been previously shown to be modified by 25(OH)D in a study with a longer follow-up period.<sup>7</sup> However, although the lack of observed effect for measures of brain volume may be the result of a relatively short follow-up time in our study, since other studies including longer follow-up have observed a protective effect of vitamin D, we cannot rule out that brain atrophy may be less sensitive to 25(OH)D levels than the development of new lesions. In addition, participants were at different stages in the disease course, yet the range of disease duration at recruitment in this study was relatively short (median duration, 3 years; interquartile range, 1-7 years). Exposure to UV light is also an

important determinant of serum 25(OH)D levels<sup>11,22,23</sup> and has immunologic effects not mediated by vitamin D that could potentially affect MS outcomes.<sup>22</sup> Nonetheless, the inverse association between serum 25(OH)D levels and MS outcomes in regions with low levels of UV light during most of the year and in patients with more severe disease who are less likely to spend time outdoors support a role for vitamin D. All patients in the present study received interferon beta-1b, and, although uniform treatment is an important advantage, our results may not be generalizable to patients who receive other drugs. Finally, as in all observational studies, confounding by an unmeasured factor related to both serum 25(OH)D levels and MS progression cannot be excluded. However, adjustment for known confounders did not materially influence effect estimates.

Results of this study are consistent with the known immunomodulatory role of vitamin D and also support evidence from clinical trials<sup>24-29</sup> that have shown dose-dependent effects of vitamin D on the immune system, including a reduced proliferation by peripheral blood T cells in response to MS-related antigens and an increased proportion of interleukin 10<sup>+</sup> CD4<sup>+</sup> T cells.

Several ongoing randomized trials are assessing the effect of supplementary vitamin D in patients with relapsing-remitting MS and will provide important evidence in establishing causality. Ethical concerns, however, prevent the randomization to placebo of patients with vitamin D insufficiency and, thus, the experimental demonstration of the effects of vitamin D supplementation in these patients. Therefore, our study provides crucial complementary evidence on the importance of correcting vitamin D insufficiency in MS and, in addition, contributes to identifying the optimal 25(OH)D level.

## Conclusions

The findings of this large prospective investigation comprising patients from 5 continents and a broad range of serum 25(OH)D levels suggest that adequate vitamin D status is an important determinant of MS activity not only early in the disease course but also several years after the diagnosis. These results also suggest that individuals with MS may benefit from vitamin D levels above those currently considered by many to be sufficient in healthy adults.

### ARTICLE INFORMATION

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**Study concept and design:** Pohl, Ascherio.

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