ARTICLE

Vitamin D, smoking, EBV, and long-term cognitive performance in MS

11-year follow-up of BENEFIT

Marianna Cortese, MD, PhD, Kassandra L. Munger, ScD, Elena H. Martínez-Lapiscina, MD, PhD, Christian Barro, MD, Gilles Edan, MD, Mark S. Freedman, MD, Hans-Peter Hartung, MD, Xavier Montalbán, MD, Frederick W. Foley, PhD, Iris Katharina Penner, PhD, Bernhard Hemmer, MD, Edward J. Fox, MD, PhD, Sven Schippling, MD, Eva-Maria Wicklein, MD, Ludwig Kappos, MD, Jens Kuhle, MD, PhD, and Alberto Ascherio, MD, DrPH, on behalf of the BENEFIT Study Group

Correspondence
Dr. Cortese
mcortese@hsph.harvard.edu

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Abstract

Objective

To investigate whether vitamin D, smoking, and anti-Epstein-Barr virus (EBV) antibody concentrations predict long-term cognitive status and neuroaxonal injury in multiple sclerosis (MS).

Methods

This study was conducted among 278 patients with clinically isolated syndrome who participated in the clinical trial BENEFIT (Betaferon/Betaseron in Newly Emerging Multiple Sclerosis for Initial Treatment) and completed the 11-year assessment (BENEFIT-11). We measured serum 25-hydroxyvitamin-D (25(OH)D), cotinine (smoking biomarker), and anti-Epstein-Barr virus nuclear antigen 1 (EBNA-1) immunoglobulin G (IgG) at baseline and at months 6, 12, and 24 and examined whether these biomarkers contributed to predict Paced Auditory Serial Addition Test (PASAT)-3 scores and serum neurofilament light chain (NfL) concentrations at 11 years. Linear and logistic regression models were adjusted for sex, baseline age, treatment allocation, steroid treatment, multifocal symptoms, T2 lesions, and body mass index.

Results

Higher vitamin D predicted better, whereas smoking predicted worse cognitive performance. A 50-nmol/L higher mean 25(OH)D in the first 2 years was related to 65% lower odds of poorer PASAT performance at year 11 (95% confidence intervals [95% CIs]: 0.14–0.89). Standardized PASAT scores were lower in smokers and heavy smokers than nonsmokers (p_{trend} = 0.026). Baseline anti–EBNA-1 IgG levels did not predict cognitive performance (p_{trend} = 0.88). Associations with NfL concentrations at year 11 corroborated these findings—a 50-nmol/L higher mean 25(OH)D in the first 2 years was associated with 20% lower NfL (95% CI: −36% to 0%), whereas smokers had 20% higher NfL levels than nonsmokers (95% CI: 2%–40%). Anti–EBNA-1 antibodies were not associated with NfL.

Conclusions

Lower vitamin D and smoking after clinical onset predicted worse long-term cognitive function and neuronal integrity in patients with MS.

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From the Department of Nutrition (M.C., K.L.M, A.A.), Harvard T.H. Chan School of Public Health, Boston, MA; Department of Global Public Health and Primary Care (M.C.), University of Bergen, Bergen, Norway; Department of Neurology (E.H.M.-L.), Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain; Departments of Medicine, Biomedicine and Clinical Research (C.B., L.K., J.K.), Neurologic Clinic and Policlinic, University Hospital Basel, University of Basel, Basel, Switzerland; CHU Hôpital Pontchaillou (G.E.), Rennes, France; University of Ottawa and Ottawa Hospital Research Institute (M.S.F.), Ottawa, Canada; Department of Neurology (H.-P.H.), Medical Faculty, Heinrich-Heine Universität, Düsseldorf, Germany; St. Michael's Hospital (X.M.), University of Toronto, Canada and Multiple Sclerosis Center of Catalonia (Cemcat) (X.M.), Vall d'Hebron University Hospital, Barcelona, Spain; Ferkauf Graduate School of Psychology (F.W.F.), Yeshiva University, New York, NY; Department of Neurology (I.K.P.), Medical Faculty, Heinrich-Heine Universität, Düsseldorf and COGITO Center for Applied Neurocognition and Neuropsychological Research (I.K.P.), Düsseldorf, Germany; Technical University of Munich (B.H.), School of Medicine and Munich Cluster for Systems Neurology (SyNergy) (B.H.), Munich, Germany; Central Texas Neurology Consultants (E.J.F.), Round Rock, TX; Neuroimmunology and Multiple Sclerosis Research (S.S.), Department of Neurology, University Hospital Zurich, University of Zurich and Center for Neuroscience Zurich (S.S.), Federal Institute of Technology (ETH), Zurich, Switzerland; Bayer AG (E.-M.W.), Berlin, Germany; Department of Epidemiology (A.A.), Harvard T.H. Chan School of Public Health, Boston, MA and Channing Division of Network Medicine (A.A.); and Department of Medicine (A.A.), Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

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Coinvestigators are listed at links.lww.com/WNL/B80.

Glossary

BMI = body mass index; **CI** = confidence interval; **CIS** = clinically isolated syndrome; **CV** = coefficient of variation; **EBV** = Epstein-Barr virus; **EBNA-1** = Epstein-Barr virus nuclear antigen 1; **EDSS** = Expanded Disability Status Scale; **IgG** = immunoglobulin G; **INF β-1b** = interferon beta-1b; **MS** = multiple sclerosis; **NfL** = neurofilament light chain; **OR** = odds ratio; **PASAT** = Paced Auditory Serial Addition Test; **VCA** = viral capsid antigen.

Cognitive impairment is a common and debilitating symptom of multiple sclerosis (MS),[1] substantially affecting patients' quality of life.[2,3] Cognitive impairment is associated with both white matter inflammatory lesions and gray matter pathology, such as cortical lesions and brain atrophy,[4,5] and is thus also a manifestation of neurodegenerative pathology.[1] Because there is no established treatment, the search for modifiable factors that prevent or slow cognitive decline is especially important.[6–9]

Low vitamin D, cigarette smoking, and elevated antibodies against Epstein-Barr virus (EBV) nuclear antigen 1 (EBNA-1), which are established MS risk factors,[10] have also been associated with a clinically and radiologically more active and faster progressing disease in some,[11–24] although not all, studies.[16,24–30] However, whether these MS risk factors specifically predict patients' long-term cognitive status has not been explored.

We have previously reported that among participants in the clinical trial BENEFIT (Betaferon/Betaseron in Newly Emerging Multiple Sclerosis for Initial Treatment), those with higher vitamin D levels at 1 or 2 years after recruitment had fewer active lesions and less brain atrophy during 5 years of follow-up.[11] In this study, we extended the follow-up to 11 years and examined whether vitamin D, smoking, and anti–EBNA-1 antibodies early after the first manifestation of relapsing-remitting MS contributed to predict long-term cognitive function and neuroaxonal integrity independent of disease-modifying treatment.

Methods

Study population and design

BENEFIT (clinicaltrials.gov: NCT00185211) was a multicenter double-blind phase 3 clinical trial of early vs delayed treatment with interferon beta-1b (INF β-1b) in 468 patients with clinically isolated syndrome (CIS), a first clinical episode suggestive of MS. Preplanned, rater-blinded follow-up was 5 years, later followed by observation up to 8.7 years, and then by assessment at year 11 to evaluate long-term effects of early treatment. The current investigation was conducted among the 278 patients who completed the 11-year examination (BENEFIT-11: NCT01795872). These patients comprise 71.3% of all patients at the sites eligible to participate in BENEFIT-11 and were comparable at baseline to the originally randomized trial population (medians, age: 30 vs 30 years, 70% vs 71% females, Expanded Disability Status Scale [EDSS] score: 1.50 vs 1.50, T2 lesions: 18 vs 17).[31] At year 11 assessment, 61.5% of the 278 enrolled patients were on disease-modifying treatment, and of these, about 50% were on INF β-1b,[31] about 23% were on other injectables, 15% on oral drugs, 9% on monoclonal antibodies, 5% on immunosuppressants, and <1% on immunoglobulins.

Standard protocol approvals, registrations, and patient consents

The Harvard T.H. Chan School of Public Health Institutional Review Board approved this study, and BENEFIT participants provided written informed consent. We used deidentified data.

Serum biomarkers of vitamin D, smoking, and EBV infection

Biomarkers were measured in serum collected at baseline and at months 6, 12, and 24 for all exposures, and also at months 54 and 60 and at year 11 for vitamin D. Samples were shipped to a central German laboratory within 3 days of collection and stored at −20°C before measurement.

We measured 25-hydroxyvitamin D (25(OH)D), the preferred biomarker of vitamin D nutrition status,[10] using ELISA (Immunodiagnostic Systems Inc., Fountain Hills, AZ) in samples collected within the first 24 months and chemiluminescence immunoassay (Roche Diagnostics) in later samples. From blind quality control samples, the average coefficients of variation (CVs) were 4.4% intra- and 11.7% inter-assay for samples up to month 24 and 4.0% intra- and 4.9% inter-assay for samples collected thereafter. 25(OH)D levels varied, as expected, by season, with strong correlations between season-synchronous and weak correlation between season-asynchronous levels[11] and were thus adjusted for seasonal variation, as previously described,[32] to estimate long-term average vitamin D status. Briefly, raw 25(OH)D levels were regressed on the periodic function $\sin(2\pi x/12) + (-\cos(2\pi x/12)$, where x is the month of blood collection, within strata by sex adjusting categorically for age (18–22, 23–27, 28–32, 33–37, 38–42, and >43 years) and assay batch. The residuals derived from this model (raw minus predicted levels) were added to the sex-specific mean 25(OH)D levels.

We quantified levels of immunoglobulin G (IgG) against EBNA-1, a measure of past EBV infection, and against viral capsid antigen (VCA), a measure of acute infection, if EBNA-1 antibodies are negative, using ELISA (DiaMedix Corp., Miami, FL). CVs were 0.95% intra- and 4.2% inter-assay for EBNA-1.

The nicotine metabolite cotinine, a biomarker of current/recent tobacco use that correlates with the number of cigarettes smoked,[33] was also measured by ELISA. Smokers typically have levels >25 ng/mL, whereas nonsmokers have levels <10 ng/mL.[34] CVs were 3.1%–7.2% intra- and 12.7%–13.4% interassay.

Cognitive function and neuronal integrity

Cognitive function was assessed during neurologic examinations (baseline, months 6, 12, 18, 24, 30, 36, 42, 48, 54, and 60, and year 11) by the Paced Auditory Serial Addition Test (PASAT) on a scale of 0–60. The test was also administered twice before baseline (at screening and between screening and baseline) to minimize practice effects from baseline.[35] The test does not assess all aspects of cognition affected in MS, but is validated to capture the most common MS-specific cognitive issues with processing speed, attention, and working memory.[6] Using a single-molecule array (Simoa),[36] serum neurofilament light chain (NfL) concentrations were determined at baseline, year 1, and year 11 at the Laboratory of the University Hospital Basel, Departments of Neurology and Biomedicine, Switzerland. NfL is a sensitive biomarker of neuroaxonal injury, strongly associated with NfL in CSF.[37]

Statistical analyses

We conducted statistical analyses in SAS 9 (SAS Institute Inc, Cary, NC). In the primary analyses, we examined whether serum concentrations of 25(OH)D, cotinine, and anti–EBNA-1 IgGs measured within the first 24 months after CIS contributed to predict cognitive performance and neuroaxonal integrity at year 11. This long lag was meant to minimize reverse causality, that is, that the disease course itself affected exposure status and could induce spurious associations.

To assess cognitive performance, we used linear regression with a standardized (z-) PASAT score at year 11 as the outcome (mean = 52.5, SD = 9.0, 1 unit on the z-PASAT corresponds to 1 SD) and reported regression coefficients (β) and 95% confidence intervals (CIs). Furthermore, we used logistic regression to estimate the odds of scoring worse/more poorly defined as a PASAT score at or below the median (56 points) at year 11 to optimize statistical power with regard to deteriorating but still high-functioning patients in this study population. We reported odds ratios (ORs) and 95% CIs. The analyses of 25(OH)D were repeated to examine the change in the PASAT from month 6 to year 11 as the outcome, by adjusting the models for the PASAT score at month 6.

To examine the neuroaxonal integrity, the associations of 25(OH)D, cotinine, and anti-EBNA-1 IgG within the first 24 months with NfL concentrations at year 11 were assessed using linear regression. NfL levels were log transformed to improve normality. As regression coefficients for NfL reflect changes on the log scale, they were back transformed to reflect changes on a multiplicative scale (e.g., an estimate of 1.10 corresponds to 10% increase in NfL) and reported as percent difference in median NfL in the exposure compared with the reference group.

To quantify vitamin D exposure, all season-adjusted 25(OH)D levels up to month 24 were averaged, except for baseline concentration, which may have been influenced by the first clinical episode.[12] In secondary analyses, we examined whether the results differed for mean 25(OH)D within the first 60 months. Using several measurements allowed us to classify with more precision the vitamin D exposure over time, which is likely to be the relevant exposure. These means were assessed continuously in 50 nmol/L steps and in quintiles as in previous BENEFIT investigations.[11]

With regard to smoking exposure, individuals with baseline cotinine >25 ng/mL were compared with those with levels <10 ng/mL. Furthermore, patients consistently >25 ng/mL at baseline and at months 6, 12, and 24 were classified as smokers and those <10 ng/mL as nonsmokers. These categories were also chosen a priori based on previous BENEFIT investigations.[25] In a subanalysis, we further distinguished heavy smokers who had cotinine levels consistently ≥193 ng/mL (median among smokers). Smokers and heavy smokers were compared with nonsmokers. If a measurement was missing for a specific time point, the last available value was carried forward. The use of multiple cotinine measurements decreases the risk of misclassification of smoking status. To further decrease the risk of misclassification, participants with levels of 10–25 ng/mL or levels changing from <10 to >25 ng/mL across measurements were kept as a separate group in categorical analyses (similar to a missing indicator) but were not reported in the results, as their exposure could not be clearly determined.

Finally, anti–EBNA-1 IgG (baseline and months 6, 12, and 24) and anti–VCA IgG levels (months 6 and 12) were examined in quartiles derived from the antibody distribution at each time point, using the bottom quartile as the reference, as in previous BENEFIT studies.[25] For categorical exposures, we reported the p for trend across categories using the median within each category as a continuous variable to assess dose-response relationships.

We adjusted all multivariable models for baseline age in 5-year groups (18–22, 23–27, 28–32, 33–37, 38–42, and >43 years), sex, treatment allocation at baseline (INF β-1b or placebo), steroid treatment during CIS (yes-no), multifocal symptom onset (yes-no), number of baseline T2-weighted MRI lesions (2–4, 5–8, and ≥9, categorically), and baseline body mass index (BMI) (continuously). We also assessed whether results differed when adjusting the multivariable models categorically for the region of trial participation (Canada, Scandinavia, and Northern or Southern Europe). If an exposure was significantly associated with the outcome(s), we included it as a covariate in the models of the other exposures.

Because fatigue and depression can influence cognitive performance,[38] we moreover examined whether 25(OH)D, cotinine, and anti-EBNA-1 IgG were associated with the PASAT score at year 11 after further adjusting the primary analyses both for the total fatigue score on the Fatigue Scale for Motor

and Cognitive Functions (<43: no fatigue, ≥43–<63: mild/moderate fatigue, ≥63: severe fatigue)[38] and for the depression score on the Center for Epidemiologic Studies Depression scale (no, mild, and severe depression) assessed at year 11.[39] Fatigue was reported by 54% and depressive symptoms by 31.3% of the patients at year 11.[31]

Data availability

The datasets analyzed in the current study are not publicly available because of restricted access, but further information about the datasets is available from the corresponding author on reasonable request.

Results

Selected baseline characteristics of the 278 BENEFIT-11 participants according to 25(OH)D, cotinine, and anti–EBNA-1 levels are shown in table 1. The most notable differences were a lower BMI and fewer baseline T2 lesions among patients with higher 25(OH)D and a higher proportion of males among individuals with higher anti–EBNA-1 titers and smokers. Participants had on average 2.6 relapses during follow-up (range: 0–15), and 13.8% reported intake of vitamin D supplements from baseline to year 11. The mean PASAT score was 52.5 (SD ±9), and the mean NfL concentration was 30 pg/mL (SD ±30.5 pg/mL) at year 11. Neither NfL at baseline (Spearman, $r = -0.10$, $p = 0.13$) nor at year 1 ($r = -0.11$, $p = 0.17$) correlated with the PASAT at year 11. NfL at year 1 ($r = 0.42$, $p < 0.0001$) correlated more strongly with NfL at year 11 than baseline NfL ($r = 0.25$, $p = 0.0002$).

Long-term cognitive function

Individuals with higher 25(OH)D during the first years following a CIS were less likely to score below the median in the PASAT performed at year 11. In analyses using 25(OH)D as a continuous variable, a 50-nmol/L higher mean level within the first 24 months after CIS was associated with 65% lower odds of obtaining a below median PASAT score at year 11 (n = 219: multivariable OR (OR_{adj}) = 0.35, 95% CI: 0.14–0.89, $p = 0.027$). We obtained similar results when adjusting also for the PASAT at month 6 to assess the association between 25(OH)D levels and PASAT score change from month 6 to year 11 ($OR_{adj} = 0.23$, 95% CI: 0.07–0.73, $p = 0.013$). In categorical analyses, patients with mean 25(OH)D in the top quintile within the first 24 months were less likely to score below the median PASAT score at year 11 compared with those in the bottom quintile, and there was a statistically significant inverse trend across the categories (figure; adjusting also for the PASAT at month 6 to assess the PASAT score change during follow-up: $OR_{adj} = 0.33$, 95% CI: 0.10–1.09 comparing the top with the bottom quintile, $p_{trend} = 0.015$). Associations were similar for mean 25(OH)D within the first 60 months (figure). The results of linear regression analyses were also consistent with better cognitive function in patients with higher mean 25(OH)D-levels, but did not reach statistical significance ($\beta_{adj} = 0.20$, 95% CI: −0.27 to 0.68 for a 50-nmol/L increment in mean 25(OH)D within the first 24 months, $\beta_{adj} = 0.25$, 95% CI: −0.19 to 0.69 for the same increment over the first 60 months).

Overall, smoking tended to predict worse long-term cognitive function, although the associations did not reach statistical significance in all analyses. The OR for a PASAT score below the median for baseline cotinine levels >25 ng/mL compared with <10 ng/mL was 1.64 (95% CI: 0.88–3.06, $p = 0.12$). Similar results were obtained using average cotinine levels over the first 24 months (OR = 1.69, 95% CI: 0.88–3.25, $p = 0.12$). In linear models, smokers tended to have an up to 0.6 SDs worse long-term cognitive performance corresponding to clinically meaningful 5.4 points on the PASAT, most pronounced among patients considered heavy smokers (cotinine consistently >193 ng/mL, median among smokers) (table 2).

Anti–EBNA-1 IgG antibodies (at baseline and at months 6, 12, and 24) and anti–VCA IgG antibodies (at months 6 and 12) were not associated with PASAT scores in any analysis (table 3).

The multivariable models yielded similar results as the age- and sex-adjusted analyses. The associations between each exposure and the PASAT score did not meaningfully change when we mutually adjusted the multivariable models also for mean 25(OH)D in the first 24 months (those with smoking or anti–EBNA-1 IgG as main exposures) and/or smoking based on cotinine levels over the first 24 months (those with vitamin D or anti–EBNA-1 IgG as main exposures). Adding region or fatigue and depression indicators into the models did also not lead to any relevant differences in the results.

Long-term neuroaxonal integrity

The associations with long-term neuronal integrity corroborated the main findings regarding long-term cognitive performance. A 50-nmol/L higher mean 25(OH)D level in the first 24 or 60 months was associated with a 20% lower serum NfL (95% CI: −35% to −1%), indicating less neuroaxonal loss at year 11 (table 4). Comparing the top with the bottom quintile of mean 25(OH)D also yielded moderate inverse associations (table 4). Furthermore, smoking within the first 24 months from onset was associated with a 20% higher NfL at year 11 (95% CI: 2%–40%) (table 4). Finally, quartiles of anti–EBNA-1 IgG indices at any of the assessed time points were not linked to 11-year NfL concentrations (table 4). Multivariable estimates were similar to the age and sex–only adjusted estimates. Additional adjustments for 25(OH)D and/or cotinine did not change the results.

Discussion

Higher mean 25(OH)D levels after clinical MS onset predicted a better cognitive function as assessed by the PASAT and lower NfL levels 11 years into the disease course,

Table 1 Baseline characteristics of the 278 BENEFIT-11 participants by quintiles of mean 25(OH)D within the first 24 months,[a] quartiles of baseline EBNA-1 IgG antibody titer,[b] and baseline cotinine levels[c]

	Mean 25(OH)D quintiles, nmol/L			Baseline EBNA-1 quartiles		Baseline cotinine, ng/mL	
	1	3	5	1	4	<10	>25
Median	30.2	48.9	73.3	3.9	4.9	2.6	162.6
Range	15.8–35.5	44.3–53.0	65.3–106.9	1.2–4.1	4.7–5.3	1.6–8.8	30.2–323.4
No. (%)	53 (19.8)	54 (20.1)	53 (19.8)	67 (25.2)	66 (24.8)	175 (64.1)	93 (34.1)
Age, median (Q1–3), y	31 (25–39)	29 (24–34)	32 (26–38)	32 (26–37)	30 (25–35)	32 (25–38)	29 (24–34)
Male, %	23.9	44.2	34.0	19.7	39.5	26.8	39.8
INF β-1b treatment, %	52.1	54.3	65.5	59.7	55.6	62.1	53.7
Multifocal onset, %	47.3	49.2	46.2	57.1	44.2	54.4	46.8
Steroid use, %	67.4	74.3	68.7	75.7	62.8	70.9	73.3
T2 lesions, median (Q1–3)	23 (11–43)	19 (9–37)	11 (4–31)	20 (7–49)	21 (7–37)	18 (6–37)	19 (9–41)
BMI, median (Q1–3), kg/m^2	23.8 (21.0–26.6)	23.4 (21.2–26.6)	22.1 (20.1–24.5)	22.6 (21.0–26.2)	23.9 (21.4–26.3)	23.4 (21.0–26.5)	23.5 (21.3–26.7)
PASAT mo6, mean (SD)[d]	54.6 (6.4)	54.9 (6.1)	55.1 (6.7)	54.1 (7.8)	53.9 (7.7)	54.8 (6.9)	53.4 (7.9)
NfL y1, mean (SD), pg/mL[d]	29.5 (21.7)	29.5 (21.4)	25.3 (11.9)	24.2 (10.6)	26.4 (13.4)	26.3 (17.9)	30.7 (25.2)

Abbreviations: 25(OH)D = 25-hydroxyvitamin D; BMI = body mass index; EBNA-1 = Epstein-Barr virus nuclear antigen 1; IgG = immunoglobulin G; INF β-1b = interferon beta-1b; mo6 = month 6; NfL = neurofilament light chain; PASAT = Paced Auditory Serial Addition Test; Q1–3 = 25th–75th percentile; y1 = year 1.
[a] Mean 25(OH)D concentration of measurements at months 6, 12, and 24.
[b] Among EBNA-1 IgG-positive individuals (266 of 277 with a baseline measurement of EBNA-1 IgG).
[c] Cotinine >25 ng/mL indicative of active smoking and <10 ng/mL of nonsmoking; 5 participants had an equivocal baseline level of 10–25 ng/mL.
[d] PASAT scores at month 6 and serum NfL concentrations at year 1 across categories of main exposures.

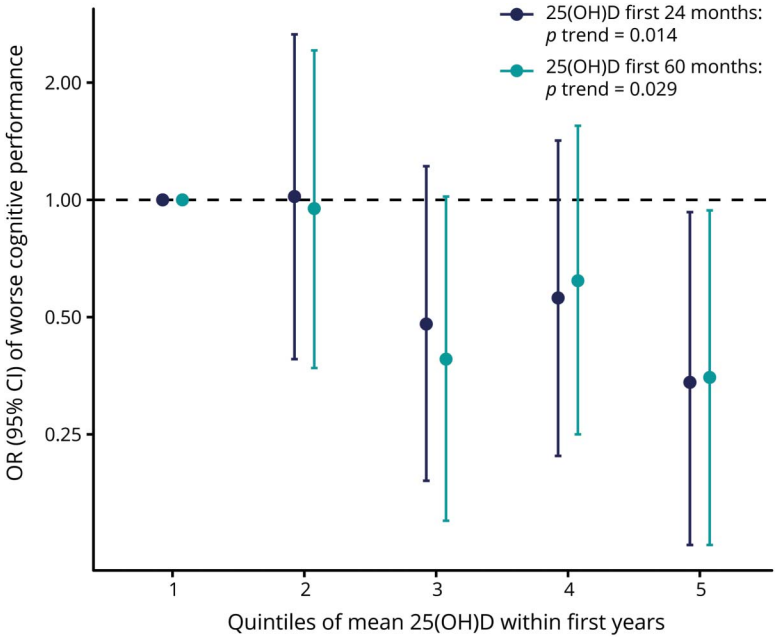
suggesting that adequate vitamin D levels could contribute to long-term neuroprotection in individuals with MS. Smokers tended to have worse long-term cognitive scores on the PASAT and higher NfL levels than nonsmokers. In contrast, EBV serology markers did not predict cognitive performance or NfL concentrations. These results are consistent with the suggestion that vitamin D supplementation and smoking cessation early after MS onset might protect long-term cognitive function and central neuroaxonal integrity, independent of disease-modifying treatment.

Our findings are in line with those of previous studies that suggested an effect of vitamin D and smoking and no effect of EBV on the EDSS.[11,14,18,25] The EDSS, however, is little sensitive to cognitive impairment,[40] which was specifically addressed in our study. Only preliminary evidence was otherwise available from 2 small studies, one cross-sectional, in which smoking was associated with worse cognition in MS,[41] and the other longitudinal, but with only 3 months of follow-up, supporting a beneficial effect of vitamin D on cognitive function.[42] Furthermore, a remarkably high proportion of ever and persistent smokers was observed in a cohort of patients with MS with predominant severe cognitive impairment.[43] Cognitive function was also not reported in the so far inconclusive vitamin D supplementation trials in individuals with MS.[26] The awaited results of several larger, thus better-powered trials may be more informative,[44] although a favorable effect on progression might only become apparent beyond a typical 2-year trial period.

Our findings on cognitive impairment are substantiated by our results on neuroaxonal injury providing biomarker evidence of ongoing pathology in the CNS. Both neuroinflammatory and neurodegenerative processes can increase NfL levels[37] and could contribute to our results. Cortical lesions and gray matter loss are more strongly associated with cognitive decline than white matter lesions,[4] but ultimately, we cannot evaluate from NfL elevations only which pathology was driving the results. Intriguingly, vitamin D supplementation was associated with decreased NfL levels in a 2-year randomized clinical trial among untreated patients with MS.[45] In another study, smoking was linked to brain atrophy,[19] which is, in turn, related to neuroaxonal injury and cognitive impairment.[5] Our findings add to the evidence that NfL may be a promising biomarker for clinical MS research.

Vitamin D might have neuroprotective properties or be a prognostic marker of long-term cognition and neuroaxonal integrity. The direct or immune-mediated biological mechanisms of long-term neuroprotection can only be hypothesized. In the short term, vitamin D might contribute to maintaining immune homeostasis as suggested in a previous study.[46] Cognitive reserve modifies the expression of cognitive

Figure Odds ratio (OR) and 95% confidence intervals (CIs) of poorer cognitive performance on the PASAT (at or below a median score of 56) 11 years after clinically isolated syndrome according to quintiles of mean 25-hydroxyvitamin D (25(OH)D) within the first 24 and 60 months from symptom onset



ORs are adjusted for baseline age and sex, treatment allocation at baseline (interferon-beta yes-no), multifocal symptom onset (yes-no), T2-weighted MRI lesions at baseline (categorically: 2–4, 5–8, and ≥9), body mass index at baseline (continuously), and treatment with steroids during first attack (yes-no). PASAT = Paced Auditory Serial Addition Test.

dysfunction following neurodegeneration,[47] and higher vitamin D could, alternatively, be a marker of a higher cognitive reserve. Cigarette smoking has also been linked to brain atrophy in the general population and could be directly neurotoxic,[48] leading, for example, to production of nitric oxide in the CSF, which is thought to promote neurodegenerative processes.[15] An

Table 2 Serum cotinine levels, a biomarker of smoking, and long-term cognitive performance as assessed by the PASAT 11 years after CIS

		z-PASAT score 11 years after CIS[a]	
Cotinine levels, ng/mL	**No.**	**Sex- and age-adjusted β (95% CI)[b]**	**Multivariable β (95% CI)[c]**
At baseline[d]			
<10	140	Ref.	Ref.
>25	76	−0.30 (−0.61, 0.02)	−0.29 (−0.62, 0.04)
All measurements[e]			
<10	125	Ref.	Ref.
>25	70	−0.35 (−0.67, −0.02)	−0.33 (−0.67, 0.01)
<10	125	Ref.	Ref.
>25 to ≤193	61	−0.30 (−0.65, 0.04)	−0.29 (−0.64, 0.07)
>193	9	−0.60 (−1.35, 0.15)	−0.59 (−1.35, 0.17)
p_{trend}[f]		0.020	0.026

Abbreviations: CI = confidence interval; CIS = clinically isolated syndrome; PASAT = Paced Auditory Serial Addition Test; β = linear regression coefficient.
[a] Linear regression of smoking status determined by serum biomarker cotinine on standardized PASAT z-scores (mean of zero, SD of 1) 11 years after CIS.
[b] Adjusted categorically for baseline age (18–22, 23–27, 28–32, 33–37, 38–42, and >43 years) and sex.
[c] Apart from baseline age and sex, also adjusted for treatment allocation at baseline (interferon-beta yes-no), multifocal symptom onset (yes-no), T2-weighted MRI lesions at baseline (categorically: 2–4, 5–8, and ≥9), body mass index at baseline (continuously), and treatment with steroids during first attack (yes-no).
[d] A baseline serum cotinine level (missing = 3) of >25 ng/mL indicates active cigarette smoking, and <10 ng/mL indicates nonsmoking.
[e] Serum cotinine levels at baseline and at months 6, 12, and 24 used to classify nonsmokers (all measures <10 ng/mL), smokers (>25 ng/mL), and heavy smokers (>193 ng/mL). Last available measurement carried forward for missing levels.
[f] Probability value for linear trend across the medians within each category.

Table 3 Odds ratio (OR) and 95% confidence intervals (CIs) of worse long-term cognitive performance on the PASAT 11 years after CIS according to quartiles of anti-EBNA-1 and -VCA antibodies[a]

		PASAT score ≤56 points (median) 11 years after CIS	
	No.	Sex- and age-adjusted OR (95% CI)[b]	Multivariable OR (95% CI)[c]
Anti-EBNA-1 IgG level			
At baseline (BL)			
Quartile			
1	56	Ref.	Ref.
2	55	1.26 (0.58–2.75)	1.27 (0.58–2.79)
3	50	1.33 (0.60–2.97)	1.23 (0.54–2.80)
4	55	1.11 (0.51–2.42)	1.06 (0.48–2.35)
p_{trend}[d]		0.75	0.88
At month 24			
Quartile			
1	49	Ref.	Ref.
2	47	0.83 (0.35–1.94)	0.82 (0.34–1.95)
3	48	0.53 (0.23–1.22)	0.50 (0.21–1.19)
4	52	1.02 (0.45–2.31)	1.01 (0.43–2.38)
p_{trend}		0.69	0.68
Anti-VCA IgG level			
At mo 6			
Quartile			
1	49	Ref.	Ref.
2	47	0.76 (0.33–1.73)	0.76 (0.32–1.80)
3	51	1.13 (0.49–2.60)	1.07 (0.46–2.53)
4	50	0.87 (0.38–1.99)	0.86 (0.36–2.08)
p_{trend}		0.92	0.92

Abbreviations: CI = confidence interval; CIS = clinically isolated syndrome; EBNA-1 = Epstein-Barr virus nuclear antigen 1; IgG = immunglobulin G; OR = odds ratio; PASAT = Paced Auditory Serial Addition Test; VCA = viral capsid antigen.
[a] Odds of the PASAT score at or below the median (56 points) 11 years after CIS comparing higher quartiles of EBNA-1 and VCA IgG antibody titers to bottom quartile using logistic regression.
[b] Adjusted categorically for baseline age (18–22, 23–27, 28–32, 33–37, 38–42, and >43 years) and sex.
[c] Apart from baseline age and sex, also adjusted for treatment allocation at baseline (interferon-beta yes-no), multifocal symptom onset (yes-no), T2-weighted MRI lesions at baseline (categorically: 2–4, 5–8, and ≥9), body mass index at baseline (continuously), and treatment with steroids during first attack (yes-no).
[d] Probability value for linear trend across the medians within the quartiles.

indirect effect through acceleration of comorbidities like vascular dementia is another possibility.[48] Differences could, to some extent, already have been present at CIS if smoking patients had been smoking before CIS, which is likely. It is thus less informative to examine PASAT changes after baseline with respect to smoking. Whichever the mechanism, our findings suggest that vitamin D and smoking are independent predictors of long-term cognitive performance.

The strengths of our study include the longitudinal design, the systematic long follow-up of participants across study sites, all recruited at CIS and receiving standard treatment, and the availability of repeated measurements of serum biomarkers to consider exposure changes over time, decrease the risk of misclassification, and exclude reverse causality as an explanation for our findings. Given that there is no established treatment of cognitive decline and vitamin D supplementation is safe even at high doses, supplementation could routinely be considered among patients with CIS and MS, especially those with insufficient levels.[44] To encourage and offer patients help to quit smoking remains crucial.

Our study has some limitations. The study population consists of white Caucasians, which limits the generalizability of

Table 4 Serum 25-hydroxyvitamin D (25(OH)D), cotinine, and EBNA-1 antibody levels and long-term neuroaxonal injury as measured by serum NfL concentrations in patients with CIS

	No.	NfL level 11 years after CIS	
		Sex- and age-adjusted % difference (95% CI)[a,b]	Multivariable adjusted % difference (95% CI)[a,c]
Mean 25(OH)D levels			
First 24 mo			
50 nmol/L increments	217	−14% (−31 to +7%)	−20% (−36% to 0%)
P		0.19	0.05
Quintile			
1	39	Ref.	Ref.
2	44	−10% (−28 to +13%)	−11% (−29 to +11%)
3	48	−10% (−28 to +12%)	−15% (−32 to +7%)
4	46	−8% (−27 to +16%)	−13% (−31 to +9%)
5	40	−11% (−30 to +13%)	−17% (−35 to +6%)
p_{trend}[d]		0.46	0.18
First 60 mo			
50 nmol/L increments	217	−15% (−31 to +5%)	−20% (−35% to −1%)
P		0.12	0.04
Quintile			
1	40	Ref.	Ref.
2	44	−7% (−26 to +17%)	−6% (−25 to +18%)
3	45	−5% (−24 to +20%)	−8% (−27 to +15%)
4	49	−6% (−25 to +18%)	−9% (−27 to +14%)
5	39	−16% (−34 to +6%)	−20% (−37% to +1%)
p_{trend}		0.19	0.073
All cotinine levels[e], ng/mL			
<10	124	Ref.	Ref.
>25	68	+21% (+3 to +41%)	+20% (+2 to +40%)
p		0.019	0.025
<10	124	Ref.	Ref.
>25 to ≥193	61	+21% (+3 to +43%)	+21% (+3 to +43%)
>193	7	+14% (−24 to +70%)	+7% (−28 to +59%)
p_{trend}		0.027	0.043
EBNA-1 IgG levels			
At baseline			
Quartile			
1	53	Ref.	Ref.
2	50	−7% (−24 to +14%)	−8% (−24 to +12%)
3	54	+22% (−1 to +48%)	+21% (−1 to +48%)
4	58	−1% (−18 to +21%)	−1% (−18 to +20%)

Continued

Table 4 Serum 25-hydroxyvitamin D (25(OH)D), cotinine, and EBNA-1 antibody levels and long-term neuroaxonal injury as measured by serum NfL concentrations in patients with CIS *(continued)*

		NfL level 11 years after CIS	
	No.	Sex- and age-adjusted % difference (95% CI)[a,b]	Multivariable adjusted % difference (95% CI)[a,c]
p_{trend}		0.57	0.59
At month 24			
Quartile			
1	46	Ref.	Ref.
2	51	−9% (−26 to +13%)	−8% (−25 to +14%)
3	50	0% (−19 to +23%)	−1% (−19 to +23%)
4	49	+6% (−14 to +30%)	+8% (−12 to +34%)
p_{trend}		0.57	0.45

Abbreviations: CI = confidence interval; CIS = clinically isolated syndrome; EBNA-1 = Epstein-Barr virus nuclear antigen 1; IgG = immunoglobulin G; NfL = neurofilament light chain.
[a] NfL levels were log transformed to improve normality. Linear regression coefficients, reflecting changes on a log scale, were back transformed to reflect changes on a multiplicative scale and reported as % difference in median NfL levels between the exposure and the reference group. Confidence intervals including 0% difference are not statistically significant using a probability cutoff of 0.05.
[b] Adjusted categorically for baseline age (18–22, 23–27, 28–32, 33–37, 38–42, and >43 years) and sex.
[c] Apart from baseline age and sex, also adjusted for treatment allocation at baseline (interferon-beta yes-no), multifocal symptom onset (yes-no), T2-weighted MRI lesions at baseline (categorically: 2–4, 5–8, and ≥9), body mass index at baseline (continuously), and treatment with steroids during first attack (yes-no).
[d] Probability value for linear trend across the medians within the categories.
[e] Levels of serum cotinine, a biomarker of current/recent tobacco use, at baseline and at months 6, 12, and 24 used to classify nonsmokers (all measures <10 ng/mL), smokers (>25 ng/mL), and heavy smokers (>193 ng/mL). Last available level carried forward for missing levels.

our findings to other racial/ethnic groups. We could not assess whether even higher vitamin D levels (~100 nmol/L) have an additional favorable effect on the disease course. Moreover, we might have underestimated the effect of smoking, as ever-smokers were misclassified as nonsmokers if they stopped smoking at or before CIS. Furthermore, we could not adjust our analyses for education or physical activity. These or other factors could confound the associations between vitamin D/smoking and cognitive decline. However, evidence of a beneficial effect of exercise on cognitive performance is preliminary and could be explained by a favorable impact on mood and/or fatigue.[9,49,50] Moreover, about one-third of the patients were treated with disease-modifying drugs other than INF β-1b at year 11; however, we had no information about when they switched to which treatment during follow-up and could therefore not adjust the analyses for treatment differences among the patients. In addition, the PASAT is not an exhaustive measure of cognition in MS, and the use of a neuropsychological test battery including tests for different cognitive skills would have given a more complete picture of cognitive performance, but the PASAT does capture the aspects most commonly affected in MS, that is, processing speed and memory.[6] Performance in the PASAT is prone to practice effects.[6] Nevertheless, the tests administered before month 6 in BENEFIT were not used in this study. Instead, we used the scores from tests administered thereafter (numerous test administrations before the main outcome, PASAT at year 11) to exclude influences of initial and thus large practice effects on the PASAT performance.[35] Any practice effect is further unlikely to fully explain our findings, as the extent of the practice effect is probably not related to the exposures unless these truly have an effect. Like every cognitive test, PASAT scores do not convey how the patient is managing cognitive tasks in real life.[6] Furthermore, ceiling effects might have masked small PASAT changes among high-scoring individuals who experienced a decline in functioning although still scoring high,[35] but we addressed this issue by examining performance below a specific threshold to capture individuals who were most affected in this study population. Also, the association between 25(OH)D and NfL levels was modest and at the limit of statistical significance. These associations, however, were most likely attenuated by the long lag between the last assessment of 25(OH)D (24 months) and NfL measurement (11 years), which was introduced to minimize the possibility of reverse causation.

In summary, lower vitamin D levels and smoking after clinical onset predicted worse long-term cognitive function and neuronal integrity in patients with MS. These results suggest that correcting vitamin D insufficiency and abstaining from cigarette smoking after clinical MS onset might protect long-term cognitive function and CNS integrity.

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Disclosure

M. Cortese, K. L. Munger, E. H. Martínez-Lapiscina, C. Barro, G. Edan, and M. S. Freedman report no disclosures. H.-P. Hartung reports personal fees for serving on a steering committee of Bayer HealthCare during the conduct of the study. X. Montalban, F. W. Foley, I. K. Penner, B. Hemmer, E. J. Fox, and S. Schippling report no disclosures. E.- M. Wicklein is a salaried employee of Bayer AG. L. Kappos, J. Kuhle, and A. Ascherio report no disclosures. Go to Neurology.org/N for full disclosures.

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Appendix Authors

Name	Location	Contribution
Marianna Cortese, MD, PhD	Harvard T.H. Chan School of Public Health, Boston	Analysis and interpretation of the data and drafting and revising the manuscript for content
Kassandra L. Munger, ScD	Harvard T.H. Chan School of Public Health, Boston	Obtaining funding, analysis and interpretation of the data, and revising the manuscript for content
Elena H. Martínez-Lapiscina, MD, PhD	University of Barcelona, Barcelona	Interpretation of the data and revising the manuscript for content
Christian Barro, MD	University of Basel, Basel	Interpretation of the data and revising the manuscript for content
Gilles Edan, MD	CHU Hôpital Pontchaillou, Rennes	Interpretation of the data and revising the manuscript for content
Mark S. Freedman, MD	Ottawa Hospital Research Institute, Ottawa	Interpretation of the data and revising the manuscript for content
Hans-Peter Hartung, MD	Heinrich-Heine University, Düsseldorf	Interpretation of the data and revising the manuscript for content
Xavier Montalbán, MD	University of Toronto, Toronto	Interpretation of the data and revising the manuscript for content
Frederick W. Foley, PhD	Yeshiva University, New York	Interpretation of the data and revising the manuscript for content
Iris Katharina Penner, PhD	COGITO Center for Applied Neurocognition and Neuropsychological Research, Düsseldorf	Interpretation of the data and revising the manuscript for content
Bernhard Hemmer, MD	Technical University of Munich, Munich	Interpretation of the data and revising the manuscript for content
Edward J. Fox, MD, PhD	Central Texas Neurology Consultants, Round Rock	Interpretation of the data and revising the manuscript for content
Sven Schippling, MD	University of Zurich, Zurich	Interpretation of the data and revising the manuscript for content
Eva-Maria Wicklein, MD	Bayer AG, Berlin	Major role in acquisition of the data, interpretation of the data, and revising the manuscript for content
Ludwig Kappos, MD	University of Basel, Basel	Interpretation of the data and revising the manuscript for content
Jens Kuhle, MD, PhD	University of Basel, Basel	Major role in acquisition of the data, interpretation of the data, and revising the manuscript for content
Alberto Ascherio, MD, DrPH	Harvard T.H. Chan School of Public Health, Boston	Obtaining funding, study concept and design, major role in acquisition of data, analysis and interpretation of data, and revising the manuscript for content

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