

The Effect of Smoking on Long-term Gray Matter Atrophy and Clinical Disability in Patients with Relapsing-Remitting Multiple Sclerosis

Ingrid Anne Lie, MD, Kristin Wesnes, MD, PhD, Silje S. Kvistad, MD, PhD, Iman Brouwer, MASc, Stig Wergeland, MD, PhD, Trygve Holmøy, MD, PhD, Rune Midgard, MD, PhD, Alla Bru, MD, Astrid Edland, MD, Randi Eikeland, MD, PhD, Sonia Gosal, MD, Hanne F. Harbo, MD, PhD, Grethe Kleveland, MD, Yvonne S. Sørenes, MD, Nina Øksendal, MD, Frederik Barkhof, MD, PhD, Hugo Vrenken, PhD, IR, Kjell-Morten Myhr, MD, PhD, Lars Bø, MD, PhD, and Øivind Torkildsen, MD, PhD

Correspondence

Dr. Lie
ingrid.lie@uib.no

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Abstract

Background and Objectives

The relationship between smoking, long-term brain atrophy, and clinical disability in patients with multiple sclerosis (MS) is unclear. Here, we assessed long-term effects of smoking by evaluating MRI and clinical outcome measures after 10 years in smoking and nonsmoking patients with relapsing-remitting MS (RRMS).

Methods

We included 85 treatment-naïve patients with RRMS with recent inflammatory disease activity who participated in a 10-year follow-up visit after a multicenter clinical trial of 24 months. Smoking status was decided for each patient by 2 separate definitions: by serum cotinine levels measured regularly for the first 2 years of the follow-up (during the clinical trial) and by retrospective patient self-reporting. At the 10-year follow-up visit, clinical tests were repeated, and brain atrophy measures were obtained from MRI using FreeSurfer. Differences in clinical and MRI measurements at the 10-year follow-up between smokers and nonsmokers were investigated by 2-sample *t* tests or Mann-Whitney tests and linear mixed-effect regression models. All analyses were conducted separately for each definition of smoking status.

Results

After 10 years, smoking (defined by serum cotinine levels) was associated with lower total white matter volume ($\beta = -21.74, p = 0.039$) and higher logT2 lesion volume ($\beta = 0.22, p = 0.011$). When defining smoking status by patient self-reporting, the repeated analyses found an additional association with lower deep gray matter volume ($\beta = -2.35, p = 0.049$), and smoking was also associated with a higher score (higher walking impairment) on the log timed 25-foot walk test ($\beta = 0.050, p = 0.039$) after 10 years and a larger decrease in paced auditory serial addition test (attention) scores ($\beta = -3.58, p = 0.029$).

From the Department of Clinical Medicine (I.A.L., K.-M.M., L.B., Ø.T.), University of Bergen; Neuro-SysMed, Department of Neurology, Haukeland University Hospital (I.A.L., K.W., S.S.K., S.W., K.-M.M., Ø.T.), Bergen; St. Olav's University Hospital (K.W.), Trondheim; Department of Immunology and Transfusion Medicine (S.S.K.), Haukeland University Hospital, Bergen, Norway; Department of Radiology and Nuclear Medicine (I.B., F.B., H.V.), MS Center Amsterdam, Amsterdam Neuroscience, Amsterdam UMC, location VUmc, The Netherlands; Norwegian Multiple Sclerosis Registry and Biobank (S.W.), Department of Neurology, Haukeland University Hospital, Bergen; Institute of Clinical Medicine (T.H., H.F.H.), University of Oslo; Department of Neurology, Akershus University Hospital (T.H.), Lørenskog; Department of Neurology (R.M.), Molde Hospital; Department of Neurology (A.B.), Stavanger University Hospital; Department of Neurology (A.E.), Vestre Viken Hospital Trust, Drammen; Department of Research and Education (R.E.), Sørlandet Hospital Trust, Kristiansand; Faculty of Health and Sport Science (R.E.), University of Agder, Grimstad; Department of Neurology (S.G.), Østfold Hospital Kalnes, Grålum; Department of Neurology (H.F.H.), Oslo University Hospital; Department of Neurology (G.K.), Innlandet Hospital Lillehammer; Department of Neurology (Y.S.S.), Haugesund Hospital; Department of Neurology (N.Ø.), Nordland Hospital Trust, Bodø, Norway; Institutes of Neurology and Healthcare Engineering (F.B.), University College London, Great Britain; and Norwegian Multiple Sclerosis Competence Centre (L.B.), Department of Neurology, Haukeland University Hospital, Bergen, Norway.

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Glossary

BBB = blood-brain barrier; BL = baseline; EDSS = Expanded Disability Status Scale; eTIV = estimated total intracranial volume; GM = gray matter; MS = multiple sclerosis; PASAT = paced auditory serial addition test; RRMS = relapsing-remitting MS; SPSS = Statistical Product and Service Solutions; T25FW = timed 25-foot walk test; VD = vascular-disease; WM = white matter.

Discussion

Smoking was associated with brain atrophy and disability progression 10 years later in patients with RRMS. The findings imply that patients should be advised and offered aid in smoking cessation shortly after diagnosis, to prevent long-term disability progression.

Smoking is a known negative prognostic factor in patients with multiple sclerosis (MS), associated with higher disability¹ and higher risk of conversion to progressive disease phenotypes.^{1,2} The causal relationship between these associations is not clear, and several pathophysiologic mechanisms have been proposed, for example: cigarette smoke triggering a proinflammatory cascade, inducing autoimmunity and heightened inflammatory activity; facilitating entry of immune cells to the CNS by disruption of the blood-brain barrier (BBB); epigenetic changes; and direct neurotoxicity due to mitochondrial damage.^{3,4}

Studies examining the relation between smoking and inflammatory disease activity have reported inconsistent findings. Although some studies found smoking to be associated with higher relapse rates⁵ and lesion loads,^{1,6,7} 2 studies using cotinine, an alkaloid metabolite of nicotine,⁸ to define smoking status, did not.^{9,10}

In MS, smoking has cross-sectionally been shown to be associated with lower total brain volume,^{1,6,11-13} but longitudinal relationships have been less well studied and with varying results.^{1,7,9} Furthermore, few studies have considered the relation between smoking and gray matter (GM) atrophy, again with conflicting results.^{6,11,13}

To better understand the possible adverse prognostic effects of smoking in MS, we aimed to investigate the relation between smoking and long-term brain lesion load, atrophy, and clinical outcome measures, first by comparing smokers and nonsmokers, defined by both patient self-reporting and cotinine levels, and second by studying a possible dose effect, using mean cotinine levels in smoking patients with MS.

Methods

Participants

The patients included in this study participated in a 10-year follow-up visit, after a multicenter trial on ω -3 fatty acids in MS (the OFAMS-study). In the original trial, a total of 92 patients with relapsing-remitting MS (RRMS) were followed up for 24 months; a detailed description of the study is provided elsewhere.¹⁴ During the OFAMS study period, patients attended regular follow-up visits at their local study site, undergoing biochemical, radiologic, and clinical examinations, including the Expanded Disability Status Scale (EDSS), timed 25-foot walk test (T25FW), the

dominant hand and nondominant hand 9-hole peg test, and the paced auditory serial addition test (PASAT). Ten years after the trial concluded, all available (87) participants were invited to a follow-up visit, of which 85 accepted.¹⁵ At the 10-year follow-up visit, the patients repeated the radiologic and clinical examinations. Between the OFAMS-study and the 10-year follow-up visit, the participants had received routine clinical treatment and care.

Standard Protocol Approvals, Registrations, and Patient Consents

The OFAMS-study and the 10-year follow-up were approved by the Regional Committee for Medical and Health Research Ethics in Western Norway Regional Health Authority (OFAMS-study: clinicaltrials.gov, Identifier: NCT00360906). All participants gave their written informed consent.

Cotinine Measurement

Serum samples were stored at -80°C until analysis and performed simultaneously for all samples from each patient. As previously described,¹⁰ serum cotinine levels were measured by liquid chromatography tandem mass spectrometry at Bevit AS (Bergen, Norway). Laboratory technicians were blinded to patient clinical status. Serum cotinine levels were analyzed from samples collected during the OFAMS-study at baseline (BL) and months 6, 12, 18, and 24.

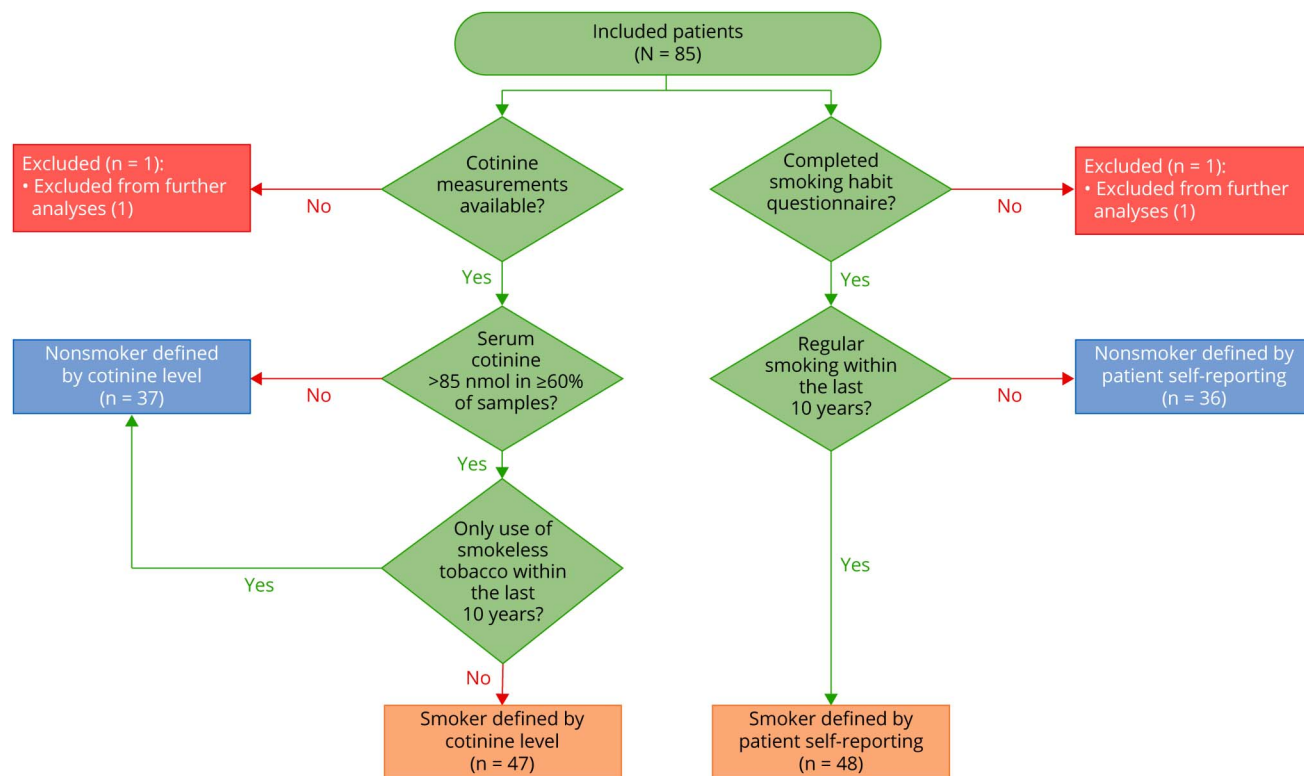
Definitions of Smokers and Nonsmokers

The effect of smoking status on long-term MRI and clinical outcome measures was analyzed using 2 definitions (described below) of smoking status separately: by (1) serum cotinine levels, with the findings presented in the main text and (2) patient self-reporting, with the findings presented in the supplemental material.

Smoking Defined by Cotinine Levels

Cotinine levels >85 nmol/L indicate recent tobacco use¹⁶ and are regarded to distinguish tobacco users from nontobacco users in the general population.¹⁷ Patients were categorized into 2 groups according to serum cotinine level: smokers were defined as patients with serum cotinine level >85 nmol/L in $\geq 60\%$ of the samples and nonsmokers were defined as patients with serum cotinine levels ≤ 85 nmol/L in $\geq 60\%$ of the samples. Based on previous studies not finding nicotine or smokeless tobacco to be associated with MS disease progression,^{18,19} patients who at the 10-year follow-up visit

Figure 1 Flowchart Illustrating the Classification Process According to the 2 Definitions of Smoking



reported “snuff” use (oral tobacco), but no smoking for the past 10 years, were defined as nonsmokers (Figure 1).

Smoking Defined by Patient Self-Reporting

At the 10-year follow-up visit, patients answered a questionnaire about habits of tobacco use, including smoking and snuff use. Patients who reported to have smoked regularly within the past 10 years were defined as smokers while patients who did not report regular smoking, or reported only snuff use (6 patients), were defined as nonsmokers (Figure 1).

Risk Factors and Presence of Peripheral, Cardiovascular, or Cerebrovascular Disease

Vascular risk factors and established peripheral, cardiovascular, and cerebrovascular disease are independently associated with brain imaging changes.²⁰ As smoking is a known risk factor for such conditions,²¹ patient-reported hypertension, dyslipidemia, hypercoagulable disorder, and symptomatic cardiovascular or cerebrovascular disease and/or events was designated as a dichotomous vascular disease (VD) factor and included as a covariate in the final analyses.

MRI Data and Analysis

The 10-year Follow-up Visit

Imaging was performed at the different study sites, on a 3 Tesla (T) MRI scanner if available, alternatively using a 1.5T MRI scanner, with a standard head coil. The acquisition protocol included the following MRI sequences: a T2-weighted 3D sagittal fluid attenuated inversion recovery

(FLAIR) (resolution: $1 \times 1 \times 1$ mm³, echo time (TE)/repetition time (TR)/inversion time (TI) = 386/5000/1.65–2.2 ms) and a postcontrast T1-weighted 3D sagittal magnetization-prepared rapid gradient echo sequence (resolution: $1 \times 1 \times 1$ mm³, TE/TR/TI = 2.28/1800/900 ms, flip angle 8°). Acquisition details across sites are presented in eTable 1, links.lww.com/NXI/A728.

Lesion Segmentation

Lesion segmentation was performed on FLAIR images using lesion segmentation tool (version 2.0.15; applied-statistics.de/lst.html).²² To optimize lesion filling, gadolinium-enhancing regions (both lesions and other regions) were first removed, by applying an upper intensity threshold at the 98th percentile. Next, the FMRIB Software Library (version 5.0.10²³) was used to fill in abnormal voxels in these preprocessed T1-weighted images using the `lesion_filling` tool.²⁴ Then, only the filled lesion voxels were pasted back into the original postcontrast 3D T1-weighted images to create the final lesion filled images.

Morphological Reconstruction

Cortical reconstruction and parcellation for (local) cortical volume and thickness measurement and subcortical segmentation were performed with FreeSurfer version 7.1.1, a freely available software package for academic use, available through online download.²⁵ The technical details of FreeSurfer procedures have been previously described.^{26,27} The use of FreeSurfer on postcontrast 3D T1-weighted images as applied here, was recently validated.²⁸

Table 1 Demographic and Clinical Characteristics

	BL	10-y follow-up visit	BL	10-y follow-up visit
Smoking status defined by cotinine level	Nonsmokers (37)		Smokers (47)	
Age in y, mean (SD)	37.5 (6.8)	49.4 (6.9)	37.9 (9.7)	49.7 (9.7)
Sex, female, N (%)	22 (59.5)		32 (68.1)	
Time from diagnosis, mean in y (SD)	2.8 (3.5)	14.8 (3.6)	2.2 (3.0)	14.3 (3.1)
Disease phenotype (N)	RRMS (37)	RRMS (35), SPMS (2)	RRMS (47)	RRMS (42), SPMS (5)
EDSS, mean (SD)	1.8 (0.8)	2.6 (1.6)	2.0 (0.9)	2.9 (1.6)
Mean cotinine level (SD)^a			850.6 (454.5)	
Smoking status defined by patient self-reporting	Nonsmokers (36)		Smokers (48)	
Age in y, mean (SD)	37.5 (7.6)	49.4 (7.7)	37.9 (9.2)	49.7 (9.2)
Sex, female, N (%)	22 (61.1)		33 (68.8)	
Time from diagnosis, mean in y (SD)	2.8 (3.6)	14.9 (3.7)	2.3 (3.0)	14.3 (3.1)
Disease phenotype (N)	RRMS (36)	RRMS (35), SPMS (1)	RRMS (48)	RRMS (42), SPMS (6)
EDSS, mean (SD)	1.8 (0.8)	2.6 (1.6)	2.0 (0.9)	2.9 (1.6)

Abbreviations: BL = baseline; EDSS = Expanded Disability Status Scale; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

^a Mean serum cotinine level calculated from measurements obtained from BL to month 24.

Quality control was performed by visual inspection, discarding cases with large segmentation errors. Minor to moderate segmentation errors of specific anatomic regions were found in all scans and are previously shown to occur more frequently and to a more severe degree in postcontrast images.²⁸ As these errors were so commonly occurring, mostly with the same effect (overestimation of GM volume/cortical thickness²⁸), we chose to not apply any manual corrections for these errors in our analyses.

The Desikan-Killiany atlas²⁹ was used to extract cortical thickness measures. The mean cortical thickness of the left and right hemisphere was averaged to calculate the overall mean cortical thickness. Furthermore, total cerebral GM and white matter (WM) volume and total deep GM and thalamus volume (average of left and right hemisphere) were obtained. Because of frequent sub-optimal segmentation of the temporal pole (previously found in a minor to moderate degree in almost 50% of postcontrast scans²⁸), this region was excluded when calculating the total GM volume.

Statistical Analysis

Statistical analyses were performed using Statistical Product and Service Solutions (SPSS) for macOS (Version 25; SPSS, Chicago, IL) and R software (V.4.0.5).

Kolmogorov-Smirnov tests and visual inspection of the histograms were used to assess the normality of the variables.

The primary outcome measures were MRI and clinical measurements obtained cross-sectionally at the 10-year follow-up visit and the difference in clinical measurements from month 24 to the 10-year follow-up.

As a first exploratory analysis, the difference in outcome measures between smokers and nonsmokers was analyzed by 2-sample *t* tests for normally distributed variables; otherwise, Mann-Whitney tests were used.

The relationship of smoking status and mean cotinine level with MRI and clinical outcome measures was then further investigated by a linear mixed-effect regression model, correcting for age, sex, vascular disease, estimated total intracranial volume (eTIV) (eTIV included as a covariate in analyses regarding brain volume measurements), BL EDSS, and time from diagnosis. To correct for scanner variability, MRI scanner was entered as a random effect. Assumptions for linear regression were checked for each final model; if the assumptions were not satisfied, log-linear transformation was performed (e.g., logT2 lesion volume). For variables with values below 1, a constant was added before log transformation.

Data Availability

Data not provided within this article may be shared (anonymized) by request from a qualified investigator.

Results

Patient Characteristics

We included the 85 patients who participated in the 10-year follow-up. Each patient was classified as a smoker or nonsmoker, by the 2 different definitions of smoking: (1) by cotinine levels measured during the OFAMS-study and (2) by retrospective patient self-reporting at the 10-year follow-up

Table 2 MRI and Clinical Measures in Smokers and Nonsmokers (Defined by Cotinine Level) at the 10-Year Follow-up Visit and Change in Clinical Measures Between Month 24 and the 10-Year Follow-up Visit

MRI/clinical measure	Nonsmokers (mean, SD)	Smokers (mean, SD)	Mean difference	95% CI	<i>p</i> Value
Total GM volume (mL)	643.62 (55.76)	621.83 (47.51)	21.785	-3.220, 46.791	0.087
Total WM volume (mL)	465.33 (54.98)	435.76 (42.33)	29.577	5.961, 53.192	0.015
Total deep GM volume (mL)	57.73 (4.98)	54.59 (5.50)	3.139	0.586, 5.691	0.017
Thalamus volume (mL)	8.08 (1.02)	7.67 (1.00)	0.41	-0.08, 0.90	0.096
Mean Cth (mm)	2.55 (0.13)	2.52 (0.12)	0.03	-0.03, 0.09	0.329
T2 lesion volume (mL) ^a	3.11 (3.07)	6.63 (9.68)			0.014
T2 lesion count	19.70 (7.61)	21.62 (9.85)	-1.918	-6.115, 2.278	0.365
EDSS ^a	2.5 (1.5)	2.0 (1.6)			0.306
Change in EDSS ^a	0.5 (2.0)	0.0 (1.13)			0.505
T25FW ^a	3.88 (1.29)	4.43 (1.47)			0.031
Change in T25FW ^a	-0.01 (1.30)	0.31 (1.23)			0.202
D9-HPT ^a	20.02 (6.59)	21.31 (5.68)			0.167
Change in D9-HPT ^a	2.41 (3.11)	3.11 (4.65)			0.480
ND9-HPT ^a	20.68 (6.59)	23.23 (7.27)			0.130
Change in ND9-HPT ^a	3.14 (4.80)	4.08 (6.11)			0.307
PASAT	49.06 (8.40)	45.11 (10.68)	3.95	-0.43, 8.32	0.077
Change in PASAT	-3.91 (7.20)	-7.26 (6.70)	3.35	0.13, 6.57	0.042

Abbreviations: Cth = cortical thickness; D9-HPT = dominant hand 9-hole peg test; EDSS = Expanded Disability Status Scale; GM = gray matter; ND9-HPT = nondominant hand 9-hole peg test; PASAT = paced auditory serial addition test; T25FW = timed 25-foot walk; WM = white matter.

^a Difference analyzed by Mann-Whitney *U* test, median, and interquartile range reported.

Bold text indicates statistically significant *p* values.

(Figure 1). Samples available for cotinine analyses were missing for one patient, and another patient did not complete the questionnaire concerning tobacco use, leaving 84 patients to be classified as smoker or nonsmoker by each definition. Including the 2 patients missing either cotinine measurements or the questionnaire, 9 patients were classified differently based on the 2 definitions. Forty-seven patients were smokers and 37 nonsmokers defined by cotinine levels. By patient self-reporting, 48 patients were smokers and 36 nonsmokers. Of the 48 smokers defined by patient self-reporting, 47 reported to smoke 10 years ago. Additional patient self-reported smoking habits are listed in supplemental eTable 2, links.lww.com/NXI/A728.

The mean follow-up time from the BL and month 24 visit to the 10-year follow-up visit was 12.0 (± 0.6) and 10.0 (± 0.6) years, respectively. Table 1 summarizes the clinical characteristics of the included patients.

Difference in MRI and Clinical Outcome Measures Between Smokers and Nonsmokers

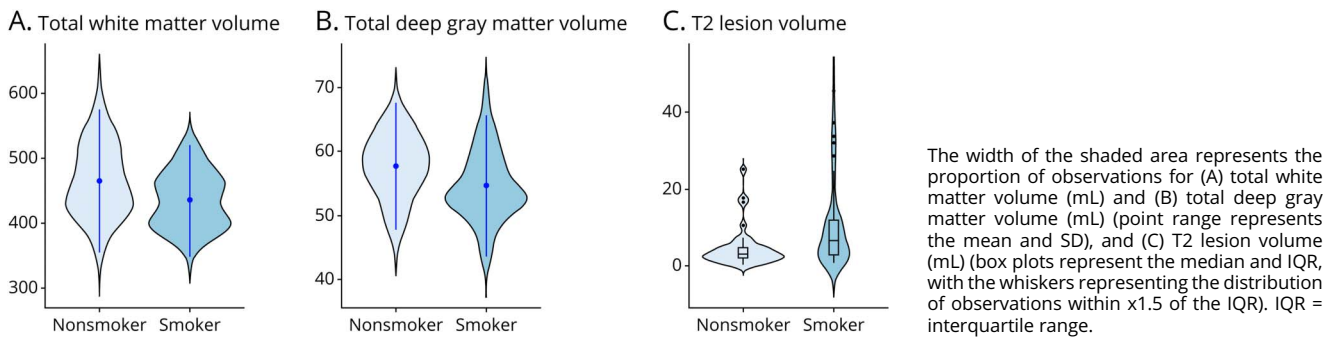
The results of the exploratory *t* tests are shown in Table 2 (smoking defined by cotinine levels) and supplemental eTable 3, links.lww.com/NXI/A728 (smoking defined by patient self-

reporting). In brief, total WM ($p = 0.015$) and deep GM ($p = 0.017$) volumes were significantly smaller, and total T2 lesion volumes were significantly larger ($p = 0.014$) in smokers defined by cotinine level (figure 2). For the clinical measures, smokers had a higher score (more disability) on the T25FW test ($p = 0.031$) after 10 years and a larger decrease in attention scores measured by the PASAT test ($p = 0.042$) between month 24 and the 10-year follow-up visit (figure 3). The results were similar for smoking defined by patient self-reporting (eTable 3).

Smoking Status and Long-term MRI and Clinical Outcome Measures

The results from the linear mixed-model investigating the relationship between smoking and long-term MRI and clinical measures are shown in Table 3 (smoking defined by cotinine levels) and supplemental eTable 4, links.lww.com/NXI/A728 (smoking defined by patient self-reporting). Smoking defined by cotinine level was associated with lower total WM volume ($\beta = -21.74$, $p = 0.039$) after 10 years and with higher total logT2 lesion volume ($\beta = 0.22$, $p = 0.011$). Similar results were found for smoking defined by patient self-reporting (eTable 4), additionally associated with lower deep GM volume ($\beta = -2.35$, $p = 0.049$) after 10 years. There were no significant associations between smoking and clinical disability when

Figure 2 Distribution Plots of MRI Measurements at the 10-Year Follow-up Visit in Nonsmokers and Smokers



defined by cotinine levels (Table 3), but when defined by patient self-reporting, smoking was associated with a higher score on the logT25FW test ($\beta = 0.050$, $p = 0.039$) after 10 years and a larger decrease in PASAT scores ($\beta = -3.58$, $p = 0.029$) (eTable 4).

Dose-Effect Relationship on Long-term MRI and Clinical Outcome Measures

The results from the mixed-effect model assessing the relation between mean cotinine levels in smokers (defined by cotinine level) and MRI and clinical outcome measures are shown in supplemental eTable 5, links.lww.com/NXI/A728. In a bivariate model, higher cotinine levels were significantly associated with lower mean cortical thickness, but not after adjusting for age, sex, VD, BL EDSS, and time from diagnosis.

Discussion

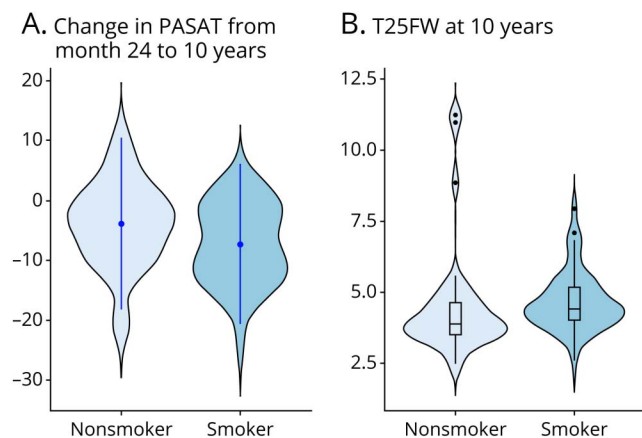
We found that patients with MS who smoked had lower total WM and deep GM volumes and higher T2 lesion volumes after 10 years. Smokers also accumulated more disability and incurred a larger decrease in attention scores measured by the T25FW and PASAT test, respectively. The results obtained by defining smokers by cotinine levels collected during the initial 2-year period were highly comparable with those obtained when defining smokers by retrospective patient self-reporting. Furthermore, the associations were still significant when correcting for vascular risk factors and established cardiovascular disease. These findings suggest that smoking has a negative long-term influence on prognosis and disease progression in patients with MS.

The association between smoking and higher lesion load is partly in line with previous research,^{1,7} supporting the notion that smoking could heighten inflammatory activity.³ However, smoking and increased lesional activity have not been consistently related,^{9,10} as also shown in a previous study investigating the same patient cohort as in this current work. In the previous study, no association was found between tobacco use (defined by cotinine levels) and the occurrence of new or enlarging lesions during the trial period of 24 months.¹⁰ The discrepant findings in this same study population may be a result of the

different outcome measures used, that is, lesion volumes vs the less sensitive dichotomous measure of new or expanding lesions present or not present, especially considering the more limited follow-up time of 2 years. Moreover, it is unknown how smoking may affect pathologic processes within the occurring lesions. Lesions with persistent subtle inflammation, called chronic active or smoldering lesions, are associated with low-grade BBB leakage,³¹ higher atrophy rates,³² and have been shown to develop in patients with RRMS and slowly expand over years.³³ In this study, we were unable to investigate whether smokers had a higher fraction of smoldering lesions, but testing that hypothesis could provide insight to the discrepant associations found for the occurrence of new lesions and total lesion volume and to the overall worse prognosis seen in smoking patients with MS.³

After 10 years, we found that deep GM and total WM volume were lower in patients who smoked. Previous longitudinal studies on smoking/nonsmoking patients with MS have mainly assessed whole-brain atrophy and have not reported consistent results.^{1,7,9} Cross-sectional studies assessing GM atrophy showed a similar lack of consensus.^{6,11,13} In studies investigating non-MS populations, smoking is associated with atrophy most evidently in the frontal and temporal lobe, cingulate gyrus, and the cerebellum^{33,34} while the associations with subcortical GM are more variable.^{33,34} Furthermore, neither studies on MS nor those on non-MS populations have found smoking to be related to lower WM volume.^{6,7,11,13,33,34} In early MS, atrophy in the deep GM has previously been shown to develop at a relatively high rate^{35,36} compared with other GM regions, and to be closely related to WM lesions.^{36,37} Although the causal mechanisms are still not sufficiently clear, a spatiotemporal relationship between WM lesions and subsequent deep GM atrophy progression through neuroaxonal degradation³⁸ seems likely, explaining at least part of the neurodegenerative process. Together with the higher lesion volume, our findings of lower deep GM and total WM volumes in smoking patients with MS may suggest that the pathologic changes are driven by increased inflammatory damage in the WM, followed by secondary degeneration in regions either consisting of WM or highly connected through

Figure 3 Distribution Plots of Clinical Measurements in Nonsmokers and Smokers



The width of the shaded area represents the proportion of observations for (A) change in PASAT score from month 24 to 10 years (point range represents the mean and SD) and (B) T25FW test score at 10 years (box plots represent the median and IQR, with the whiskers representing the distribution of observations within $\times 1.5$ of the IQR). IQR = interquartile range; PASAT = paced auditory serial addition test; T25FW = timed 25-foot walk.

WM tracts.³⁹ The hypothesis that the main neurodegenerative pathway in MS is driven by neuroaxonal injury has also been proposed in a recent study, which observed higher levels of neurofilament light chain in smokers.⁴⁰

Although not significant after correcting for age, sex, vascular risk factors, BL EDSS, and time since diagnosis, our initial bivariate analysis found that higher cotinine levels in smokers were associated with lower cortical thickness after 10 years. This is similar to previous literature on non-MS populations, finding a dose-dependent relationship between smoking and cortical atrophy.^{34,41} The different smoking-associated atrophy patterns seen when comparing smoking and nonsmoking patients with MS (lower deep GM and total WM volume), and cotinine levels in smoking patients (lower cortical thickness), may suggest that smoking affects atrophy progression both through MS-specific disease mechanisms and directly in a dose-dependent manner. It is, however, important to highlight that our sample size for the dose-effect analyses was small and that the current data did not allow us to investigate these possible mechanisms directly.

At the 10-year follow-up visit, smokers had a higher degree of walking impairment measured by the T25FW test, indicating higher risk of disability accrual. The lack of association between smoking defined by cotinine levels and change in EDSS score over the follow-up has previously been reported in this current patient population.¹⁵ In our study, we confirmed this finding, for both definitions of smoking. The results are partly in line with previous research, much of which reported a relation between smoking and higher EDSS or Multiple Sclerosis Severity Score in cross-sectional analyses,^{1,6} but more variably longitudinally.^{1,9,40,42,43} A possible cause of the inconsistent results for EDSS is the known low sensitivity to

change for this measurement,⁴⁴ suggesting that more targeting tests should be used to capture disability progression in specific functions. Overall, the associations with disability accrual were modest in our study, and after correcting for relevant covariates, it was only found for one of the 2 definitions of smoking (patient self-reporting). In future studies, these longitudinal relations should be further investigated in larger patient populations.

In our study, patients who smoked also had a higher decrease in the PASAT score from month 24 to the 10-year follow-up. This finding is in line with previous studies, where smokers tended to perform worse cognitively.^{40,45} Attention, information processing, and working memory are cognitive domains commonly affected in patients with MS,⁴⁶ but impairment on these domains has also been shown in smoking non-MS populations.³⁴ This may imply that smoking patients with MS are at additive risk of developing cognitive impairment, through mechanisms specific to both MS and smoking. This suggests an important clinical consequence to minimize long-term GM atrophy and clinical decline; people with MS who smoke should be encouraged and assisted to quit smoking.

Our study is not without limitations. Brain atrophy and lesion load were measured cross-sectionally at the 10-year follow-up visit. In future research, long-term measurements should be corrected for values at baseline if available, or be investigated by longitudinal analyses, to conclude on atrophy progression in smoking patients with MS.

Obtaining volume and cortical thickness measures in post-contrast images by FreeSurfer is not the standard approach. Recent work using data from a subgroup of this patient cohort has demonstrated excellent consistency between values obtained from precontrast and postcontrast 3D T1-weighted images,²⁸ although minor to moderate segmentation errors, especially in the temporal lobe were more common in post-contrast images. In this current study, we did not apply manual corrections for these errors, and the volume of the temporal lobe was excluded from the total GM volume to limit the possible bias introduced by the larger variability in measurements extracted from this region. In future studies, corrections of these segmentation errors should be considered, especially if evaluating regional atrophy measures.

Owing to its stability in plasma over time (half-life of approximately 20 hours), cotinine has become the preferred biomarker to quantify long-term nicotine exposure.⁸ However, the use of cotinine levels as a proxy for smoking is potentially biased by other sources of nicotine. This is an important limitation because neither nicotine nor smokeless tobacco have shown to induce inflammation in MS¹⁹ or increase the risk of the disease.¹⁸ In our study, patients who at the 10-year follow-up reported use of smokeless tobacco (e.g., snuff) exclusively (4 patients) were, therefore, classified as nonsmokers, regardless of their measured cotinine levels. Furthermore, the results obtained by defining smoking by

Table 3 Effect of Smoking Status (Defined by Cotinine Level) on MRI and Clinical Measures at the 10-Year Follow-up Visit and Change in Clinical Measures Between Month 24 and the 10-Year Follow-up Visit

MRI/clinical measure	N	Beta	St. Error	95% CI	p Value
Total GM volume (mL)	67	-12.025	9.419	-29.349, 5.299	0.208
Total WM volume (mL)	67	-21.739	10.254	-40.599, -2.880	0.039
Total deep GM volume (mL)	67	-2.109	1.127	-4.183, -0.036	0.068
Thalamus volume (mL)	67	-0.352	0.221	-0.758, 0.054	0.118
Mean Cth (mm)	67	-0.031	0.025	-0.076, 0.015	0.214
LogT2 lesion volume (mL) ^a	64	0.224	0.084	0.069, 0.379	0.011
T2 lesion count	64	2.095	2.302	-2.163, 6.352	0.368
EDSS	67	0.140	0.289	-0.396, 0.676	0.630
Change in EDSS	65	-0.234	0.301	-0.792, 0.324	0.441
LogT25FW ^a	66	0.012	0.024	-0.032, 0.056	0.622
LogChange in T25FW ^a	64	0.013	0.020	-0.024, 0.051	0.511
LogD9-HPT ^a	64	0.035	0.023	-0.007, 0.078	0.130
LogChange in D9-HPT ^a	62	0.044	0.049	-0.047, 0.135	0.377
LogND9-HPT ^a	64	0.022	0.021	-0.017, 0.061	0.300
LogChange in ND9-HPT ^a	62	0.064	0.062	-0.051, 0.179	0.310
PASAT	64	-2.572	2.167	-6.581, 1.436	0.242
Change in PASAT	62	-2.654	1.565	-5.543, 0.236	0.097

Abbreviations: Cth = cortical thickness; D9-HPT = dominant hand 9 hole-peg test; EDSS = Expanded Disability Status Scale; GM = gray matter; ND9-HPT = nondominant hand 9-hole peg test; PASAT = paced auditory serial addition test; T25FW = timed 25-foot walk; WM = white matter.

^a Dependent variable log transformed because of nonnormality (log-linear transformation).

Bold text indicates statistically significant *p* values.

cotinine levels were comparable with those using patient self-reporting to define smoking habits. The associations with MRI atrophy and clinical measures were somewhat stronger when using the definition based on patient self-reporting, most notably for clinical disability. This may be explained by this definition also capturing patients who smoked regularly in time periods during the follow-up, after the 2 years of the clinical trial. In this study, serum cotinine measurements were not available after the first 2 years of follow-up or at the 10-year follow-up visit. In future works, the effect of cotinine levels measured regularly over a longer follow-up period should be explored, especially to determine the effect of smoking duration and cessation. Nevertheless, the overall comparable results suggest that serum cotinine levels provide an objective and reliable option for defining smoking habits and especially to investigate dose-dependent relationships.

Several comorbid conditions may independently influence brain tissue changes (including gray and WM atrophy and localized WM hyperintensities)²⁰ and can be caused or exacerbated by smoking.²¹ We attempted to limit the effect of these complex interrelations by correcting for vascular risk factors and established cardiovascular and cerebrovascular disease. MS disease-related factors, such as disease-modifying

therapies, are also likely to affect brain atrophy. In this data set, however, this was not possible to statistically consider because the patients had used a variety of therapies at different times and duration over the follow-up.

Smoking was associated with lower deep GM and total WM volume and higher T2 lesion volume after 10 years in patients with RRMS. Patients who smoked had higher physical and cognitive disability accrual, measured by the T25FW and PASAT test, respectively. The findings suggest that smoking patients with MS should be advised and offered aid in smoking cessation as early as possible in the disease course.

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References

1. Healy BC, Ali EN, Guttman CR, et al. Smoking and disease progression in multiple sclerosis. *Arch Neurol*. 2009;66(7):858-864.

2. Degelman ML, Herman KM. Smoking and multiple sclerosis: a systematic review and meta-analysis using the Bradford Hill criteria for causation. *Mult Scler Relat Disord*. 2017;17:207-216.
3. Rosso M, Chitnis T. Association between cigarette smoking and multiple sclerosis: a review. *JAMA Neurol*. 2020;77(2):245-253.
4. Wingerchuk DM. Smoking: effects on multiple sclerosis susceptibility and disease progression. *Ther Adv Neurol Disord*. 2011;5(1):13-22.
5. Petersen ER, Søndergaard HB, Laursen JH, et al. Smoking is associated with increased disease activity during natalizumab treatment in multiple sclerosis. *Mult Scler*. 2018;25(9):1298-1305.
6. Zivadinov R, Weinstock-Guttman B, Hashmi K, et al. Smoking is associated with increased lesion volumes and brain atrophy in multiple sclerosis. *Neurology*. 2009;73(7):504-510.
7. Horakova D, Zivadinov R, Weinstock-Guttman B, et al. Environmental factors associated with disease progression after the first demyelinating event: results from the multi-center SET study (P05.134). *Neurology*. 2013;80(7 suppl):P05.134.
8. Florescu A, Ferrence R, Einarson T, Selby P, Soldin O, Koren G. Methods for quantification of exposure to cigarette smoking and environmental tobacco smoke: focus on developmental toxicology. *Ther Drug Monit*. 2009;31(1):14-30.
9. Munger KL, Fitzgerald KC, Freedman MS, et al. No association of multiple sclerosis activity and progression with EBV or tobacco use in BENEFIT. *Neurology*. 2015;85(19):1694-1701.
10. Kvistad S, Myhr KM, Holmøy T, et al. No association of tobacco use and disease activity in multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm*. 2016;3(4):e260.
11. Kappus N, Weinstock-Guttman B, Hagemeyer J, et al. Cardiovascular risk factors are associated with increased lesion burden and brain atrophy in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2016;87(2):181-187.
12. Durhan G, Diker S, Has AC, et al. Assessment of the effect of cigarette smoking on regional brain volumes and lesion load in patients with clinically isolated syndrome. *Int J Neurosci*. 2016;126(9):805-811.
13. Graetz C, Groger A, Luessi F, et al. Association of smoking but not HLA-DRB1*15:01, APOE or body mass index with brain atrophy in early multiple sclerosis. *Mult Scler*. 2019;25(5):661-668.
14. Torkildsen O, Wergeland S, Bakke S, et al. omega-3 fatty acid treatment in multiple sclerosis (OFAMS Study): a randomized, double-blind, placebo-controlled trial. *Arch Neurol*. 2012;69(8):1044-1051.
15. Wesnes K, Myhr KM, Riise T, et al. Low vitamin D, but not tobacco use or high BMI, is associated with long-term disability progression in multiple sclerosis. *Mult Scler Relat Disord*. 2021;50:102801.
16. Connor Gorber S, Schofield-Hurwitz S, Hardt J, Levasseur G, Tremblay M. The accuracy of self-reported smoking: a systematic review of the relationship between self-reported and cotinine-assessed smoking status. *Nicotine Tob Res*. 2009;11(1):12-24.
17. Biochemical verification of tobacco use and cessation. *Nicotine Tob Res*. 2002;4(2):149-159.
18. Hedström AK, Bäärnhielm M, Olsson T, Alfredsson L. Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis. *Neurology*. 2009;73(9):696-701.
19. Jiang W, St-Pierre S, Roy P, Morley BJ, Hao J, Simard AR. Infiltration of CCR2+Ly6Chigh proinflammatory monocytes and neutrophils into the central nervous system is modulated by nicotinic acetylcholine receptors in a model of multiple sclerosis. *J Immunol*. 2016;196(5):2095-2108.
20. Friedman JI, Tang CY, de Haas HJ, et al. Brain imaging changes associated with risk factors for cardiovascular and cerebrovascular disease in asymptomatic patients. *JACC Cardiovasc Imaging*. 2014;7(10):1039-1053.
21. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. *J Am Coll Cardiol*. 2004;43(10):1731-1737.
22. Schmidt P, Gaser C, Arsic M, et al. An automated tool for detection of FLAIR-hyperintense white-matter lesions in Multiple Sclerosis. *NeuroImage*. 2012;59(4):3774-3783.
23. FMRIB Software Library. fmrib.ox.ac.uk/fsl.
24. Battaglini M, Jenkinson M, De Stefano N. Evaluating and reducing the impact of white matter lesions on brain volume measurements. *Hum Brain Mapp*. 2012;33(9):2062-2071.
25. FreeSurfer. surfer.nmr.mgh.harvard.edu/.
26. Fischl B. FreeSurfer. *Neuroimage*. 2012;62(2):774-781.
27. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *NeuroImage*. 1999;9(2):179-194.
28. Lie IA, Kerkling E, Wesnes K, et al. The effect of gadolinium-based contrast-agents on automated brain atrophy measurements by FreeSurfer in patients with multiple sclerosis. *Eur Radiol*. 2022;32(5):3576-3587.
29. Desikan RS, Ségonne F, Fischl B, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage*. 2006;31(3):968-980.
30. Hochmeister S, Grundtner R, Bauer J, et al. Dysferlin is a new marker for leaky brain blood vessels in multiple sclerosis. *J Neuropathol Exp Neurol*. 2006;65(9):855-865.
31. Absinta M, Sati P, Masuzzo F, et al. Association of chronic active multiple sclerosis lesions with disability in vivo. *JAMA Neurol*. 2019;76(12):1474-1483.
32. Dal-Bianco A, Grabner G, Kronnerwetter C, et al. Long-term evolution of multiple sclerosis iron rim lesions in 7 T MRI. *Brain*. 2021;144(3):833-847.
33. Elbejjani M, Auer R, Jacobs DR, et al. Cigarette smoking and gray matter brain volumes in middle age adults: the CARDIA Brain MRI sub-study. *Transl Psychiatry*. 2019;9(1):78.

34. Gallinat J, Meisenzahl E, Jacobsen LK, et al. Smoking and structural brain deficits: a volumetric MR investigation. *Eur J Neurosci.* 2006;24(6):1744-1750.
35. Eshaghi A, Prados F, Brownlee WJ, et al. Deep gray matter volume loss drives disability worsening in multiple sclerosis. *Ann Neurol.* 2018;83(2):210-222.
36. Bergsland N, Horakova D, Dwyer MG, et al. Subcortical and cortical gray matter atrophy in a large sample of patients with clinically isolated syndrome and early relapsing-remitting multiple sclerosis. *AJNR Am J Neuroradiol.* 2012;33(8):1573-1578.
37. Henry RG, Shieh M, Amirbekian B, Chung S, Okuda DT, Pelletier D. Connecting white matter injury and thalamic atrophy in clinically isolated syndromes. *J Neurol Sci.* 2009;282(1-2):61-66.
38. Klaver R, De Vries HE, Schenk GJ, Geurts JJ. Grey matter damage in multiple sclerosis: a pathology perspective. *Prion.* 2013;7(1):66-75.
39. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol.* 2015;15(9):545-558.
40. Cortese M, Munger KL, Martínez-Lapiscina EH, et al. Vitamin D, smoking, EBV, and long-term cognitive performance in MS. *Neurology.* 2020;94(18):e1950.
41. Karama S, Ducharme S, Corley J, et al. Cigarette smoking and thinning of the brain's cortex. *Mol Psychiatry.* 2015;20(6):778-785.
42. Manouchehrinia A, Tench CR, Macted J, Bibani RH, Britton J, Constantinescu CS. Tobacco smoking and disability progression in multiple sclerosis: United Kingdom cohort study. *Brain.* 2013;136(pt 7):2298-2304.
43. Koch M, van Harten A, Uyttenboogaart M, De Keyser J. Cigarette smoking and progression in multiple sclerosis. *Neurology.* 2007;69(15):1515-1520.
44. Meyer-Moock S, Feng YS, Maeurer M, Dippel FW, Kohlmann T. Systematic literature review and validity evaluation of the expanded disability status scale (EDSS) and the multiple sclerosis functional composite (MSFC) in patients with multiple sclerosis. *BMC Neurol.* 2014;14:58.
45. Ozcan ME, Ince B, Bingöl A, et al. Association between smoking and cognitive impairment in multiple sclerosis. *Neuropsychiatr Dis Treat.* 2014;10:1715-1719.
46. Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. *Lancet Neurol.* 2008;7(12):1139-1151.