Smoking is associated with increased lesion volumes and brain atrophy in multiple sclerosis

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ABSTRACT

Background: Cigarette smoking has been linked to higher susceptibility and increased risk of progressive multiple sclerosis (MS). The effects of smoking on MRI characteristics of patients with MS have not been evaluated.

Objectives: To compare the MRI characteristics in cigarette smoker and nonsmoker patients with MS.

Methods: We studied 368 consecutive patients with MS (age $44.0 \pm \text{SD } 10.2$ years, disease duration 12.1 ± 9.1 years) comprising 240 never-smokers and 128 (34.8%) ever-smokers (currently active and former smokers). The average number of packs per day smoked ($\pm \text{SD}$) was 0.95 ± 0.65 , and the mean duration of smoking was 18.0 ± 9.5 years. All patients obtained full clinical and quantitative MRI evaluation. MRI measures included T1, T2, and gadolinium contrast-enhancing (CE) lesion volumes (LVs) and measures of central, global, and tissue-specific brain atrophy. The associations between smoking status and MRI measurements were assessed in regression analysis.

Results: Smoking was associated with increased Expanded Disability Status Scale (EDSS) scores ($p = 0.004$). The median EDSS scores (interquartile range) in the ever-smoker group and the active-smoker group were both 3.0 (2.0), compared with 2.5 (2.5) in never-smokers. There were adverse associations between smoking and the lesion measures including increased number of CE lesions ($p < 0.001$), T2 LV ($p = 0.009$), and T1 LV ($p = 0.003$). Smoking was associated with decreased brain parenchymal fraction ($p = 0.047$) and with increases in the lateral ventricle volume ($p = 0.001$) and third ventricle width ($p = 0.023$).

Conclusions: Smoking is associated with increased blood-brain barrier disruption, higher lesion volumes, and greater atrophy in multiple sclerosis. *Neurology*® 2009;73:504–510

GLOSSARY

BPF = brain parenchymal fraction; **CE** = contrast-enhancing; **CIS** = clinically isolated syndromes; **EDSS** = Expanded Disability Status Scale; **GMF** = gray matter fraction; **LV** = lesion volume; **LVV** = lateral ventricle volume; **MS** = multiple sclerosis; **P-P** = probability-probability; **PPMS** = primary progressive multiple sclerosis; **r_p** = partial correlation; **RRMS** = relapsing-remitting multiple sclerosis; **SE** = standard error of slope; **SPMS** = secondary progressive multiple sclerosis; **3VW** = third ventricle width; **WMF** = white matter fraction.

Considerable evidence indicates that both genetic and environmental factors are involved in multiple sclerosis (MS) susceptibility and disease progression.¹ Cigarette smoking is one of the most compelling environmental risk factors linked to the development and worsening of MS. The other environmental risk factors implicated include sun exposure/vitamin D deficiency and Epstein–Barr virus infections.^{2,3}

Recent studies have shown a relationship between cigarette smoking and MS,^{1,4} although some earlier studies did not find evidence for a link.^{5–7} Subjects who smoked 20 to 40 cigarettes a day had twice the risk of developing MS compared with nonsmokers,⁴ and smoking may interact with the progression of MS.^{4,8–10} Smoking was recently shown to be a risk factor for

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From the Buffalo Neuroimaging Analysis Center (R.Z., K.H., N.A., M.S., M.D., S.H., J.D.), The Jacobs Neurological Institute (R.Z., B.W.-G., M.R.), and Baird Multiple Sclerosis Center (B.W.-G.), Department of Neurology; and Department of Pharmaceutical Sciences (M.R.), State University of New York, Buffalo, NY.

Supported by the National Multiple Sclerosis Society (RG3743 and a Pediatric MS Center of Excellence Center Grant). The funding sources had no role in the research, analysis, and interpretation or in the preparation of the manuscript.

Disclosure: Author disclosures are provided at the end of the article.

conversion from clinically isolated syndromes (CIS) to clinically definite MS; the interval to the first relapse occurred sooner in smokers and the hazard ratio for conversion over 3 years for smokers was 1.8 compared with nonsmokers.¹¹ Smoking worsens the prognosis for progressive disease,¹² and the hazard ratio of secondary progression was 3.6 for ever-smokers compared with never-smokers.⁸ Cigarette smoking can also exacerbate MS, both chronically and acutely.^{2,9}

MRI provides the best available surrogate markers for monitoring the MS disease process and for assessing the effects of treatments and can therefore complement the currently available epidemiologic evidence for the role of smoking in MS disease progression. Numerous studies have shown MRI measures to be generally more sensitive than clinical measures such as the Kurtzke Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Functional Composite because it can provide evidence for subclinical disease activity. A diverse array of MRI metrics capable assessing pathologic processes ranging from transient blood–brain barrier breakdown and lesion dynamics to more irreversible atrophy is available. The mechanisms that relate cigarette smoking to MS risk or progression are not well understood, and the extent of anatomic brain damage associated with smoking in patients with MS has not been characterized. The goal of this study is to compare the MRI characteristics of smokers and non-smokers with MS to better understand the role of cigarette smoking in MS and further identify underlying mechanisms that relate to MS disease progression.

METHODS Study population. Consecutive patients with MS at the Baird Multiple Sclerosis Center were prospectively enrolled at the time of their routine clinical follow-up visit, and the patients with CIS were enrolled at the time of their first visit.

The inclusion criteria were MRI examination and smoking-related history obtained at the time of the patient's clinical visit (± 30 days), age 18–80 years, and EDSS score 0 to 8.5. Exclusion criteria consisted of relapse and steroid treatment in the 1 month preceding study entry, preexisting medical conditions known to be associated with brain pathology (e.g., neurodegenerative disorders, cerebrovascular disease, positive history of alcohol abuse), and insufficient quality of the MRI scan for quantitative analysis.

Based on the self-reported smoking questionnaire, subjects were classified as never-smokers or ever-smokers (including both currently active smokers and former smokers). The smoking

questionnaire was based on the smoking-related questions in the New York State Multiple Sclerosis Consortium (a regional affinity group of 16 MS treatment centers throughout New York State) questionnaire and included additional questions that were developed for this study. The questionnaire collected data on smoking history and current smoking habits, including number of cigarettes smoked per day and numbers of years of smoking. The active smokers were classified those who smoked more than 10 cigarettes per day in the 3 months before the study start, and the former smokers were those who had smoked cumulatively for at least 6 months sometime in the past. Of the 368 enrolled patients, there were 240 never-smokers and 128 ever-smokers (of these, 96 were active smokers and 32 were former smokers). The demographics are summarized in table 1.

The clinical assessments included patient's age at MRI scan, disease type,¹³ duration of the disease, disability examination assessed by EDSS, and the presence, type, and duration of disease-modifying therapy.

Standard protocol approvals, registrations, and patient consents. The study data were obtained under a protocol approved by the Human Subjects Institutional Review Board of the University at Buffalo. Written informed consent was obtained from all patients participating in the study.

MRI acquisition and analysis. Image acquisition. Quantitative MRI analysis was available for all patients. Patients underwent brain MRI using a 1.5-T General Electric Signa 4x/Lx scanner. The following scans were acquired: axial dual fast spin-echo T2/proton density-weighted image, 3-dimensional spoiled-gradient recalled T1-weighted image, spin-echo T1-weighted image with and without gadolinium contrast, and fast fluid attenuated inversion recovery. The acquisition parameters are summarized in appendix e-1 on the *Neurology*[®] Web site at www.neurology.org.

Image analysis. The MRI analysts were blinded to patients' clinical characteristics and clinical status. The following MRI measures were performed: T1, T2, and gadolinium contrast-enhancing (CE) lesion volumes (LVs), and measures of central, global, and tissue-specific brain atrophy (tables 1 and 2).

Lesion measures. The T2 and T1 LVs were measured using a semiautomated edge detection contouring–thresholding technique previously described.¹⁴

Global atrophy measures. Statistical Parametric Mapping (SPM5, Institute of Neurology, Queen Square, London, UK) was used.¹⁵ The MRI scans were aligned, stripped of extracranial tissue (e.g., soft tissue, skull, orbit), and segmented into brain parenchyma and CSF compartments.¹⁶ The brain parenchymal fraction (BPF), a measure of normalized brain volume, was computed by dividing the total brain parenchyma by total intracranial volume. Similarly, gray matter fraction (GMF) and white matter fraction (WMF) were obtained by dividing each of these variables by total intracranial volume. Images were weighted by their 3-dimensional lesion masks to exclude the lesions from the normalization images.¹⁷ The scan–rescan reliability was 0.4% for BPF, 0.3% for GMF, and 0.9% for WMF.¹⁸

Central atrophy measures. In addition to global atrophy, central brain atrophy measures were calculated: lateral ventricle volume (LVV) and third ventricle width (3VW), as previously described.^{19,20}

Data analysis. The program SPSS-16 (SPSS Inc., Chicago, IL) was used. The cube root transformation was applied to T2, T1, and CE LVs.²⁰ The LVV and 3VW, which are MRI measures of central atrophy, were logarithm (base 10) transformed. These variables ex-

Table 1 Clinical and demographic characteristics of the cohort

Characteristic	All patients	Never-smokers	Ever-smokers	Active smokers	p Value,* never vs ever
n	368	240	128		
Females:males	291:77 (79)	190:50 (79)	101:27 (79)	73:20 (79)	1.0
Race, %					
White	92.6	91.9	93.8	92.8	
Black	6.1	6.6	5.3	6.0	
Other	1.3	1.5	0.9	1.2	
MS type					
RRMS	252 (68.6)	164 (68.6)	88 (68.8)	63 (67.7)	
CIS	21 (5.7)	14 (5.9)	7 (5.5)	6 (6.5)	
SPMS	84 (22.9)	53 (22.2)	31 (24.2)	22 (23.7)	
PPMS	10 (2.8)	8 (3.3)	2 (1.6)	2 (2.2)	
Age, y	44.0 ± 10.2	43.9 ± 10.0	44.3 ± 10.7	42.7 ± 10.3	0.71
Education, y	14.3 ± 2.2	14.5 ± 2.3	13.8 ± 2.0	13.7 ± 2.0	0.011
Disease duration, y	12.0 ± 9.1	11.9 ± 9.1	12.3 ± 9.2	12.4 ± 9.5	0.72
Treatment duration, y	3.83 ± 2.8	3.95 ± 2.8	3.59 ± 2.7	3.78 ± 2.8	0.26
MRI characteristics					
% with CE lesions	32.6	30.9	35.9	40.2	
CE lesion number	0.87 ± 3.1	0.72 ± 2.5	1.2 ± 4.1	0.92 ± 1.5	
CE LV, mL	0.20 ± 1.4	0.10 ± 0.37	0.39 ± 2.3	0.23 ± 0.69	
T2 LV, mL	14.2 ± 15.0	13.4 ± 14.9	15.7 ± 15.1	15.6 ± 14.9	
T1 LV, mL	2.2 ± 3.5	2.0 ± 3.6	2.5 ± 3.3	2.4 ± 3.3	
GMF	0.406 ± 0.034	0.407 ± 0.032	0.405 ± 0.037	0.406 ± 0.041	
WMF	0.395 ± 0.026	0.396 ± 0.024	0.392 ± 0.031	0.390 ± 0.034	
BPF	0.801 ± 0.033	0.803 ± 0.034	0.797 ± 0.031	0.797 ± 0.033	
3VW, mm	3.7 ± 2.4	3.5 ± 2.3	4.0 ± 2.5	4.0 ± 2.7	
LVV, mL	19.1 ± 14.8	18.3 ± 14.4	20.7 ± 15.3	20.7 ± 16.2	

Data are number (percentage) or mean ± SD.

*The *p* values are from an independent samples *t* test, except for the frequency of male and female ratio, which was analyzed with the Fisher exact test. The *p* values for MRI characteristics were determined using regression analysis and are summarized in table 2.

MS = multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; CIS = clinically isolated syndromes; SPMS = secondary progressive multiple sclerosis; PPMS = primary progressive multiple sclerosis; CE = contrast-enhancing; LV = lesion volume; GMF = gray matter fraction; WMF = white matter fraction; BPF = brain parenchymal fraction; 3VW = third ventricle width; LVV = lateral ventricle volume.

hibited large deviations from normality in probability–probability (P-P) plots. Deviations from normality, linearity, and homoscedasticity in the dependent variable can adversely affect the normality of the distribution of residuals required for regression analysis. The transformation procedures were effective at reducing the skewness of MRI parameters as assessed with P-P plots.

In multivariate linear regression analysis, the MRI variable of interest was the dependent variable; the independent variables were age, sex, disease and treatment durations, presence of progressive MS, and smoking status.

The number of CE lesions (dependent variable) was analyzed using a Poisson log-linear regression model wherein age, disease duration, and treatment duration were covariates and smoking status, sex, and presence of progressive MS were factors. Poisson regression is appropriate for count and count rate variables and has been previously used for modeling CE lesion activity and relapse rates in MS.²¹

The EDSS is an ordinal scale, and the polytomous universal model ordinal regression method with age, disease duration, and treatment duration as covariates and smoking status, sex, and presence of progressive MS as factors was used for analysis.

Because of the multiple testing involved in the analysis of MRI and clinical data, we used a conservative $\alpha = 0.01$ to assess significance. A statistical trend was assumed if $p \leq 0.05$.

RESULTS Study population. Demographic, clinical, and MRI characteristics are summarized in table 1. The never-smoker and ever-smoker groups did not differ in age, disease duration, or treatment duration. The proportion of females was 79% in both groups, and the proportion of patients with progressive MS was 74% in both groups. There was a modest differ-

Table 2 Multiple regression results for MRI measurements with ever-smoker/never-smoker smoking status correcting for female sex, age, disease and treatment duration, and presence of progressive MS

MRI measurement	Overall model			Ever-smoker/never-smoker variable			
	F	R ²	p	Slope	SE	r _p	p
Enhancing lesion volume	3.79	0.053	0.001	0.90	0.44	0.12	0.043
T2 lesion volume	9.74	0.15	<0.001	2.3	0.86	0.15	0.009
T1 lesion volume	12.9	0.19	<0.001	1.9	0.63	0.17	0.003
GMF	20.9	0.29	<0.001	−0.003	0.003	−0.048	0.41
WMF	0.42	−0.012	0.87	−0.004	0.003	−0.067	0.25
BPF	24.1	0.32	<0.001	<0.006	0.003	−0.12	0.047
LVV	12.8	0.19	<0.001	0.078	0.030	0.15	0.009
3VW	11.0	0.16	<0.001	0.062	0.027	0.13	0.024

MS = multiple sclerosis; SE = standard error of slope; r_p = partial correlation; GMF = gray matter fraction; WMF = white matter fraction; BPF = brain parenchymal fraction; LVV = lateral ventricle volume; 3VW = third ventricle width.

ence in years of education (14.5 ± 2.3 years in never-smokers vs 13.8 ± 2.0 years in ever-smokers, $p = 0.011$ in independent sample t test). The ever-smoker group smoked an average ($\pm$ SD) of 0.95 ± 0.65 packs per day, and the mean ($\pm$ SD) duration was 18.0 ± 9.5 years.

The proportion of patients using disease-modifying therapies was 90% in the never-smoker group and 89% in the ever-smoker group; 74% of never-smokers and 73% of ever-smokers were using interferon- β therapy; 10% of never-smokers and 13% of ever-smokers were using glatiramer acetate.

EDSS. The median (interquartile range) EDSS score for the ever-smoker group was 3.0 (2.0), compared with 2.5 (2.5) for the never-smoker group. In ordinal regression analysis, the association of EDSS score ($\chi^2 = 140$, $p < 0.001$) with never-smoker/ever-smoker smoking status variable (Wald parameter = 8.29) had $p = 0.004$, which is less than the adjusted $\alpha = 0.01$ required for significance.

Lesion-related MRI measures. The mean values of the key lesion-related (CE lesion number, T2 LV, T1 LV) and central atrophy (LVV) MRI measures are summarized in the figure. The mean number of CE lesions for the ever-smoker group was 1.2 ± 4.1 , compared with 0.72 ± 2.5 in the never-smoker group (figure, A). Poisson regression analysis indicated that the ever-smoker status was associated with greater number of CE lesions (Wald χ^2 for smoking status variable = 17.0, $p < 0.001$). In regression analysis, the association between CE LV with smoking status had a positive slope of 0.90 (SE = 0.44) and $p = 0.043$. These results indicate that ever-smokers are at higher risk for developing more numerous CE lesions.

The regression results summarized in table 2 demonstrate that smoking is associated with increased T2 LV and T1 LV.

Global and central atrophy measures. The regression results for GMF and BPF, which are considered global and tissue-specific atrophy measures, and LVV and 3VW, which are central atrophy measures, are shown in table 2. There was no evidence for an association between smoking and GMF. The overall model for WMF was not significant ($p = 0.87$) and was considered uninformative. However, the association between BPF with smoking status had a negative slope of -0.006 (SE = 0.003) and $p = 0.047$, indicating that smoking is associated with greater global atrophy. Smoking was associated with an increase in the LVV ($p = 0.001$) and with increased 3VW; the latter association had $p = 0.023$. These changes are consistent with increased central atrophy in smokers.

These regression analyses were also conducted using the never-smoker, former smoker, and active smoker categories of the smoking status variable. The associations of EDSS score ($p = 0.005$ for nonsmokers), CE LV ($p = 0.039$), T2 LV ($p = 0.023$), T1 LV ($p = 0.011$), LVV ($p = 0.021$), and 3VW ($p = 0.040$) with the never-smoker/former smoker/active smoker smoking status variable were qualitatively similar to those obtained with the ever-smoker/never-smoker smoking status variable. There was no evidence for an association with the number of packs smoked per day and with former smoker/active smoker status among the ever-smokers.

DISCUSSION In patients with MS, smoking was associated with higher increased lesion burden and greater brain atrophy. Our results indicate that a wide range of quantitative brain MRI markers is affected by smoking in patients with MS.

MS ever-smokers in our study had higher EDSS scores than never-smokers (median EDSS score 3.0 in ever-smokers vs 2.5 in never-smokers, $p \leq 0.001$). This finding supports previous reports showing that cigarette smoking increases the risk for disease progression.^{8,12}

In our cohort, we observed an association between smoking and an increased number of CE lesions. Nicotine, a major component of cigarettes, has been shown to increase microvascular blood flow on the brain and to raise the influx of permeable solutes across the blood–brain barrier in rats.²² This is important because leakage of the blood–brain barrier has been suggested as a factor in initiating the development of MS, and may be relevant to the recent report that cigarette smoking increases the risk of converting from CIS to clinically definite MS.¹¹ Cigarette smoking causes a transient worsening of motor

Figure Mean values for the number of contrast-enhancing lesions, T2 lesion volume, T1 lesion volume, and lateral ventricle volume

Summary of the mean values for the number of contrast-enhancing lesions (A), T2 lesion volume (B), T1 lesion volume (C), and lateral ventricle volume (D) in the never-smoker, ever-smoker, and active smoker groups. The error bars are standard errors.

functions in patients with MS compared with healthy controls.²³

We discuss some limitations of our study. Some of the shortcomings are related to the nature of self-reported questionnaire used. For example, we have not been able to consider secondhand smoke. Children exposed to parental smoking were found to have a higher MS risk: the duration of exposure affected the level of risk²⁴ and the incidence of MS increased with the cumulative exposure to smoking. Another potential criticism is our definition of the ever-smoker group. Most epidemiologic studies, e.g., National Health and Nutrition Examination Survey III, require exposure to 100 or more cigarettes,²⁵ which may involve an exposure period of 3 to 12 months of active smoking, depending on individual-specific changes in smoking patterns in response to the evolution of nicotine dependence. Given the reduced social acceptance and stigma associated with smoking in the United States, we expected that subjects would harbor some reservation regarding

smoking behaviors of a limited nature, and we selected the cutoff of 6 months based on reasoning that subjects would be likely to recall and more willingly provide information regarding a 6-month period of cigarette smoking. A different, less stringent definition of past smoking could in theory influence the distribution of sample size between the ever-smoker and never-smoker groups.

Patients with MS exhibit a higher frequency of behaviors including cigarette smoking, alcohol abuse/dependence, and lower levels of physical activity that are known to adversely affect health outcomes.²⁶ Smoking can also be associated with other comorbidities and with other environmental risk factors for MS, such as increased anti-Epstein-Barr virus antibodies.^{26,27} Our exclusion criteria consisted of conditions that may influence quantitative MRI analysis, including cerebrovascular disease and positive history of alcohol abuse. However, we did not obtain data on other comorbidities, such as obesity, hypertriglyceridemia, and hypertension.

African-Americans tend to have a more severe MS disease process, with rapid progression of disability and poor response to first-line disease-modifying therapies.²⁸ There is increasing evidence that smoking patterns differ between African-Americans and white Americans.²⁹ Our study did not have sufficient African-Americans and was not powered to address the contributions of racial differences in smoking behavior.

We also did not observe associations between the years of smoking or pack-years smoked. The exact reasons for the lack of association are not clear but might be related to the self-reported nature of the questionnaire. However, several other studies of smoking in MS have reported associations with the ever-smoker vs never-smoker status,^{8,11,12} but their results with the continuous variables of duration of smoking and pack-years of smoking have not been emphasized to the same extent. Despite these limitations, the advantage of the panel of MRI measures we used is that they can assess both transient pathologic processes such as blood–brain barrier breakdown and long-term, relatively irreversible processes such as cumulative neurodegeneration.

The biological basis of the potential link between smoking and MS has not yet been fully elucidated. In addition to nicotine, cigarette smoke contains hundreds of potentially toxic components, including tar, carbon monoxide, and polycyclic aromatic hydrocarbons.⁹ Two phases of cigarette smoke exist: a tar or particulate phase and a gaseous phase, both of which contain extremely high concentrations of free radicals. Several hypotheses based on biological mechanisms link cigarette smoking to MS and to other autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus.^{9,30,31} The most frequent suggested mechanisms include possible vascular effects, effects on the immune system, increased production of nitric oxide, increased frequency of respiratory infections, and a potential direct neurotoxic effect of cyanides and other components of cigarette smoking.⁹

Cigarette smoke also affects the influx and activation of neutrophils, macrophages, and monocytes,³² and in addition, it increases the expression of the activation marker Fas (CD95) on B and CD4 T-lymphocyte cell surfaces.³³ Smoking is also associated with increases in C-reactive protein, interleukin 6, and urinary thromboxane metabolites, which are important markers of inflammation in autoimmune diseases.^{34–36} Data from animal models indicate that cigarette smoking also increases expression of matrix metalloproteinase 9.³⁷ Increases in the ratio of matrix metalloproteinase 9 to the levels of its endogenous inhibitors are associated with gadolinium CE lesion activity on MRI of patients with MS.³⁸ Cigarette smoking also has antiestrogenic effects through the

formation of inactive 2-hydroxy catechol estrogens³⁹ and affects the hormonal balance in women, which may in turn affect the Th1–Th2 balance.⁴⁰

Our findings provide evidence for a link between smoking and brain injury and could be important for providing the support for antismoking education in schools and more targeted smoking cessation programs to patients with MS. The potential mechanisms are still unclear, but the growing body of epidemiologic evidence for the association of cigarette smoking and MS warrants further investigation with well-designed prospective studies with MRI measures.

AUTHOR CONTRIBUTIONS

Dr. Robert Zivadinov contributed to study design, MRI and clinical data acquisition and analysis, and manuscript preparation. Dr. Bianca Weinstock-Guttman contributed to the clinical and neurologic aspects, patient consent and recruitment, and manuscript preparation. Murali Ramanathan contributed to data analysis, result interpretation, and manuscript preparation. All other authors contributed to MRI image analysis.

ACKNOWLEDGMENT

The authors thank Wee V. Yong, PhD, Hotchkiss Brain Institute and Department of Clinical Neurosciences, University of Calgary, Alberta, Canada, for useful input for this manuscript.

DISCLOSURE

Dr. Zivadinov has received speaker honoraria and consultant fees from Teva Neurosciences, Biogen Idec, Aspreva, Pfizer, and EMD Serono; and has received research support from the National Multiple Sclerosis Society, the National Science Foundation, Biogen Idec, Teva Neuroscience, Genzyme, Aspreva, and Jog for the Jake Foundation. Dr. Weinstock-Guttman has received speaker honoraria and consultant fees from Biogen Idec, TEVA Neurosciences, EMD Serono, and Novartis; and has received research support from Biogen Idec, EMD Serono, TEVA, Cyberonics, and the National Multiple Sclerosis Society. Dr. Hashmi, Dr. Abdelrahman, Dr. Stosic, Mr. Dwyer, Ms. Hussein, and Ms. Durfee report no disclosures. Dr. Ramanathan serves as an editor for the *American Association of Pharmaceutical Scientists Journal*, receives royalties for publishing *The Pharmacy Calculations Workbook* [Pinnacle, Summit and Zenith, 2008]; and receives research support from EMD Serono, Novartis, Pfizer, the National Multiple Sclerosis Society, and the National Science Foundation.

Received February 19, 2009. Accepted in final form May 1, 2009.

REFERENCES

1. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis, part II: noninfectious factors. *Ann Neurol* 2007;61:504–513.
2. Kantarci O, Wingerchuk D. Epidemiology and natural history of multiple sclerosis: new insights. *Curr Opin Neurol* 2006;19:248–254.
3. Zivadinov R, Zorzon M, Weinstock-Guttman B, et al. Epstein-Barr virus is associated with gray matter atrophy in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2009; 80:620–625.
4. Ghadirian P, Dadgostar B, Azani R, Maisonneuve P. A case-control study of the association between socio-demographic, lifestyle and medical history factors and multiple sclerosis. *Can J Public Health* 2001;92:281–285.
5. Casetta I, Granieri E, Malagu S, et al. Environmental risk factors and multiple sclerosis: a community-based, case-control study in the province of Ferrara, Italy. *Neuroepidemiology* 1994;13:120–128.

6. Koch M, van Harten A, Uyttenboogaart M, De Keyser J. Cigarette smoking and progression in multiple sclerosis. *Neurology* 2007;69:1515–1520.
7. Riise T, Nortvedt MW, Ascherio A. Smoking is a risk factor for multiple sclerosis. *Neurology* 2003;61:1122–1124.
8. Hernan MA, Jick SS, Logroscino G, Olek MJ, Ascherio A, Jick H. Cigarette smoking and the progression of multiple sclerosis. *Brain* 2005;128:1461–1465.
9. Costenbader KH, Karlson EW. Cigarette smoking and autoimmune disease: what can we learn from epidemiology? *Lupus* 2006;15:737–745.
10. Pekmezovic T, Drulovic J, Milenkovic M, et al. Lifestyle factors and multiple sclerosis: a case-control study in Belgrade. *Neuroepidemiology* 2006;27:212–216.
11. Di Pauli F, Reindl M, Ehling R, et al. Smoking is a risk factor for early conversion to clinically definite multiple sclerosis. *Mult Scler* 2008;14:1026–1030.
12. Sundstrom P, Nystrom L. Smoking worsens the prognosis in multiple sclerosis. *Mult Scler* 2008;14:1031–1035.
13. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology* 1996;46:907–911.
14. Zivadinov R, Rudick RA, De Masi R, et al. Effects of IV methylprednisolone on brain atrophy in relapsing-remitting MS. *Neurology* 2001;57:1239–1247.
15. Ashburner J, Friston KJ. Voxel-based morphometry: the methods. *Neuroimage* 2000;11:805–821.
16. Carone DA, Benedict RH, Dwyer MG, et al. Semi-automatic brain region extraction (SABRE) reveals superior cortical and deep gray matter atrophy in MS. *Neuroimage* 2006;29:505–514.
17. Sanfilipo MP, Benedict RH, Zivadinov R, Bakshi R. Correction for intracranial volume in analysis of whole brain atrophy in multiple sclerosis: the proportion vs. residual method. *Neuroimage* 2004;22:1732–1743.
18. Zivadinov R, Uxa L, Bratina A, et al. HLA-DRB1*1501, -DQB1*0301, -DQB1*0302, -DQB1*0602, and -DQB1*0603 alleles are associated with more severe disease outcome on MRI in patients with multiple sclerosis. *Int Rev Neurobiol* 2007;79:521–535.
19. Benedict RH, Bruce J, Dwyer MG, et al. Diffusion-weighted imaging predicts cognitive impairment in multiple sclerosis. *Mult Scler* 2007;13:722–730.
20. Benedict RH, Bruce JM, Dwyer MG, et al. Neocortical atrophy, third ventricular width, and cognitive dysfunction in multiple sclerosis. *Arch Neurol* 2006;63:1301–1306.
21. Zhao Y, Traboulsee A, Petkau AJ, Li D. Regression of new gadolinium enhancing lesion activity in relapsing-remitting multiple sclerosis. *Neurology* 2008;70:1092–1097.
22. Chen JL, Wei L, Bereczki D, et al. Nicotine raises the influx of permeable solutes across the rat blood-brain barrier with little or no capillary recruitment. *J Cereb Blood Flow Metab* 1995;15:687–698.
23. Emre M, de Decker C. Effects of cigarette smoking on motor functions in patients with multiple sclerosis. *Arch Neurol* 1992;49:1243–1247.
24. Mikaeloff Y, Caridade G, Tardieu M, Suissa S. Parental smoking at home and the risk of childhood-onset multiple sclerosis in children. *Brain* 2007;130:2589–2595.
25. Tomar SL, Asma S. Smoking-attributable periodontitis in the United States: findings from NHANES III. National Health and Nutrition Examination Survey. *J Periodontol* 2000;71:743–751.
26. Marrie R, Horwitz R, Cutter G, Tyry T, Campagnolo D, Vollmer T. High frequency of adverse health behaviors in multiple sclerosis. *Mult Scler* 2009;15:105–113.
27. Nielsen TR, Pedersen M, Rostgaard K, Frisch M, Hjalgrim H. Correlations between Epstein-Barr virus antibody levels and risk factors for multiple sclerosis in healthy individuals. *Mult Scler* 2007;13:420–423.
28. Weinstock-Guttman B, Jacobs LD, Brownschidle CM, et al. Multiple sclerosis characteristics in African American patients in the New York State Multiple Sclerosis Consortium. *Mult Scler* 2003;9:293–298.
29. Pederson LL, Ahluwalia JS, Harris KJ, McGrady GA. Smoking cessation among African Americans: what we know and do not know about interventions and self-quitting. *Prev Med* 2000;31:23–38.
30. Hernan MA, Olek MJ, Ascherio A. Cigarette smoking and incidence of multiple sclerosis. *Am J Epidemiol* 2001;154:69–74.
31. Silman AJ, Newman J, MacGregor AJ. Cigarette smoking increases the risk of rheumatoid arthritis: results from a nationwide study of disease-discordant twins. *Arthritis Rheum* 1996;39:732–735.
32. Garey KW, Neuhauser MM, Robbins RA, Danziger LH, Rubinstein I. Markers of inflammation in exhaled breath condensate of young healthy smokers. *Chest* 2004;125:22–26.
33. Bijl M, Horst G, Limburg PC, Kallenberg CG. Effects of smoking on activation markers, Fas expression and apoptosis of peripheral blood lymphocytes. *Eur J Clin Invest* 2001;31:550–553.
34. Tracy RP, Psaty BM, Macy E, et al. Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and subclinical disease in healthy elderly subjects. *Arterioscler Thromb Vasc Biol* 1997;17:2167–2176.
35. Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. *Arterioscler Thromb Vasc Biol* 2002;22:1668–1673.
36. Smith CJ, Fischer TH, Heavner DL, et al. Urinary thromboxane, prostacyclin, cortisol, and 8-hydroxy-2'-deoxyguanosine in nonsmokers exposed and not exposed to environmental tobacco smoke. *Toxicol Sci* 2001;59:316–323.
37. Seagrave J, Barr EB, March TH, Nikula KJ. Effects of cigarette smoke exposure and cessation on inflammatory cells and matrix metalloproteinase activity in mice. *Exp Lung Res* 2004;30:1–15.
38. Lee MA, Palace J, Stabler G, Ford J, Gearing A, Miller K. Serum gelatinase B, TIMP-1 and TIMP-2 levels in multiple sclerosis: a longitudinal clinical and MRI study. *Brain* 1999;122(pt 2):191–197.
39. Michnovicz JJ, Naganuma H, Herschcopf RJ, Bradlow HL, Fishman J. Increased urinary catechol estrogen excretion in female smokers. *Steroids* 1988;52:69–83.
40. Cutolo M, Sulli A, Capellino S, et al. Sex hormones influence on the immune system: basic and clinical aspects in autoimmunity. *Lupus* 2004;13:635–638.