Negative interaction between smoking and EBV in the risk of multiple sclerosis: The EnvIMS study

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Abstract

Background: Results from previous studies on a possible interaction between smoking and Epstein-Barr virus (EBV) in the risk of multiple sclerosis (MS) are conflicting.

Objectives: To examine the interaction between smoking and infectious mononucleosis (IM) in the risk of MS.

Methods: Within the case-control study on Environmental Factors In MS (EnvIMS), 1904 MS patients and 3694 population-based frequency-matched healthy controls from Norway, Italy and Sweden reported on prior exposure to smoking and history of IM. We examined the interaction between the two exposures on the additive and multiplicative scale.

Results: Smoking and IM were each found to be associated with an increased MS risk in all three countries, and there was a negative multiplicative interaction between the two exposures in each country separately as well as in the pooled analysis (*P*=0.001). Among those who reported IM there was no increased risk associated with smoking (OR 0.95, 95% CI: 0.66-1.37). The direction of the estimated interactions on the additive scale was consistent with a negative interaction in all three countries (relative excess risk due to interaction (RERI): -0.98, 95% CI: -2.05-0.15, *P*=0.09).

Conclusions: Our findings indicate competing antagonism, where the two exposures compete to affect the outcome.

Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous systems whose etiology is unknown. Past exposure to smoking and Epstein-Barr virus (EBV) infection, as measured by a positive history of infectious mononucleosis (IM) and high antibody titers against Epstein-Barr nuclear antigen 1 (anti-EBNA1), have consistently been associated with an increased MS risk.[1] Still, the etiological mechanism is not established, and knowledge of how these factors interact could provide clues to possible pathways.

Results from a few previous studies on this interaction are conflicting. One study reported significantly higher titers of anti-EBNA1 in ever-smokers compared with never-smokers,[2] although this was not replicated in a later study.[3] It has been suggested that the interaction between smoking and pre-symptomatic EBNA1 titers is dependent on the age of the participants,[4] which could explain some of the discrepancies in previous results. A recent study reported that anti-EBNA1 and a positive history of IM are independently associated with MS, but found no significant pairwise interactions between either of the two measures of EBV infection and a history of smoking.[5]

Interaction tests are prone to insufficient power. A study with 80% power to detect a main effect, may only have 29% power to detect a statistically significant interaction effect of the same magnitude,[6] illustrating how interaction tests are particularly dependent on sample size. We examined the

interplay between smoking, history of IM and MS risk in the setting of a large case-control study of 5598 participants from three countries.

Methods

Study design

This study is part of the international multicentric case-control study of Environmental Factors in Multiple Sclerosis (EnvIMS). The EnvIMS study was carried out in well-defined geographic areas in Europe (Norway, Italy, Serbia and Sweden) and in Canada. It aimed to examine the effect of self-reported exposure to environmental and lifestyle risk factors in MS from early stages in life to disease onset and to disclose possible variations in risk between distinct populations using a common methodology. Details of the study design and methodology have been reported previously.[7]

The EnvIMS study received ethics approval at each collaborating centre.[7]

Study population and area

For the current analyses, data from Italy, Norway and Sweden was available. Cases and controls were aged 18 years or older at the time of selection. The cases were recruited from population-based MS registries and were diagnosed according to the McDonald[8] or the Poser[9] criteria with a clinical onset within 10 years prior to data collection. Specifically, the cases in the Italian component of EnvIMS were recruited from participating centres in Sardinia, Ferrara and San Marino, while Norwegian cases were recruited from the Norwegian MS registry and biobank.[10] In Sweden, cases were recruited

from the counties of Östergötland and Värmland using the Swedish MS-registry. Four times as many age and sex frequency-matched controls were randomly selected from the population registries of each region under study. The response rates among the cases were 70%, 43% and 74% for Norway, Italy and Sweden, respectively. The response rate among the controls was similar in Norway and Sweden (36% and 37%), but was lower in Italy (21%).

Exposure

Exposure information was collected through a self-administered questionnaire (EnvIMS-Q), developed specifically for the study and that had been evaluated for feasibility, reliability, cross-cultural validity and perceived difficulty of completion.[7, 11] It had an identical format for both cases and controls.

Smoking habits were reported as 'ever' and 'never' smoker, and the age of smoking initiation. History of infectious mononucleosis was reported as 'yes', 'no' and 'I do not remember', and the age period at which the disease was contracted ('0-6', '7-12', '13-15', '16-18', '19-24' and '25-30' in Norway and Sweden and in the age periods '0-5', '6-10', '11-15', '16-20', '21-25' and '26-30' in Italy). In Norway and Sweden, the age periods were adapted to the school system. Frequency of outdoor activity was reported as 'virtually all the time', 'quite often', 'reasonably often' and 'not that often' in the age periods '0-6', '7-12', '13-15', '16-18', '19-24' and '25-30' in Norway and Sweden and in the age periods '0-5', '6-10', '11-15', '16-20', '21-25' and '26-30' in Italy. A figure rating scale consisting of body sketches ranging from 1 to 9 was used to report body

size for the specific ages '5', '10', '15', '20', '25' and '30'. Level of education was reported on a five-point scale including '7 years or less' (Elementary School), '8-10 years' (Middle School), '11-13 years' (High School), '14 years or more' (College/University) and 'I do not know'.

Statistical analysis

The association between disease and exposure was estimated as odds ratios (OR) with 95% confidence intervals (95% CIs) using logistic regression.

Interaction on the additive scale was estimated as the relative excess risk due to interaction (RERI).[12] Smoking and IM were dichotomous variables taking 1 for exposed and 0 for unexposed, and 0 was considered the reference level. If OR_{ij} denotes the OR when smoking is set to *i* (*i*=0 or 1) and IM is set to *j* (*j*=0 or 1), then RERI = OR_{11} - OR_{10} - OR_{01} + 1, assuming that ORs approximate risk ratios and positive monotonicity for both exposures. An estimate of RERI that deviates from 0 is interpreted as evidence of an interaction. Corresponding CIs and p-values were calculated using the delta method.[13] Interaction on the multiplicative scale is estimated as the ratio of ORs: OR_{11} / (OR_{10} x OR_{01}).[12] A ratio that deviates from 1 is interpreted as evidence of an interaction. Corresponding CIs and p-values were estimated by including an interaction term, which was the cross product of smoking and IM, in the logistic regression model with smoking and IM. In the pooled analyses including all three countries, we also included country as a categorical variable to account for possible country specific differences.

Controls were randomly assigned an index age based on the distribution of age of disease onset of the cases. Events or reported behavior occurring after the age of onset or index age were not considered as exposure. Participants with missing values on age of smoking initiation and IM onset were excluded from the analyses. All analyses were adjusted for age and sex. Further, we adjusted one model for body size (categorical), outdoor activity (categorical), and level of education (categorical). The age periods most relevant to MS risk in previous studies from EnvIMS were used in this model. This included the age period '16-18' (Norway and Sweden) or '16-20' (Italy) for frequency of outdoor activity and body size at age 20. The α-level was set at 0.05.

The statistical analyses were performed in Stata statistical software for Macintosh, Version 14.1. College Station, TX: StataCorp 2015.

Results

The baseline characteristics and distribution of smoking and IM according to country are described in Table 1.

Smoking and IM were each found to be associated with MS in all three countries (data not shown). The estimates from the pooled analyses combining all three countries were OR 1.73 (95% CI: 1.54-1.95) and OR 2.14 (95% CI: 1.77-2.60) for smoking and IM, respectively.

There was a statistically significant negative interaction on the multiplicative scale in both Norway and Italy, and in the pooled analysis (Table 2). In Sweden, the point estimate was even slightly stronger, but did not reach statistical significance likely due to fewer participants (P = 0.06). The estimates remained similar after further adjustment for body size, outdoor activity and level of education (P = 0.008 in pooled analysis).

Table 3 shows the effect of the interaction providing the effect estimates for each of the two exposures variables stratified on the other. The estimates for IM were considerably lower among ever-smokers compared to never-smokers in all three countries. Further, there was no increased risk associated with smoking among participants with a positive history of IM compared to those with no reported history of IM. In fact, the effect estimates for smoking were OR 0.70 (95% CI: 0.32-1.52) in Italy and OR 0.73 (95% CI: 0.30-1.76) in Sweden among those who reported a positive history of IM.

In the group of those who reported both a positive history of IM and to be ever smokers, 71 reported to have contracted IM before smoking initiation, while 89 reported to have contracted it afterwards. Among those who reported to be ever smokers, the effect estimate of IM was higher when IM was contracted before smoking initiation (OR 2.15, 95% CI: 1.32-3.49) compared to when IM was contracted after smoking initiation (OR 1.54, 95% CI: 1.00-2.36). Among those who reported a positive history of IM, the effect estimate for smoking was slightly higher when smoking initiation happened after IM was contracted (OR 1.30, 95% CI: 0.74-2.27) compared to before (OR 0.99, 95% CI: 0.60-1.62).

The direction of RERIs was consistent with a negative interaction on the additive scale in all three countries, but the results did not reach statistical significance (RERI -0.98, 95% CI: -2.05-0.15, *P* = 0.09) (Table 2).

Discussion

We observed a negative interaction on the multiplicative scale between smoking and IM on MS risk, both in the pooled analysis and in the country specific analyses for Italy, Norway and Sweden. Also measured on the additive scale the interaction was negative, although this finding did not reach statistical significance. There was no increased risk of MS associated with smoking among those who had reported IM. The results suggest that smoking and IM affect MS risk in the absence of the other, but that they operate on shared biological pathways.

Studies on the interplay between smoking and measures of EBV are conflicting, and few studies have detected any interaction between these two factors. Previous studies vary in their methodology, study population and assessments of exposure, which may explain some of the discrepancies observed. Several studies have measured anti-EBNA1, which at higher levels may be a marker of an altered immunologic response to EBV associated with a higher MS risk.[1] However, higher levels of anti-EBNA1 do not seem to predict a positive history if IM,[14] and the two measures of EBV infection are independently associated with MS,[5] suggesting that they may reflect different aspects of an EBV-infection. Thus, the results of studies on anti-EBNA1 and smoking do not necessarily compare to our study. Further, a recent study observed a trend towards a negative interaction among younger participants and a trend towards positive interaction among older participants, as defined by age at EBV assessment, although the interactions were not statistically

significant.[4] Still, this could explain why a previous study observed a positive interaction between anti-EBNA1 and smoking, as the EBV assessment was primarily done in older participants compared to other studies.[2] Lastly, the sample size and independency between cases and controls vary between previous studies. As tests for interaction are particularly dependent on sample size, this could also explain the lack of any significant interaction in some of the previous studies.

This study is one of the largest studies on the interplay between smoking, IM and MS, and used randomly selected population-based controls. Findings of the risk factors most consistently associated with MS in earlier studies have been replicated in EnvIMS,[15-17] suggesting that the study is suitable for examining how the risk factors interact. We observed a significant interaction on the multiplicative scale, which may be a natural scale to assess interaction in a logistic regression model, as the model is exponential and thus multiplicative, and because it has been suggested that risk factors for MS operate in a multiplicative manner.[5] Further, the direction of RERIs was suggestive of a negative interaction even on the additive scale, consistent with the findings on the multiplicative scale.

The results of this study indicate competing antagonism, where two exposures compete to affect the outcome.[18] Both exposures were associated with MS risk in the absence of the other, but smoking was no longer associated with MS among those with a prior history of IM in any of the three countries. Similarly, the effect estimates for IM in our study were considerably

lower among ever-smokers compared to never-smokers. This suggests that the two exposures are operating on shared biologic pathways.

There is currently limited evidence on potential pathways that can explain our findings. While it has previously been noted that EBV activation and nicotine metabolism share several molecular pathways,[2] it is not clear whether these are relevant for a subsequent development of MS. Further, nicotine may not be the substance in tobacco smoke that increases MS risk.[19] Smoking and IM have been associated with altered numbers of specific T cells that are likely to be important for the development of MS, including reduced number of regulatory T (Treg) cells[20, 21] and increased number of CD8 T cells.[22, 23] If one or several immunological pathways are important for MS, and those specific pathways could be saturated by either exposure, we would expect to see a negative interaction consistent with our observations.

This study has some limitations. Case-control studies are susceptible to recall bias as the participants are asked to recall prior exposure information and there may be a differential misclassification by disease status. A selection bias due to non-response might also have influenced the estimates of the main effects. However, it is less likely that differential misclassification or bias due to non-response of one risk factor would vary across strata of another risk factor. Thus it is unlikely that these potential biases could fully explain our findings related to the interaction. Further, we had no biological measures of smoking or EBV but relied on self-report. Still, measures of antibodies against EBV may not distinguish between IM positive and IM negative EBV-exposed

persons, and recalled information may therefore currently be the only way to capture a prior IM. Moreover, although cotinine is a biomarker for current smokers, it does not capture prior smoking habits, and former smokers may be misclassified as non-smokers.

In conclusion, we observed a statistically significant negative interaction between smoking and IM. Each of the risk factors was found to be associated with MS, but the effect estimates for smoking was null and for IM markedly lower when the other exposure was present. This suggests that the two risk factors compete to affect the outcome and that they operate on shared biologic pathways.

Competing interests

The authors report no relevant disclosures.

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Table 1. Characteristics of Participants and Risk Factors According to Country

	Norway		Italy		Sweden		Pooled (all countries)	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
No. of subjects, n (%)	953 (35.7)	1717 (64.3)	707 (34.7)	1333 (65.3)	244 (27.5)	644 (72.5)	1904	3694
Year of Birth, y, mean (SD)	1964 (10.6)	1963 (10.8)	1970 (10.1)	1970 (10.7)	1967 (11.5)	1969 (11.5)	1967 (10.8)	1966 (11.3)
Sex (female:male)	2.3:1	2.7:1	1.9:1	2.2:1	2.3:1	3.6:1	2.1:1	2.6:1
Age of disease onset, y, mean (SD)	37.6 (10.2)	37.7 (10.2)*	33.2 (10.1)	33.7 (10.5)*	37.1 (10.7)	35.5 (10.3)*	35.9 (10.4)	35.9 (10.5)
Smoking†, n (%)	545 (58.9)	853 (50.7)	354 (52.2)	510 (40.1)	111 (47.8)	230 (37.3)	1010 (55.0)	1593 (44.6)
- Missing, n (%)	27 (2.8)	35 (2.0)	29 (4.1)	61 (4.6)	12 (4.9)	27 (4.2)	68 (3.6)	123 (3.3)
Infectious mononucleosis†, n (%)	146 (16.7)	136 (8.4)	53 (9.0)	62 (5.5)	43 (20.8)	41 (7.7)	242 (13.2)	239 (6.7)
- Missing, n (%)	27 (2.8)	43 (2.5)	33 (4.7)	68 (5.1)	15 (6.1)	26 (4.0)	75 (3.9)	137 (3.7)

SD: Standard deviation
* Randomly assigned index age based on the distribution of age of onset among cases

Table 2. Interaction Between Smoking and Infectious Mononucleosis (IM) and the Risk of Multiple Sclerosis.

	Cases, n (%)	Controls, n (%)	OR (95% CI)
Pooled (all countries)			
Smoking=0, IM=0	550 (35.0)	1676 (51.5)	1.00 (reference)
Smoking=1, IM=0	793 (50.5)	1340 (41.2)	1.86 (1.62, 2.12)
Smoking=0, IM=1	117 (7.5)	118 (3.6)	3.06 (2.31, 4.03)
Smoking=1, IM=1	111 (7.1)	118 (3.6)	2.93 (2.21, 3.89)
Norway			
Smoking=0, IM=0	217 (27.5)	726 (45.1)	1.00 (reference)
Smoking=1, IM=0	439 (55.6)	748 (46.5)	2.05 (1.69, 2.49)
Smoking=0, IM=1	58 (7.4)	64 (4.0)	2.89 (1.95, 4.27)
Smoking=1, IM=1	75 (9.5)	72 (4.5)	3.35 (2.34, 4.81)
Italy			
Smoking=0, IM=0	250 (43.5)	639 (57.5)	1.00 (reference)
Smoking=1, IM=0	274 (47.6)	413 (37.2)	1.70 (1.37, 2.10)
Smoking=0, IM=1	33 (5.7)	33 (3.0)	2.52 (1.52, 4.18)
Smoking=1, IM=1	19 (3.3)	26 (2.3)	1.83 (0.99, 3.37)
Sweden			
Smoking=0, IM=0	83 (40.3)	311 (58.6)	1.00 (reference)
Smoking=1, IM=0	80 (38.8)	179 (33.7)	1.83 (1.26, 2.65)
Smoking=0, IM=1	26 (12.6)	21 (4.0)	4.78 (2.53, 9.02)
Smoking=1, IM=1	17 (8.3)	20 (3.8)	3.26 (1.63, 6.52)

Measure of interaction on additive scale: RERI (95% CI) =
Pooled: -0.98 (-2.05, 0.15); *P* = 0.09 **Norway**: -0.58 (-2.08, 0.98); *P* = 0.48 **Italy**: -1.39 (-3.08, 0.31); *P* = 0.11 **Sweden**: -2.34 (-5.61, 1.46); *P* = 0.25
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) =
Pooled: 0.52 (0.35, 0.77); *P* = 0.001 **Norway**: 0.57 (0.34, 0.96); *P* = 0.04 **Italy**: 0.43 (0.19, 0.95); *P* = 0.04 **Sweden**: 0.40 (0.15, 1.03); *P* = 0.06
ORs are adjusted for age and sex. For smoking and IM, "0" refers to unexposed and "1" refers to exposed.
IM: Infectious mononucleosis; OR: Odds ratio; CI: Confidence interval; RERI: Relative excess risk due to interaction

Table 3. The Association Between infectious mononucleosis (IM), Smoking and MS Across Strata of Each Exposure

	OR (95% CI) for MS with a history of IM, within strata of smoking		OR (95% CI) for MS with a history of smoking, within strata of IM	
	Smoking=0	Smoking=1	IM=0	IM=1
Pooled (all countries)	3.06 (2.31, 4.03)	1.53 (1.16, 2.03)	1.86 (1.62, 2.12)	0.95 (0.66, 1.37)
Norway	2.89 (1.95, 4.27)	1.65 (1.16, 2.35)	2.05 (1.69, 2.49)	1.15 (0.71, 1.86)
Italy	2.52 (1.52, 4.18)	1.09 (0.59, 2.03)	1.70 (1.37, 2.10)	0.70 (0.32, 1.52)
Sweden	4.78 (2.53, 9.02)	1.70 (0.82, 3.53)	1.83 (1.26, 2.65)	0.73 (0.30, 1.76)

For smoking and IM, "1" refers to exposed and "0" refers to unexposed. The ORs are adjusted for age and sex
OR: Odds ratio; IM: Infectious mononucleosis