

Title: Distinct influence of different vascular risk factors on white matter brain lesions in multiple sclerosis

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ABSTRACT

Objective: To determine if VRF, i.e. smoking, arterial hypertension (HT), dyslipidemia and diabetes, have an effect on MS pathology as measured by MS typical brain lesions, we have compared brain MRIs from MS patients with and without VRF age- and sex-matched.

Methods: Brain MRIs from 5 centres were scored for the presence of Dawson's fingers (DF) and juxtacortical lesions (JCL). A regression model was built to predict the effect of each individual VRF on DF and JCL, considering age and disease duration.

Results: 92 MS cases without VRF and 106 MS with one or more VRF (80 ever-smokers, 43 hypertensives, 25 dyslipidemics and 10 diabetics) were included. Ever-smoking associated with a higher burden of DF ($\text{EXP(B)}=1.29$, 95%CI 1.10,1.51, $p<0.01$) and JCL ($\text{EXP(B)}=1.38$, 95%CI 1.21, 1.57, $p<0.01$). No other VRF had an impact on DF. Dyslipidemia associated with increased JCL ($\text{EXP(B)}=1.30$, 95%CI 1.10, 1.56, $p<0.01$) but HT did not associate with any of the outcomes.

Conclusions: Individual VRF appear to affect MS-specific lesions differently. An increase in MS lesions was mainly seen in smokers, however this VRF is most likely to be present from onset of MS, and other VRF effects may be partly mitigated by treatment. Our findings support that treating VRF and cessation of smoking may be important in the management of MS.

INTRODUCTION

Vascular risk factors (VRF) and comorbidities are associated with worse clinical outcomes in multiple sclerosis (MS)[1]. Higher T2 white matter brain lesions (WML) volumes have been seen in MS patients with concomitant arterial hypertension (HT) and smoking[2,3]. It is not clear whether or not this increase of lesion load is due to a higher burden of MS or vascular WML nor whether the effects of individual VRF on MS WML differ. Several lesion characteristics segregate more clearly with MS, namely juxtacortical U- or S-shaped, ovoid or elongated well-demarcated lesions perpendicular to the wall of lateral ventricle (Dawson's fingers)[4]. In the present study we aimed to determine if there is an excess of these 'MS-specific' lesions on conventional MRI in MS with VRF compared with MS without VRF and to explore the effect of each individual VRF on 'MS-specific' lesions.

MATERIAL AND METHODS

Study design and cohorts

A multicentre retrospective case-control study was set up to compare MS cases with and without VRF. Anonymized clinical and imaging data was obtained from five Magnetic Resonance Imaging in MS (MAGNIMS – www.magnims.eu) network centres: Graz, London, Rome, Barcelona, Amsterdam. Each centre contributed pairs of age- and sex-matched MS cases with and without VRF. The imaging and clinical data were collated centrally in Oxford, according to local ethics regulations, quality controlled and blinded for the visual scoring. MS cases were diagnosed according to the 2010 McDonald criteria and exclusion criteria were presence of previous stroke or high risk for cardioembolic stroke (e.g. atrial fibrillation, valvular heart disease), other neurological diseases or known non-MS brain lesions.

Demographic and clinical data was provided including: sex, age, disease duration and disease modifying treatment (DMT) at the time of the MRI (yes, no and if latter if they have ever had

treatment). The presence or absence of the following VRF was recorded according to previously used definitions[5,6]: 1/ HT (ever); 2/hypercholesterolemia (ever); 3/diabetes mellitus (DM); 4/ self-reported smoking status – positive if patients ever smoked >10 cigarettes a day for at least 6 months (**Table 1**). T1 and T2-Fluid Attenuated Inversion Recovery (FLAIR) – weighted brain imaging sequences obtained from 3 Tesla brain MRIs were provided, with variable sequence protocol depending on the source of the data but of established high quality.

Visual Scoring

A single investigator (RG), blinded to the VRF status, performed visual scoring of the brain MRIs. Inter and intra-rater variability was tested on a subset (N=100) by repeat blind scoring by RG and two other independent blinded evaluators (MJ, GP). MS-like WML recorded were: 1/ the number of Dawson's fingers (defined as well demarcated periventricular lesions, with elongated, ovoid or flame-like shape, perpendicular to the wall of the lateral ventricles), touching the ventricular lining, on axial T2-FLAIR; 2/ number of juxta-cortical lesions (well demarcated lesions that touched the cortex on both axial T2-FLAIR and T1); 3/ U- or S-shaped juxtacortical lesions – a subset of juxtacortical lesions with S or U shapes.

Statistical analysis

Inter-rater agreement was measured by free marginal multi-rater kappa coefficient[7]. A Poisson log-linear regression model (Model 1) was used to determine the effect of the presence of any VRF (yes/no) on the number of Dawson's fingers, considering disease duration. The same model was used to assess the relationship of VRF and the number of juxta-cortical lesions. A similar regression analysis was repeated but each individual VRF was included in the model (Model 2): ever-smoking, HT, dyslipidemia, and DM, as well as disease duration and age, firstly to predict the number of Dawson fingers and secondly the number of juxta-

cortical lesions. Results are presented as the exponentials of the regression coefficients (EXP (B)) and respective confidence intervals (CI). A conservative $p < 0.01$ was used to assess significance. All analyses were performed with SPSS version 25.

Data availability

Raw anonymised data are available for appropriate requests.

RESULTS

Clinical features of the cohorts used for lesion visual scoring and inter-rater agreement for the visual scoring

Data from 201 MS cases (93 without and 108 with VRF) were available, but three cases were excluded due to the presence of confluent lesions. Clinical features of the cohorts included are depicted in Supplemental Table 1. No significant differences were found between the MS with no VRF and the MS with any VRF regarding age, sex, disease duration, MS subtype or DMT. However, hypertensive ($N=43$, mean age 50.6 ± 9.3 years, $p=0.03$) and diabetic ($N=10$, mean age 55.5 ± 7.8 years, $p=0.002$) MS patients were significantly older compared with those without VRF ($N=92$, 47.5 ± 8.3 years).

Substantial inter-rater agreement was found for the number of: Dawson's fingers (0.75), juxtacortical lesions (0.72) and U-S shape juxtacortical (0.65) lesions.

The effects of the presence of VRF on lesions

The number of juxtacortical lesions was 27.0% higher in MS with VRF (Exp (B)=1.27, 95%CI 1.11, 1.44, $p < 0.001$) compared with MS without VRF in a Poisson model adjusting for disease duration (Model 1).

No significant differences between these two groups were found regarding the number of U- or S-shaped juxtacortical lesions nor of Dawson's fingers (Table 1, Model 1).

The effects of the presence of individual VRF on lesions

In a model to predict the number of Dawson's fingers, considering each of the VRF and adjusting for disease duration and age (Model 2), ever smoking associated with a higher burden of Dawson's fingers, while no significant association was found with the other VRF (Table 1, Model 2).

In a similar model to predict the number of juxtacortical lesions, smoking and dyslipidemia were associated with a higher burden of juxtacortical lesions and diabetes with a lower number of these lesions. No association was found between HT and juxtacortical lesion number.

None of the individual VRF was associated with the number of U- or S-shaped juxtacortical lesions (Table 1, Model 2).

All the studied 'MS-specific' lesions significantly increased with disease duration but interestingly a reverse association was seen with age.

To visually illustrate the effects of smoking alone considering the previous results, we removed patients with smoking plus other VRFs to produce a pure 'MS smokers only' group and a 'MS with non-smoking VRF only' group and compared these groups with 'MS without VRF'. Figure 1 shows that with increased disease duration there is an excess of Dawson's fingers in the 'MS smokers only' group compared with the other two groups, and an increase in juxtacortical lesions in both the 'MS smokers only' compared to the other two groups and an increase in the 'MS with non-smoking VRF only' group compared to MS without VRF.

Discussion

This study is novel in studying the effect of different VRF on lesions with features more usually associated with MS, rather than total lesion numbers which does not distinguish between vascular and MS pathology. A key finding of our study is that VRF should not be considered as a composite, as each has a different association with ‘MS-specific’ lesions. Smoking increased both Dawson’s fingers and juxtacortical lesion number, but dyslipidemia only increased the latter. HT showed no effect on MS-like lesions, while DM was associated with a lower number of juxta-cortical lesions.

The documented effect of smoking and hyperlipidemia on ‘MS-specific’ lesions supports a direct influence on MS pathology, possibly through promotion of acute inflammation [2,8–11]. In line with our results, HT [12] and DM [13] and total VRF [5] have failed to show an impact on T2 lesion volume in MS, although MS specific lesion locations was not studied, and individual VRF, including smoking, were not adjusted for. As expected disease duration had a significant effect on lesions but less expected age had an inverse effect when disease duration was included in the model. This interesting observation is in line with our *post mortem* studies showing that the number of active plaques decreases with age at death [14]. DM and age-related cerebrovascular changes may have an unforeseen negative impact on classic MS WML development.

Our results need to be interpreted with caution since the number of patients included in each individual VRF group was low (from 10 in diabetes group to 80 in the smoking group) and no information regarding VRF treatment, that may have an impact on MS WML [2,15] was available. Additionally, VRFs may be present for different lengths of time and be managed more or less effectively, for example smoking usually starts in adolescence before the onset of MS and it is not-treatable by medication, whereas HT, DM and dyslipidemia tend to develop later and are treated, thus smoking may be a higher VRF in our cohort of patients. Finally, our

study may have been underpowered to detect an association between the studied VRF and the less commonly found, but possibly more specific, U- or S-shaped juxtacortical lesions.

Despite these limitations, our study highlights that individual VRF should not be grouped together, that they appear to have an effect on MS-like lesions (not just brain lesions per se), and reinforces the importance of supporting patients in giving up smoking. To better understand the effect of each VRF on specific types of WML, future prospective studies matching age across all groups, and risk grading the individual VRFs (e.g. pack years for smoking, time and control of individual VRFs) are warranted.

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TABLE AND FIGURE

Table 1. Summary of the effect of any VRF (Model 1) and of each individual VRF (Model 2) on the number of Dawson's fingers and juxta-cortical lesions **p<0.01

Parameter	Dawson's fingers			Juxtacortical lesions (JCL)			U-S JCL		
	Exp (B)	95%CI Exp (B)	p	Exp (B)	95%CI Exp (B)	p	Exp (B)	95%CI Exp (B)	p
Model 1									
VRF (N=106)	1.05	0.89,1.22	0.55	1.27	1.11,1.44	<0.01**	0.81	0.57,1.15	0.26
Disease duration	1.03	1.02,1.03	<0.01**	1.02	1.01,1.02	<0.01**	1.02	1.00,1.03	0.03*
Model 2									
Smoking ^a (N=80)	1.29	1.10,1.51	<0.01**	1.38	1.21,1.57	<0.01**	1.14	0.79,1.64	0.47
Hypertension ^b (N=43)	0.86	0.70,1.106	0.17	0.85	0.71,1.01	0.06	0.61	0.36, 1.02	0.06
Dyslipidaemia ^c (N=25)	0.97	0.77,1.23	0.80	1.30	1.10,1.56	<0.01**	0.85	0.48,1.53	0.59
Diabetes ^d (N=10)	0.66	0.41,1.07	0.09	0.34	0.20,0.58	<0.01**	0.35	0.09,1.46	0.15
Disease duration	1.04	1.03,1.04	<0.01**	1.02	1.02,1.03	<0.01**	1.02	1.00,1.04	0.03*
Age	0.97	0.96,98	<0.01**	0.98	0.97,0.99	<0.01**	0.99	0.97,1.02	0.63

a Cases were considered to be smokers if they had ever smoked >10 cigarettes a day for at least 6 months; **b** Hypertension was diagnosed if blood pressure >149/90 mmHg or on antihypertensive treatment; **c** Hypercholesterolemia was diagnosed if total cholesterol >5.0 mmol (193 mg/dl) and/or hypertriglyceridemia (ever) (triglycerides >1.8 mmol/l (150 mg/dl) or on antidyslipidemia treatment; **d** diabetes mellitus was diagnosed according to WHO definitions[6] (diabetes symptoms plus: HbA_{1c} ≥ 6.5% (48mm/mol) or a random venous plasma glucose concentration ≥ 11.1 mmol/l or a fasting plasma glucose concentration ≥ 7.0 mmol/l or two hour plasma glucose concentration ≥ 11.1 mmol/l two hours after 75 g anhydrous glucose in an oral glucose tolerance test) or on antidiabetic treatment

Figure 1. Dawson's Fingers (a) and Juxtacortical lesion (b) counts against disease duration in MS per VRF group: without VRF (blue line), smoking only (grey line) and VRF other than smoking (red line).