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Title: Vascular disease and multiple sclerosis: a post-mortem study exploring their relationships

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Abstract

Vascular comorbidities have a deleterious impact on multiple sclerosis clinical outcomes but it is unclear whether this is mediated by an excess of extracranial vascular disease (i.e. atherosclerosis) and/or of cerebral small vessel disease or worse multiple sclerosis pathology. To address these questions, a study using a unique *post mortem* cohort wherein whole-body autopsy reports and brain tissue were available for interrogation was established. Whole body autopsy reports were used to develop a global score of systemic vascular disease that included aorta and coronary artery atheroma, cardiac hypertensive disease, myocardial infarction and ischemic stroke. The score was applied to 85 multiple sclerosis cases (46 females, age range 39 to 84 years, median 62.0 years) and 68 control cases. Post-mortem brain material from a subset of the multiple sclerosis (n=42; age range 39 to 84 years, median 61.5 years) and control (n=39) cases was selected for detailed neuropathological study. For each case, formalin-fixed paraffin-embedded tissue from the frontal and occipital white matter, basal ganglia and pons was used to obtain a global cerebral small vessel disease score that captured the presence and/or severity of arteriolosclerosis, periarteriolar space dilatation, hemosiderin leakage, microinfarcts, and microbleeds. The extent of multiple sclerosis-related pathology (focal demyelination and inflammation) was characterised in the multiple sclerosis cases. Regression models were used to investigate the influence of disease status on systemic vascular disease and cerebral small vessel disease scores and, in the multiple sclerosis group, the relationship between multiple sclerosis-related pathology and both vascular scores. We show that: 1) systemic cardiovascular burden, and specifically atherosclerosis, is lower and cerebral small vessel disease is higher in multiple sclerosis cases that die at younger ages compared with controls; 2) the association between systemic vascular disease and cerebral small vessel disease is stronger in multiple sclerosis compared with controls; 3) periarteriolar changes, including periarteriolar space dilatation, hemosiderin deposition and inflammation, are key features of multiple sclerosis pathology outside the classic demyelinating lesion. Our data argues against a common primary trigger for atherosclerosis and multiple sclerosis but suggest that an excess burden of cerebral small vessel disease in multiple sclerosis may explain the link between vascular co-morbidity and accelerated irreversibility disability.

Key words: Multiple Sclerosis, cerebral small vessel disease, atherosclerosis

Abbreviations: IS – Ischemic stroke, MI - myocardial infarction; PSD - periarteriolar space dilatation, PHL - periarteriolar hemosiderin leakage, WM - White Matter

Introduction

Multiple sclerosis is a chronic CNS demyelinating disease in which inflammation, blood brain barrier disruption, and neurodegeneration are cardinal features. There is marked heterogeneity in multiple sclerosis clinical severity (Thompson *et al.*, 2018), with some patients reaching clinical disability milestones much sooner than others (Vukusic and Confavreux, 2007; Tutuncu *et al.*, 2013; Confavreux and Vukusic, 2014; McKay *et al.*, 2017). People with multiple sclerosis who have vascular comorbidities, such as diabetes, hypertension, hypercholesterolemia and heart disease, have accelerated disability accumulation, requiring a walking aid sooner (Marrie *et al.*, 2010) and have lower brain volumes than their counterparts without comorbidities (Pichler *et al.*, 2019). The mechanism by which vascular risk factors impact disability in multiple sclerosis is not known.

It is well established that vascular risk factors influence the burden of systemic vascular disease, most commonly atherosclerosis, through a complex interplay of genes and environmental factors (Webber *et al.*, 2012; Qiu and Fratiglioni, 2015; Head *et al.*, 2017). However, whether people with multiple sclerosis have a higher burden of systemic vascular disease compared to people without multiple sclerosis, has been difficult to ascertain. Epidemiological studies use broad case definitions (Marrie *et al.*, 2014) and only a few small studies, showing inconsistent results, have performed arterial imaging in multiple sclerosis patients (Jakimovski *et al.*, 2019). Further, attempts to link vascular risk factors or systemic vascular disease to cerebral small vessel disease have relied on surrogate *in vivo* imaging markers, such as white matter (WM) signal change, perivascular space dilatation, and ischaemic change (Pantoni, 2010; Gouw *et al.*, 2011, Wardlaw *et al.*, 2013*a*, *b*; Cuadrado-Godia *et al.*, 2018), which are difficult to distinguish from imaging signatures related to the pathological processes associated with multiple sclerosis. These limitations have precluded a meaningful assessment of the relationship between systemic vascular disease and cerebral small vessel disease in the multiple sclerosis brain during life.

Post-mortem studies provide an opportunity to dissect the relative contributions of cerebral small vessel disease and multiple sclerosis-related pathology. A recently developed standardised post-mortem scoring system of cerebral small vessel disease has allowed reliable and reproducible assessment of signatures (Skrobot *et al.*, 2016), including myelin rarefaction, small arteriolar changes (i.e. arteriolosclerosis, periarteriolar hemosiderin deposition (PHL)), and presence of ischaemia and microhaemorrhages. Surprisingly, despite the relevance of vascular co-morbidity on clinical outcome and of the blood-brain barrier on the evolution of the multiple sclerosis lesions (Spencer *et al.*, 2018), the extent of cerebral small vessel disease has not been evaluated in multiple sclerosis (Geraldes *et al.*, 2017). Further, how cerebral small vessel disease relates to objective measures of systemic vascular disease in multiple sclerosis remains elusive. Of course, objective measures of systemic vascular disease would be best derived from whole-body autopsy data, which are typically neglected in people with multiple sclerosis who donate their brains for research.

We hypothesised that multiple sclerosis associates with more severe systemic and cerebral vascular disease. To evaluate this, we had access to an internationally unique post-mortem cohort wherein whole-body autopsy reports and brain tissue were available for assessment of systemic vascular disease and cerebral small vessel disease, respectively, so that their severity and relationships could be explored in multiple sclerosis and controls. First, we show that multiple sclerosis has vascular changes that extend beyond the venule, affecting the arteriole, even in the absence of vascular comorbidity. Second, we provide evidence of a complex, age-dependent relationship between systemic vascular disease and cerebral small vessel disease that differs in multiple sclerosis compared to controls. Finally, we demonstrate that an excess burden of cerebral small vessel disease in multiple sclerosis may explain the link between vascular co-morbidity and accelerated irreversible disability.

Methods

Material and methods

Study population

A human autopsy cohort of pathologically confirmed multiple sclerosis cases and matched controls from the Oxford Brain Bank (1971-2007) were selected for study (**Fig.1** for detailed

information on cohort selection). Each case had comprehensive whole-body autopsy reports (including the vascular system) and post-mortem brain material to study the nature and extent of systemic vascular disease and cerebral small vessel disease, respectively (**Table 1 and Fig. 1**). None of the multiple sclerosis cases were exposed to disease modifying treatments. The study was approved by the local ethics research committee (REC 15/SC/0639) and complied with UK Human Tissue Act regulations.

Assessment of systemic vascular disease from autopsy reports

Autopsy report data was reviewed to develop a standardised questionnaire that captured routinely collected systemic vascular disease-related pathology elements (**Fig. 1)**. Two independent raters (ME, RG) applied this questionnaire to score the extent of atherosclerosis (in aorta, coronary and cervical (carotid and vertebral) arteries), and end organ damage (presence of left ventricular hypertrophy (LVH), myocardial infarction (MI), macroscopic large vessel ischaemic stroke (IS), and/or renal hypertensive disease (RH)). Disagreements in any score were adjudicated by a third, independent rater (GD). Atheroma severity assessment was based on commonly used descriptors in the autopsy reports to devise a semi-quantitative score: not present (0) – 'normal', 'free of atheroma'; mild (0.33) – 'slight', 'expected atheroma for age', 'fatty infiltration only'; moderate (0.66) – 'non-significant stenosis' i.e. luminal narrowing of 10-49%; severe (1.0) – 'occlusion', 'significant narrowing', and/or 'ulcerated plaque/thrombus'. The severity of intracranial (Circle of Willis) large artery atherosclerosis/atheroma was assessed using a semi-quantitative score (from 0 to 3) (Esiri *et al.*, 1997).

LVH was scored by a composite of heart weight and documented evidence of LVH (Grajek *et al.*, 1993), as follows: 0 - < 400 g and no LVH present; 0.5 - < 400 g and mild to moderate LVH present or LVH not described but heart weight > 400 g; and 1 - > 400 g and mild to moderate LVH present or with severe LVH documented independent of heart weight.

Neuropathological evaluation

Assessment of cerebral small vessel disease

Brain material from a subset of multiple sclerosis cases (n=42, age range 39 to 84, median age 61.5 years, median disease length of 20 years) and controls (n=39, age range 40 to 85,

median age 58.0 years) was used to measure cerebral small vessel disease using established methods (Skrobot *et al.*, 2016) (**Fig. 1**). Specifically, adjacent formalin-fixed, paraffin-embedded 10 μm-thick adjacent sections, taken from four brain regions, were used as follows: frontal WM at the level of the frontal horn of the lateral ventricle; occipital WM at the level of the occipital horn; basal ganglia (lenticular nucleus or caudate nucleus); and pons at the level of the facial colliculus. Sections from each brain region were stained with haematoxylin-eosin and immunostained with primary antibodies for myelin, microglia, and smooth muscle actin (**Supplemental Table 1**). Sections were scanned at 200X magnification using Aperio Scanoscope and images were analysed with ImageScope software. Cerebral small vessel disease was assessed in the available WM areas of the frontal and occipital tissue sections 2 mm from the ventricular surface and in the entire area of the pons and basal ganglia. Cerebral small vessel disease features were scored according to recently published criteria (Skrobot *et al.*, 2016): 1. arteriolosclerosis, 2. fibrinoid necrosis and microaneurysms, 3. periarteriolar space dilatation (PSD), 4. PHL, 5. microinfarcts/lacunar infarcts, 6. large infarcts, and 7. microhaemorrhages (**Fig. 1**). Features 1 through 4 were scored if present in three or more clearly delineated, separate arterioles found in a cross-sectional orientation, and with a lumen diameter of 20 - 150 μm. Arterioles were identified by morphological features on haematoxylin-eosin, and positive immunolabelling with anti-smooth muscle antibody to delineate a continuous wreath of concentric smooth muscle (Keith *et al.*, 2017).

Periarteriolar inflammation was scored as present when five or more white blood cells were observed in the periarteriolar space of three or more arterioles in the area assessed. Scores were obtained by one rater (RG) with inter-rater reliability assessed in a subset of cases (n=20) by an experienced neuropathologist (ME) and a third rater (DJ). All raters were blinded for systemic vascular disease scoring results. For the multiple sclerosis cases, only non-plaque tissue was assessed (i.e. > 1 mm from plaque edge).

Quantification of demyelination and inflammation in multiple sclerosis

Total plaque and active plaque numbers were used as outcome measures of multiple sclerosis-related focal demyelination and inflammation. Multiple sclerosis plaques were defined as well-demarcated areas of complete myelin loss assessed by proteolipid protein. Plaques from each brain region were counted and summed to obtain a total plaque number

per case. To evaluate the reliability of plaque number as a measure of global multiple sclerosis-related focal demyelination, the total number of plaques was related to additional measures of plaque burden including: the macroscopic global WM burden (semi-quantitative score (mild, moderate, severe) applied to 1 cm thick formalin-fixed brain slices); and the percentage of tissue occupied by plaque (plaque area obtained from frontal/occipital WM divided by total WM area sampled, and from the pons/basal ganglia divided by total area sampled). The stage of demyelination was determined for each plaque using CD68 immunohistochemistry with active and mixed active/inactive plaques contributing to the total active plaque score (Kuhlmann *et al.*, 2017).

Myelin rarefaction, defined as weakly stained/pale and loose appearing myelinated WM fibres (Skrobot *et al.*, 2016), was scored 2 mm away from the periventricular lining in all sampled brain areas in multiple sclerosis (outside plaque) and control cases. A positive score was attributed to the presence of WM loosening or pallor as detected by proteolipid protein immunohistochemistry. A total myelin rarefaction score (scored 1 mm outside plaque in the multiple sclerosis cases) was obtained for each case by combining the myelin rarefaction scores of the four studied brain regions.

Statistical analysis

The distribution of the data and model assumptions were assessed analytically and graphically. Chi-squared or Fisher's exact tests, correlation coefficients (r) and parametric and non-parametric tests were applied, as appropriate. Continuous data are presented as mean ± standard deviation (SD) where normally distributed and as median and interquartile range (IQR) or range where non-normally distributed. Categorical data is presented as counts or percentages. Inter-rater agreement was measured by free marginal multi-rater kappa coefficient (Randolph, 2005) and interpreted as defined below (Landis and Koch, 1977): 0 – less than chance agreement, 0.01-0.2 – slight agreement, 0.21-0.4 – fair agreement, 0.41-0.6 – moderate agreement, 0.61-0.8 – substantial agreement, 0.81-0.99 – almost perfect agreement.

Individual vascular-related variables were expected to correlate highly with each other making Type 1 errors likely. Therefore, pre-determined systemic vascular disease composite variables were pre-defined as follows (**Fig. 1**):

- atheroma score – average of individual atheroma variables from the different scored arteries.
- end organ damage score – average of individual end organ variables (i.e. MI)
- Systemic vascular disease score (SysVasc score) – average of Atheroma and End Organ Damage Scores

Individual vascular-related variables that were recorded in more than 80% of the autopsy reports, and had substantial or above inter-rater agreement were used to develop the above composite variables.

Total scores for each of the pathological cerebral small vessel disease features were obtained by adding the scores derived from each of the four brain regions analysed. The sum of each of the total scores contributed to a global cerebral small vessel disease index (Global cSVD Index) (**Fig. 1**).

Both inter-rater agreement and internal consistency (as measured by Cronbach's alpha), were used as reliability measures of the SysVasc Score and the Global cSVD Index, while the validity of these measures was assessed by determining their association with age at death, vascular causes of death and, for the Global cSVD Index, WM myelin rarefaction.

Regression plots and linear models were used to determine the influence of multiple sclerosis on vascular scores, adjusting for age at death. To account for a possible bias in our control group towards higher SysVasc scores, a sensitivity analysis excluding death due to vascular causes, namely MI or IS was undertaken. Binary regression models were used to explore the effect of multiple sclerosis on each individual cerebral small vessel disease pathology. Regression models were fitted to evaluate the influence of multiple sclerosis on the Global cSVD Index whilst controlling the SysVasc score on groups stratified above and below the median age at death. This stratified analysis was preferred to a whole group model - including age at death and SysVasc - since a high collinearity between these variables was expected and differences between young and old at death multiple sclerosis pathology could be anticipated (i.e. older cases with less active plaques) (Frischer *et al.*, 2015; Yates *et al.*, 2015). Poisson loglinear models were used to evaluate the influence of SysVasc Score and Global cSVD Index on count variables with a Poisson distribution (i.e. total number of plaques, number of active plaques), in the above and below median age-at-death groups.

Results are presented as regression coefficients (B) or their exponentials (EXP(B)); 95% confidence intervals (CI) are shown. All tests of hypothesis were carried out using two-sided tests and p-values of less than 0.05 were considered significant. SPSS version 23 and GraphPad Prism 7 were used to generate statistical analyses and plots.

Sample size calculation

In the absence of previous studies assessing the burden of systemic and cerebral vascular disease in multiple sclerosis, the sample calculation for the comparison of vascular changes between multiple sclerosis and control cases took into account the confidence intervals (CI) of each of the individual cerebral small vessel disease pathologies included in the most recent consensus paper on cerebral small vessel disease scoring (Skrobot *et al.*, 2016), using G*Power calculator version 3.1. We aimed to include a minimum of 38 multiple sclerosis and 38 control cases.

Data availability

Raw data are available upon appropriate request.

Results

Cohort characterisation

A total of 111 multiple sclerosis cases were available for study and compared to 90 controls, which were selected on the basis of being age- and sex-matched to the multiple sclerosis group. Of these 201 cases, a total of 85 cases of multiple sclerosis (39 males, 46 females; age range of 39 to 84 years; median = 62.0 years) (**Table 1**) and 68 control cases (34 males, 34 females; age range of 40 to 85 years, median = 58.0 years) (**Table 1**), were selected for further study, as outlined in **Fig. 1**. Given the impact of age at death on pathological outcomes as previously described (Frischer *et al.*, 2015; Yates *et al.*, 2015), all cases were

segregated into groups above and below the median age (i.e. 60 years), and are hereafter referred to as the 'older' and 'younger' age at death groups, respectively.

Systemic vascular disease assessment

Age and sex did not differ between groups, however year of death did: multiple sclerosis = 1984±9.6; controls = 1988.9±9.7 (p=0.007). As for causes of death, infection was more frequent in multiple sclerosis (23.5%) compared to controls (5.3%) (p=0.001), while cancer (p=0.004), MI, or IS deaths (p=0.048) were more common in controls (**Table 1**). Other causes of death did not differ between groups (**Supplemental Table 2**).

Cerebral small vessel disease assessment

There were no differences between multiple sclerosis and control cases in regards of age, sex, year of death or post-mortem delay (multiple sclerosis = 54.8 (±31.2) hours, controls = 45.8 (±23.9) hours, p=0.152) in the subset of cases used to assess cerebral small vessel disease (**Table 1**). Cancer was a less common cause of death, and infection more common, in multiple sclerosis cases compared with controls (p <0.001 for both comparisons) (**Table 1**). Deaths due to IS and MI were more common in those older than 60 years of age – a feature common to multiple sclerosis (3/22 cases) and controls (5/18 cases). In two cases of multiple sclerosis, death was attributed to multiple sclerosis specifically. Both died before the age of 60 years.

Table 1. Demographic features of multiple sclerosis patients and controls

	Systemic vascular disease assessment					**Systemic and cerebral small vessel disease assessment**			
	Multiple Sclerosis (n=85)	**Controls** (n=68)	**p**	**Multiple Sclerosis without MI or IS deaths** (n=79)	**Controls without MI or IS deaths** (n=56)	**p**	**Multiple Sclerosis** (n=42)	**Controls** (n=39)	**p**
Female n (%)	46 (54.1)	34 (50.0)	0.630	41(51.9)	29 (51.8)	0.99	57.1 (24)	56.4 (22)	0.95
Mean age at death in years (SD)	61.1 (± 11.9)	58.5 (± 12.3)	0.190	60.9 (12.0)	57.7(12.5)	0.18	60.6 (±12.9)	58.8 (±12.9)	0.53
Median age at death in years (range)	62.0 (39-84)	58.0 (40-85)		61.0 (39-84)	57 (40-85)		61.5 (39-84)	58.0 (40-85)	
Year at death median (IQR)	1984 (9.6)	1988 (9.7)	0.007**	1984 (9.4)	1989 (9.9)	0.006	1981 (20)	1984 (16)	0.493
Causes of death n (%)									
Pulmonary embolism/ DVT	10 (11.8)	6 (8.8)	0.61	10 (12.7)	6 (10.7)	0.73	11.9 (5)	10.3 (4)	ns
Infection	42 (49.4)	12 (17.6)	0.001**	42 (53.2)	12 (22.2)	<0.001**	40.5 (17)	12.8 (5)	<0.001**
Cancer	5 (5.9)	16 (23.5)	0.004**	5 (6.3)	16 (28.6)	0.001**	0	28.2 (11)	<0.001**
MI and/or IS	6 (7.1)	12 (17.6)	0.048	NA	NA	0.06	7.1 (3)	17.9 (7)	ns
Multiple Sclerosis	3 (3.5)	NA		3 (3.8)	NA		4.8 (2)	NA	
Other	19 (22.3)	22 (32.4)	0.27	19 (24.1)	22 (39.3)	ns	35.7 (15)	30.8 (12)	ns

DVT – deep venous thrombosis, IQR – interquartile range, IS – Ischemic stroke, MI – Myocardial infarction, NA - not applicable/not available, SD – standard deviation. Mann-Whitney test for age at death, disease duration, year at death and Chi-square test for sex and causes of death *p<0.05, **p<0.01

SysVasc Score: a tool to assess systemic vascular disease from autopsy reports

Causes of death and scores for aorta and coronary atheroma categories did not differ between pathologists (**Supplemental Table 3**). Substantial or greater agreement was found between raters scoring coronary (Cohen's kappa 0.7) and aorta atheroma (Cohen's kappa 0.7), MI (Cohen's kappa 0.7), IS (Cohen's kappa 0.6) and LVH (Cohen's kappa 0.8). Given that information on cervical artery atheroma was not systematically recorded, and as inter-rater agreement on renal hypertensive disease was low (Cohen's kappa 0.3; likely a result of other renal pathologies such as infection and hydronephrosis confounding interpretation), these variables were excluded. Cronbach's alpha was 0.76 for the 2 items of Atheroma Score (aorta and coronary atheroma) and 0.65 for the 5 items of SysVasc Score (aorta, coronary atheroma, MI, IS, LVH); as such, these scores were used as a global measure of atheroma and systemic vascular burden, respectively (**Figure 1**). By comparison, Cronbach's alpha was 0.4 for the End Organ damage Score (MI, IS, LVH) (correlations between the individual variables of the SysVasc Score are included at **Supplemental Table 4**). For this reason, MI, IS and LVH were presented separately and the predefined End Organ Damage Score was not used as a measure of overall end organ damage.

SysVasc score correlated with age at death ($r=0.37$, $p<0.001$) (**Figure 2 C**). Further, SysVasc score was higher in all cases where the cause of death related to vascular disease (i.e. MI, IS and/or pulmonary embolism/deep venous thrombosis) compared with those where death was not related to vascular disease (Sys Vasc score in vascular-related deaths = 0.51± 0.04 versus non-vascular-related deaths = 0.26± 0.03, $p<0.01$) (**Figure 2 A**).

Systemic vascular disease in multiple sclerosis and controls

Individual systemic vascular disease features

Moderate-to-severe atheroma and LVH was found in approximately one third of multiple sclerosis cases (atheroma: aorta 29.4%, coronary 34.1%; LVH 28.2%), while MI (15.3%) and large artery IS (8.2%) were less common. LVH was less common in multiple sclerosis compared to control cases, whereas MI and IS did not differ between groups (**Table 2**). To determine the influence of multiple sclerosis on the presence of LVH, a binary regression model was applied to cases without MI or IS, which revealed that controls were 3.6-fold more likely to have LVH than multiple sclerosis cases, after adjusting for age at death and

sex (EXP(B)=3.6, 95%CI 1.7,7.8, p=0.001). Atheroma scores did not differ between groups in the univariate analysis (**Table 2**).

Table 2. Systemic vascular disease in multiple sclerosis cases and controls

	Multiple sclerosis (n=85)	**Controls (n=68)**	**p**	**Multiple Sclerosis without MI and/or IS death (n=79)**	**Controls without MI and/or IS death (N=56)**	**p**
Aorta atheroma n **(%)**			0.2			0.23
Not present	20 (23.5)	13 (19.1)		20 (25.3)	12 (22.4)	
Mild	30 (35.3)	21 (30.9)		30 (38.0)	19 (33.9)	
Moderate	13 (15.3)	19 (27.9)		9 (11.4)	14 (25.0)	
Severe	12 (14.1)	11 (16.2)		12 (15.2)	8 (14.3)	
NA	10 (11.8)	4 (5.9)			3 (5.4)	
Aorta atheroma score mean(SD)	0.41 (± 0.34)	0.48 (± 0.34)	0.19	0.39(±0.34)	0.44 (± 0.33)	0.33
Coronary atheroma n(%)						
Not present	30 (35.3)	21 (30.9)	0.81	30 (38.0)	19 (33.9)	0.81
Mild	26 (30.6)	21 (30.9)		25 (31.6)	19 (33.9)	
Moderate	17 (20.0)	12 (17.6)		17 (21.5)	10 (17.9)	
Severe	12 (14.1)	14 (20.6)		7 (8.9)	8 (14.3)	
Coronary atheroma score mean(SD)	0.37 (± 0.35)	0.42 (± 0.37)	0.43	0.3 (±0.32)	0.37 (± 0.34)	0.58
Atheroma score mean (SD)	0.39 (± 0.31)	0.45 (± 0.31)	0.25	0.36(±0.29)	0.41 (±0.31)	0.41
Myocardial infarction n(%)	13 (15.3)	10 (14.7)	0.83	8 (10.1)	5 (8.9)	0.82
Ischaemic stroke n (%)	7 (8.2)	9 (13.2)	0.36	6(7.6)	4 (7.1)	0.92
LVH n (%)			<0.01**			<0.01*
Not present	61 (71.1)	31(45.6)		59 (74.7)	27 (48.2)	
Mild to moderate	7(8.2)	10 (11.8)		6 (7.6)	10 (17.9)	
Severe	17 (20.0)	26(38.2)		14 (17.7)	18(32.1)	
NA		1 (1.5)			1 (3.6)	0.03*
Heart weight mean (SD)	356 (± 82.3)	406 (± 120)	<0.01**	352 (±80.9)	388.6 (± 112.3)	
SysVasc Score mean (SD)	0.27 (± 0.24)	0.35 (±0.24)	0.06	0.25(±0.22)	0.29 (±0.23)	0.17

SD – standard deviation, LVH – left ventricular hypertrophy. Chi-square test for presence of MI and IS; Mann-Whitney test for Atheroma and SysVasc Scores;

Kruskal Wallis test for all other variables *p<0.05, **p<0.01

SysVasc and atheroma scores

SysVasc scores correlated with age at death in multiple sclerosis (r=0.59, p<0.001), but not in controls (r=0.19, p=0.18) (**Figure 2 C**). Considering age at death, multiple sclerosis cases had a lower burden of SysVasc compared to controls (B=-0.09, 95%CI -0.16,-0.02, p=0.01), with similar results being found after exclusion of cases who died of MI or IS (B=-0.08, 95%CI -0.146,-0.005, p=0.03). The relationship between age at death and SysVasc differed in multiple sclerosis compared to controls (**Figure 2 C and Supplemental Table 5 for full model results**). Similar results were found after excluding MI and IS as causes of death (**Supplemental Table 5**). SysVasc score was lower in multiple sclerosis (median=0.08, interquartile range=0.25) than in control (median=0.33, interquartile range=0.42) when death occurred at a young age (≤60 years), whereas no significant differences were seen between multiple sclerosis (median=0.33, interquartile range=0.35) and controls (median=0.33, interquartile range=0.42) when death occurred at older ages (>60 years).

Regression of the Atheroma Score on age at death in cases with and without MI and IS yielded a similar pattern of results (**Supplemental Figure 1 and Supplemental Table 5**).

Cervical and intracranial large artery atheroma

Comparing cases of multiple sclerosis to control, no difference emerged in atheroma scores for the cervical arteries (34 multiple sclerosis cases, 27 controls, EXP(B)=1.03, 95%CI 0.25,4.15, p=0.97, adjusting for age at death) or intracranial large arteries (34 multiple sclerosis cases, 30 controls, (EXP(B) = 2.6, 95%CI 0.82, 16.4, p=0.09, adjusting for age at death).

Global cSVD Index: a tool to assess cerebral small vessel disease

Reliability between raters was good for most variables, i.e. arteriolosclerosis (Cohen's kappa 0.7), perivascular hemosiderin leakage (Cohen's kappa 0.88), and perivascular space dilatation (Cohen's kappa 0.46), and was fair for microbleeds and infarcts (Cohen's kappa 0.35). Cronbach's alpha indicated good internal consistency (0.61) for the variables included in the Global cSVD Index (total arteriolosclerosis, total PSD, total PHL, total fibrinoid necrosis, total infarcts, and total microbleeds).

The Global cSVD Index ranged from 1 to 11 in multiple sclerosis cases (median 5.5, IQR 1-7.25) and from 0 to 12 in controls (median 4 IQR 1-7). In support of the score's validity, the Global cSVD Index increased with age at death in the whole cohort (n= 81, r=0.39, p<0.001) (**Figure 2 D**). It was also higher in multiple sclerosis cases and controls where death was attributable to vascular disease (n=19, median 8, IQR 3-10) compared to those that died of the "other" causes (n=27, median 3, IQR 2-6), p=0.02) even after adjusting for age at death (B =2.12, 95%CI 0.15,4.1, p=0.04) (**Figure 2 B**), and associated with diffuse myelin rarefaction (r=0.38, p=0.001).

Cerebral small vessel disease in multiple sclerosis and controls

Individual cerebral small vessel disease features

PSD (**Figure 3 A-C**), arteriolosclerosis, PHL(**Figure 3 F**), and microbleeds were the most commonly observed pathological features, whereas microinfarcts were rarely seen, both in multiple sclerosis or control cases. Macroinfarcts and fibrinoid necrosis/microaneurysms were only observed in controls (**Supplemental Table 6**). Multiple sclerosis cases were four times more likely to have PSD (EXP(B)=3.9, 95%CI 1.17,13.2, p=0.03) and PHL (EXP(B)=4.14, 95%CI 1.55,11.0, p=0.005), but microinfarcts (EXP(B)=0.11, 95%CI 0.01, 1.03, p=0.05) were more common in controls. A diagnosis of multiple sclerosis did not influence arteriolosclerosis (EXP(B)=1.02, 95% CI 0.41, 2.56, p=0.96) or microbleeds (EXP(B)=0.83, 95%CI 0.32,2.14, p=0.7).

Global cSVD Index considering SysVasc

Overall Global cSVD Index did not differ between multiple sclerosis cases and controls (median 5.5, IQR 1-7.25 versus 4 IQR 1-7, p=0.146). However, the relationship between the Global cSVD Index and the SysVasc Score differed between groups: a positive correlation emerged between the two in multiple sclerosis (r=0.46, p=0.002), but not in control (r=0.22, p=0.17) (**Figure 4 A**).

A stratified analysis above and below the median age at death (60 years) was performed, a strategy justified by the observation of a different relationship between age at death and SysVasc Score in multiple sclerosis compared with controls. In cases with younger age at death (≤60 years) multiple sclerosis disease status impacted on the Global cSVD Index

(B=2.03, 95%CI0.14, 3.92, p=0.036), as demonstrated by the higher regression line in multiple sclerosis compared with controls (**Figure 4 B and Table 3**). In contrast multiple sclerosis disease status did not impact the Global cSVD Index (B =-0.50, 95%CI -2.5, 1.6, p=0.63), in cases with an older age at death (>60 years) (**Figure 4 C and Table 3**).

Table 3. Numerical results of the multivariate linear regression analysis to predict Global cSVD Index considering the SysVasc score above and below the median age at death

Model	Parameter	B	95%CI B	p
Age <=60years	**Intercept**	2.03	0.34,3.72	0.020*
	Multiple Sclerosis	2.03	0.14, 3.93	0.036*
	Systemic VD score	3.69	-0.39,7.77	0.075
F –statistic: 3.1 on 2 and 38 DF, p=0.056				
Age >60years	**Intercept**	4.46	2.24,6.68	<0.001**
	Multiple Sclerosis	-0.50	-2.61,1.61	0.634
	Systemic VD score	3.96	-0.34, 8.26	0.070
F –statistic: 1.75 on 2 and 37 DF, p=0.189				

DF – degrees of freedom, coefficient of determination (R^2) Model (age <=60) = 0.140, R^2 Model (age >61) = 0.09; **p<0.01,* p<0.05

Periarteriolar inflammation and characteristic PSD

In multiple sclerosis, peri-arteriolar inflammation outside plaques was higher in at least one brain region compared with control (76.2% versus 59.0%, respectively, p=0.01) (**Figure 3G**) . Multiple sclerosis cases were 3.4 times more likely to have periarteriolar inflammation compared with controls, adjusted for age at death, sex, and SysVasc score (EXP(B)= 3.44, 95%CI 1.51, 7.83, p=0.003).

In multiple sclerosis, periarteriolar inflammation correlated with PSD (r_s=0.27, p=0.01) only, with no association found between periarteriolar inflammation and any other feature of cerebral small vessel disease. Furthermore, enlarged perivascular spaces more commonly had clearly demarcated limits and contained strands of fibrous material, resembling veils, in multiple sclerosis than did controls in the in frontal WM (multiple sclerosis: 7/42 cases, 16.6% versus controls: 2/39 cases, 5.1%), and in the occipital WM (multiple sclerosis: 12/42, 23.8% versus controls: 2/39, 5.1%) (**Figure 3 D, E**). A binary regression model showed that multiple sclerosis disease status (EXP(B)=6.59, 95%CI 1.85,23.48, p=0.004) was associated with the presence of 'periarteriolar veils' in the frontal or occipital WM considering age at death and SysVasc.

Characterisation of multiple sclerosis-related plaques

The vast majority of multiple sclerosis cases (38/42, 90.5%) had one or more plaques in each of the four sampled brain regions, with a median of five plaques (range 0-25) plaques. Plaques were more commonly found in the pons and frontal WM (**Supplemental Table 7**). The total number of plaques was strongly correlated with the percentage of total plaque area (r=0.822, p<0.001) and also associated with the macroscopic WM lesion burden assessment (r=0.49, p=0.003).

Relationships between cerebral vascular disease and multiple sclerosis-related pathology

The relationships between Global cSVD Index and the total number of plaques and of active plaques were assessed in above and below the median age at death groups, adjusting for SysVasc score (**Table 4A**). Global cSVD Index increased the total number of plaques (EXP (B)=1.10, 95%CI 1.05,1.15, p<0.001) and the number of active plaques (EXP(B)=1.15, 95%CI 1.09,1.21, p<0.001) also increased: an observation restricted to the in the younger age at death

(≤60 years) group (**Table 4A**). In this model, as SysVasc score increased the total plaque number and the number of active plaques decreased in both age groups.

Table 4. Relationships cerebral small vessel disease and multiple sclerosis related pathology
**p<0.01,* p<0.05

		Total number of plaques			Number of active plaques		
A. Poisson loglinear regression stepwise model							
		EXP B	**95%CI**	**p**	**EXP B**	**95%CI**	**p**
Age ≤60	SysVasc Score	0.24	0.10,0.55	<0.001**	0.29	0.12,0.73	0.009*
	Global cSVD Index	1.10	1.05,1.15	<0.001**	1.15	1.09,1.21	<0.001**
		Likelihood Chi-square – 24.9, 2 on 17 DF, p<0.001			Likelihood Chi-square – 27,6, 2 on 17 DF, p<0.001		
Age >60	SysVasc Score	0.11	0.04,0.28	<0.001**	0.04	0.01,0.14	<0.001**
	Global cSVD Index	1.07	0.97,1.18	0.153	0.97	0.86,1.10	0.64
		Likelihood Chi-square – 22.1, 2 on 19 DF, p<0.001			Likelihood Chi-square – 35.6, 2 on 19 DF, p<0.001		
B. Summary of the exponential of the regression coefficients individual cSVD pathologies, adjusted for age at death and SysVasc in separate Poisson models							
		EXP B*	**95%CI**	**p**	**EXP B***	**95%CI**	**p**
Age ≤60	**Arteriolosclerosis**	1.64	1.14,2.35	0.007*	2.02	1.32,3.11	0.01*
	PSD	1.63	1.21,2.19	0.001**	1.47	1.03,2.09	0.03*
	PHL	1.48	1.08,2.01	0.013*	2.28	1.54,3.39	<0.001**
	Periarteriolar inflammation	1.61	1.17,2.23	0.004**	1.51	1.04,2.21	0.03*
Age >60	**Arteriolosclerosis**	0.75	0.46,1.22	0.25	0.55	0.28,1.08	0.08
	PSD	1.77	0.94,3.32	0.08	1.36	0.57,3.23	0.48
	PHL	1.63	1.01,2.64	0.04*	1.19	0.65,2.18	0.56
	Periarteriolar inflammation	1.67	1.05,2.65	0.03*	1.42	0.80,2.51	0.23

The relationship between the total number of plaques and the number of active plaques individual and the individual cerebral small vessel disease pathologies and was explored (**Table 4B**). The number of plaques was higher in cases with PSD (EXP(B)=1.56, 95%CI 1.22, 2.01, p=0.001), PHL (EXP(B)=1.47, 95%CI 1.15,1.88, p=0.002), and periarteriolar inflammation (EXP(B)=1.56, 95%CI 1.19,2.02, p=0.001). While no relationship was found between total plaque number and arteriolosclerosis (EXP(B)=1.27,95%CI 0.98,1.64, p=0.06), there was a positive relationship between the number of active plaques and arteriolosclerosis (EXP(B)=1.45, 95%CI 1.07,1.97, p=0.016). The number of active plaques also related to PSD (EXP(B)=1.39 95%CI 1.03,1.88, p=0.03), PHL (EXP(B)=1.71, 95%CI 1.26,2.34, p=0.001) and periarteriolar inflammation (EXP(B)=1.36, 95%CI 0.99,1.86, p=0.05). The relationships between vascular disease and multiple sclerosis-related pathology were driven by cases where death occurred at a young age (≤60 years).

Discussion

The dynamic relationship between vascular disease and multiple sclerosis may be a key determinant of multiple sclerosis heterogeneity. By studying an internationally unique cohort, for which whole-body autopsy reports and post-mortem tissue were available, we were able to devise robust and valid methods to assess systemic vascular disease (SysVasc score) and its relationship to cerebral small vessel disease in multiple sclerosis and non-neurological controls. Our data show that systemic cardiovascular burden, and specifically atherosclerosis, is lower, and cerebral small vessel disease higher, in younger multiple sclerosis cases compared with age-matched controls. The impact of systemic vascular disease on cerebral small vessel disease is stronger in multiple sclerosis compared with controls. Further, we demonstrate that periarteriolar changes, including PSD, PHL and inflammation, are pathological signatures of multiple sclerosis outside the classic demyelinated plaque. Overall, our findings shed light on the complex interaction between multiple sclerosis and vascular disease, and on how this interaction is affected by age at death.

SysVasc Score: a tool to assess systemic vascular disease from autopsy reports

Traditional post-mortem studies in multiple sclerosis only have access to brain material without whole body autopsy findings. The Oxford Brain Bank has a rich cohort of post-mortem multiple sclerosis and control cases wherein whole-body autopsy reports and brain material were available for study making this a rare resource. We created a reliable method for extracting autopsy report data to derive a systemic vascular disease (SysVasc) score. The SysVasc score closely associated with age and the presence of death from vascular causes, supporting its validity. Previous studies have quantified coronary and aorta atheroma burden from autopsy reports in the general population (Thej *et al.*, 2012; Webber *et al.*, 2012). However, a global cardiovascular score considering both atheroma and end-organ vascular related burden has not previously been proposed. The SysVasc score proved to be useful in comparing cardiovascular disease burden between multiple sclerosis and non-multiple sclerosis cases and may be a simple and useful tool to evaluate the relationship between SysVasc and brain pathology, not only in multiple sclerosis but also in other neurological disorders.

Systemic vascular disease differs in multiple sclerosis compared to controls

In our multiple sclerosis cases, a lower burden of systemic vascular disease was observed, with less severe atherosclerosis and LVH compared with controls. Our multiple sclerosis cohort did not demonstrate more MI or large artery IS compared with controls. Although an association between multiple sclerosis and cardiovascular disease has been reported in epidemiological studies (Christiansen, 2012; Marrie *et al.*, 2014, 2019; Murtonen *et al.*, 2018), these studies captured large diagnostic categories that do not differentiate between atherosclerosis and embolic vascular disorders (Christiansen *et al.*, 2010; Christiansen, 2012; Roshanisefat *et al.*, 2014). The few studies that have assessed surrogate markers of atherosclerosis (Ranadive *et al.*, 2012) or structural systemic artery changes *in vivo* (Belov *et al.*, 2018) have failed to provide convincing evidence that multiple sclerosis is linked to a higher burden of atherosclerosis.

The severity of atherosclerosis was highly dependent on age at death in our multiple sclerosis cohort. Atherosclerosis was less commonly seen in multiple sclerosis cases where death occurred before the age of 60, which is in contrast to cases where death occurred at a later age. These findings argue against a common underlying mechanism predisposing for both disorders. In highly active multiple sclerosis wherein cases die at younger ages, one must consider the presence of protective inflammation that acts against the development of atheroma and LVH. We speculate that exposure to high amounts of myelin degradation products may induce a dynamic regulation of macrophage differentiation, leading to increased atheroma clearance in the systemic arteries (Grajchen *et al.*, 2018). However, a lower prevalence of hypertension (Marrie *et al.*, 2014; Simpson *et al.*, 2014) and non-accounted for treatments - including a better control of vascular risk factors due to surveillance bias - are also plausible explanations for the observed lower SysVasc Scores observed in our younger multiple sclerosis cases. While these biases certainly apply to people with multiple sclerosis who survive many decades, their prolonged exposure to vascular risk factors and an innate-immune predominant inflammatory response, as seen in progressive multiple sclerosis (Frischer *et al.*, 2015; Mahad *et al.*, 2015), may potentiate systemic atheroma development. In older cases, the reported shift towards a more peripheral/systemic pro-atherogenic macrophage phenotypes (Cochain and Zernecke, 2017), combined with a failure of vascular wall repair, provide support for these claims (Head *et al.*, 2017). Of course, deconditioning associated with disability accumulation could also contribute to changes in cardiac architecture not accounted for in this study, and this warrants further consideration (Vis *et al.*, 2012). The fact that our multiple sclerosis cohort was not

exposed to disease modifying therapies unlike current practice adds another layer of complexity to the integration of these findings with epidemiological data.

Cerebral small vessel disease is increased in young at death multiple sclerosis cases

Similar to systemic vascular disease, age at death influenced the extent of cerebral small vessel disease in multiple sclerosis. Multiple sclerosis cases with death at younger ages (≤60 years) in which lesion burden and inflammation were greatest, exhibited higher Global cSVD Index than controls, even when controlling for the extent of systemic vascular disease burden. In these younger cases, the number and inflammatory activity of demyelinating plaques associated with several key cerebral small vessel disease pathologies, including: arteriolosclerosis, PSD, and PHL. These findings contrast with older multiple sclerosis cases where the extent of cerebral small vessel disease was related to the severity of systemic vascular disease but not the multiple sclerosis-related pathology. Cross-talk between a pro-atherogenic T-helper 1 CD4+ T cell (Th1) predominant immune response, commonly seen in early active multiple sclerosis as well as pathways that lead to arteriole damage, may explain these findings (Dendrou *et al.*, 2015; Ketelhuth and Hansson, 2016), (Wiseman *et al.*, 2016) (Sumbria *et al.*, 2016).

The impact of systemic vascular disease on cerebral small vessel disease is stronger in multiple sclerosis compared with controls

The relationship between large artery atherosclerosis and cerebral small vessel disease in the general population is unclear, let alone in multiple sclerosis where it has not previously been explored. Systemic vascular disease had a stronger association with cerebral small vessel disease in multiple sclerosis than in controls. In controls, large artery atheroma has been reported to have only a small effect on brain WM changes, an indirect marker of cerebral small vessel disease (Wardlaw *et al.*, 2014). However, other studies suggest a stronger association between cervical (Ding *et al.*, 2017), intracranial (Del Brutto and Mera, 2017), and aortic arterial atheroma (Song *et al.*, 2016) and other cerebral small vessel disease imaging markers. Our findings lend support to the notion that inflammation leads to a tighter relationship between systemic vascular disease and cerebral small vessel disease (Ding *et al.*, 2017). Accelerated cerebral small vessel disease in multiple sclerosis may contribute to a relatively hypoxic milieu that heightens vulnerability to neuronal degeneration (Frischer *et al.*, 2009; Martinez Sosa and Smith, 2017) thereby providing an explanation for the established link between vascular risk factors and accumulation of irreversible neurological disability. These findings are pertinent to

clinical practice as vascular risk factors, especially in older people with multiple sclerosis, may require more aggressive monitoring and treatment compared to their non-multiple sclerosis counterparts.

Periarteriolar changes feature multiple sclerosis pathology outside plaque

We provide evidence that vascular-related pathology in multiple sclerosis extends beyond the venule. Through the study of cerebral small vessel disease in our cohort, we differentiated small veins from arterioles, which is not possible in *in vivo* imaging studies (Wuerfel *et al.*, 2008; Kilsdonk *et al.*, 2015) and is typically neglected in multiple sclerosis post-mortem studies. In so doing, we demonstrate that arteriolar pathology is significant in multiple sclerosis outside of lesions, with larger periarteriolar spaces and more severe periarteriolar inflammation and hemosiderin deposition seen in multiple sclerosis compared with controls. Further, we found that non-lesional periarteriolar pathology associated with the number of actively inflammatory lesions in the multiple sclerosis brain. Cuffs of inflammatory cells in the Virchow-Robin spaces of venules are prominent features of acute plaques and non-lesional areas in the multiple sclerosis and experimental autoimmune encephalomyelitis (Adams *et al.*, 1985; Esiri and Gay, 1990; Agrawal *et al.*, 2013), where strands of fibrin and hemosiderin deposition may also be observed (Adams, 1988). Our findings of prominent periarteriolar inflammation and 'fibrous veils' in multiple sclerosis outside of demyelinating plaques suggests a more diffuse vasculopathy than previously described. This is further supported by the presence of hemosiderin leakage around arterioles. The conflation of these findings adds credence to the claim that multiple sclerosis impacts the entire vascular tree, which has important implications not only for development of cerebral small vessel disease but also multiple sclerosis-related pathology. Indeed, the expression of an important extracellular matrix component (laminin) receptor, predominantly restricted to the arterioles, has been shown to be upregulated in the acute and later stages of an animal model of chronic progressive multiple sclerosis (Welser *et al.*, 2017). Future work aimed at clarifying the composition of the periarteriolar inflammatory infiltrate and its interaction with the extracellular matrix would be of interest but was beyond the scope of the current study. There are also relevant *in vivo* implications for this work. Compared to controls, changes in cerebral blood flow have been found in normal appearing WM in all stages of inflammatory demyelination typical of multiple sclerosis, including clinically isolated syndrome (Law *et al.*, 2004; D'haeseleer *et al.*, 2015). Peri-arteriolar inflammation might contribute to the hemodynamic changes found in normal-appearing WM,

along with other previously described mechanisms (D'haeseleer *et al.*, 2011) (Putman and Adler, 1937; Wakefield *et al.*, 1994).

Limitations

Biases associated with post-mortem studies apply, including selection bias based on availability of material suitable for study. To this end, we were fortunate that the multiple sclerosis and control cases studied for both systemic vascular disease and cerebral small vessel disease were age- and sex-matched after appropriate inclusion and exclusion criteria were applied. In addition, people with multiple sclerosis who die at younger ages likely have much more severe inflammatory disease than is encountered in the general population, challenging the generalisation of our findings to this patient group. Further, this multiple sclerosis cohort was naïve to disease modifying therapies. While this gives a unique opportunity to investigate the natural history of the relationship between multiple sclerosis on systemic and cerebral small vessel disease, it cannot discern the impact of anti-inflammatory therapies on vascular pathology. Medication exposures, outside of multiple sclerosis-specific treatments, may have differed between multiple sclerosis and control cases but we did not have access to this information, as is common with post-mortem studies. Finally, we acknowledge that pathology studies can only provide a static view of a dynamic process that likely varies across the age and disease spectrum. Despite these limitations, this post-mortem study, with access to brain tissue supplemented by whole-body autopsy reports and brain tissue, allowed us to study systemic and cerebrovascular disease with specificity beyond the resolution of current *in vivo* techniques. This strategy has cast light onto important relationships between systemic vascular disease and cerebral small vessel disease in both multiple sclerosis and non-neurological controls and has implicated arteriolar disease as a cardinal feature of multiple sclerosis pathology.

Conclusions

In summary, we provide unequivocal evidence that systemic vascular disease is lower in younger multiple sclerosis cases but shows a steeper increase with age at death compared to matched controls. The deleterious impact of multiple sclerosis is highlighted by the tighter relationship between systemic vascular disease and cerebral small vessel disease burden and the presence of more severe periarteriolar pathology compared to controls. Our data argue against a common primary trigger for multiple sclerosis and atherosclerosis and highlights that small vessel disease in multiple sclerosis brain may be the crucial link between vascular risk

factors and worse clinical outcomes in the disease. These findings have important implications on our understanding of the pathogenesis and long-term treatment of multiple sclerosis across the lifespan.

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Competing interests

The authors have no competing interests regarding this study.

References

Adams CW. Perivascular iron deposition and other vascular damage in multiple sclerosis. J Neurol Neurosurg Psychiatry 1988; 51: 260–5.

Adams CWM, Poston RN, Buk SJ, Sidhu YS, Vipond H. Inflammatory vasculitis in multiple sclerosis. J Neurol Sci 1985; 69: 269–283.

Agrawal SM, Williamson J, Sharma R, Kebir H, Patel K, Prat A, et al. Extracellular matrix metalloproteinase inducer shows active perivascular cuffs in multiple sclerosis. Brain 2013; 136: 1760–1777.

Belov P, Jakimovski D, Krawiecki J, Magnano C, Hagemeier J, Pelizzari L, et al. Lower arterial cross-sectional area of carotid and vertebral arteries and higher frequency of secondary neck vessels are associated with multiple sclerosis. Am J Neuroradiol 2018; 39: 123–130.

Del Brutto OH, Mera RM. Enlarged perivascular spaces in the basal ganglia are independently associated with intracranial atherosclerosis in the elderly. Atherosclerosis 2017; 267: 34–38.

Christiansen CF. Risk of vascular disease in patients with multiple sclerosis: a review. Neurol Res 2012; 34: 746–53.

Christiansen CF, Christensen S, Farkas DK, Miret M, Sørensen HT, Pedersen L. Risk of arterial cardiovascular diseases in patients with multiple sclerosis: a population-based cohort study. Neuroepidemiology 2010; 35: 267–74.

Cochain C, Zernecke A. Macrophages in vascular inflammation and atherosclerosis. Pflügers Arch - Eur J Physiol 2017; 469: 485–499.

Confavreux C, Vukusic S. The clinical course of multiple sclerosis. Handb Clin Neurol 2014; 122: 343–369.

Cuadrado-Godia E, Dwivedi P, Sharma S, Ois Santiago A, Roquer Gonzalez J, Balcells M, et al. Cerebral Small Vessel Disease: A Review Focusing on Pathophysiology, Biomarkers, and Machine Learning Strategies. J Stroke 2018; 20: 302–320.

D'haeseleer M, Cambron M, Vanopdenbosch L, De Keyser J. Vascular aspects of multiple

sclerosis. Lancet Neurol 2011; 10: 657–666.

D'haeseleer M, Hostenbach S, Peeters I, Sankari S El, Nagels G, De Keyser J, et al. Cerebral hypoperfusion: a new pathophysiologic concept in multiple sclerosis? J Cereb Blood Flow Metab 2015; 35: 1406–1410.

Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Nat Rev Immunol 2015; 15: 545–558.

Ding L, Hong Y, Peng B. Association between large artery atherosclerosis and cerebral microbleeds: A systematic review and meta-analysis. Stroke Vasc Neurol 2017; 2: 7–14.

Esiri MM, Gay D. Immunological and neuropathological significance of the Virchow-Robin space. J Neurol Sci 1990; 100: 3–8.

Esiri MM, Wilcock GK, Morris JH. Neuropathological assessment of the lesions of significance in vascular dementia. J Neurol Neurosurg Psychiatry 1997: 749–753.

Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain 2009; 132: 1175–1189.

Frischer JM, Weigand SD, Guo Y, Kale N, Parisi JE, Pirko I, et al. Clinical and pathological insights into the dynamic nature of the white matter multiple sclerosis plaque. Ann Neurol 2015; 78: 710–721.

Geraldes R, Esiri MM, DeLuca GC, Palace J. Age-related small vessel disease: a potential contributor to neurodegeneration in multiple sclerosis. Brain Pathol 2017; 27: 707–722.

Gouw A a, Seewann A, van der Flier WM, Barkhof F, Rozemuller AM, Scheltens P, et al. Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. J Neurol Neurosurg Psychiatry 2011; 82: 126–35.

Grajchen E, Hendriks JJA, Bogie JFJ. The physiology of foamy phagocytes in multiple sclerosis. Acta Neuropathol Commun 2018; 6: 124.

Grajek S, Lesiak M, Pyda M, Zajac M, Paradowski S, Kaczmarek E. Hypertrophy or hyperplasia in cardiac muscle. Post-mortem human morphometric study. Eur Heart J 1993; 14: 40–7.

Head T, Daunert S, Goldschmidt-Clermont PJ. The aging risk and atherosclerosis: A fresh look at arterial homeostasis. Front Genet 2017; 8: 1–11.

Jakimovski D, Topolski M, Genovese AV, Weinstock - Guttman B, Zivadinov R. Vascular aspects of multiple sclerosis: emphasis on perfusion and cardiovascular comorbidities. Expert Rev Neurother 2019; 19: 445–458.

Keith J, Gao FQ, Noor R, Kiss A, Balasubramaniam G, Au K, et al. Collagenosis of the Deep Medullary Veins: An Underrecognized Pathologic Correlate of White Matter Hyperintensities and Periventricular Infarction? J Neuropathol Exp Neurol 2017; 76: 299–312.

Ketelhuth DFJ, Hansson GK. Adaptive Response of T and B Cells in Atherosclerosis. Circ Res 2016; 118: 668–678.

Kilsdonk I, Steenwijk M, Pouwels P, Zwanenburg J, Visser F, Luijten P, et al. Perivascular spaces in MS patients at 7 Tesla MRI: A marker of neurodegeneration? Mult Scler 2015; 21: 155–162.

Kuhlmann T, Ludwin S, Prat A, Antel J, Brück W, Lassmann H. An updated histological classification system for multiple sclerosis lesions. Acta Neuropathol 2017; 133: 13–24.

Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977; 33: 159–74.

Law M, Saindane AM, Ge Y, Babb JS, Johnson G, Mannon LJ, et al. Microvascular abnormality in relapsing-remitting multiple sclerosis: perfusion MR imaging findings in normal-appearing white matter. Radiology 2004; 231: 645–52.

Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. Lancet Neurol 2015; 14: 183–193.

Marrie RA, Rudick R, Horwitz R, Cutter G, Tyry T, Campagnolo D, et al. Vascular comorbidity is associated with more rapid disability progression in multiple sclerosis. Neurology 2010; 74: 1041–7.

Marrie RA, Garland A, Schaffer SA, Fransoo R, Leung S, Yogendran M, et al. Traditional risk factors may not explain increased incidence of myocardial infarction in MS. Neurology 2019; 92: e1624–e1633.

Marrie RA, Reider N, Cohen J, Stuve O, Trojano M, Cutter G, et al. A systematic review of the incidence and prevalence of cardiac, cerebrovascular, and peripheral vascular disease in multiple sclerosis. Mult Scler 2014; 21: 318–331.

Martinez Sosa S, Smith KJJ. Understanding a role for hypoxia in lesion formation and location in the deep and periventricular white matter in small vessel disease and multiple sclerosis. Clin Sci 2017; 131: 2503–2524.

McKay KA, Jahanfar S, Duggan T, Tkachuk S, Tremlett H. Factors associated with onset, relapses or progression in multiple sclerosis: A systematic review. Neurotoxicology 2017; 61: 189–212.

Murtonen A, Kurki S, Hänninen K, Soilu-Hänninen M, Sumelahti ML. Common comorbidities and survival in MS: Risk for stroke, type 1 diabetes and infections. Mult Scler Relat Disord 2018; 19: 109–114.

Pantoni L. Cerebral small vessel disease : from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol 2010; 9: 689–701.

Pichler A, Khalil M, Langkammer C, Pinter D, Ropele S, Fuchs S, et al. The impact of vascular risk factors on brain volume and lesion load in patients with early multiple sclerosis. Mult Scler J 2019; 25: 48–54.

Putman T, Adler A. Vascular architecture of the lesions of multiple sclerosis. Arch Neurol Psychiatry 1937; 38: 1–15.

Qiu C, Fratiglioni L. A major role for cardiovascular burden in age-related cognitive decline. Nat Rev Cardiol 2015; 12: 267–277.

Ranadive SM, Yan H, Weikert M, Lane AD, Linden MA, Baynard T, et al. Vascular dysfunction and physical activity in multiple sclerosis. Med Sci Sports Exerc 2012; 44: 238–243.

Randolph JJ. Free-Marginal Multirater Kappa (multirater K[free]): An Alternative to Fleiss' Fixed-Marginal Multirater Kappa. Joensuu Learn Instr Symp 2005: Retrieved from http://eric.ed.gov/?id=ED490661.

Roshanisefat H, Bahmanyar S, Hillert J, Olsson T, Montgomery S. Multiple sclerosis clinical course and cardiovascular disease risk - Swedish cohort study. Eur J Neurol 2014; 21: 1353-

e88.

Simpson RJ, McLean G, Guthrie B, Mair F, Mercer SW. Physical and mental health comorbidity is common in people with multiple sclerosis: nationally representative cross-sectional population database analysis. BMC Neurol 2014; 14: 128.

Skrobot OA, Attems J, Esiri M, Hortobágyi T, Ironside JW, Kalaria RN, et al. Vascular cognitive impairment neuropathology guidelines (VCING): the contribution of cerebrovascular pathology to cognitive impairment. Brain 2016; 139: 2957–2969.

Song TJ, Kim YD, Yoo J, Kim J, Chang HJ, Hong GR, et al. Association between Aortic Atheroma and Cerebral Small Vessel Disease in Patients with Ischemic Stroke. J Stroke 2016; 18: 312–320.

Spencer JI, Bell JS, DeLuca GC. Vascular pathology in multiple sclerosis: Reframing pathogenesis around the blood-brain barrier. J Neurol Neurosurg Psychiatry 2018; 89: 42–52.

Sumbria RK, Grigoryan MM, Vasilevko V, Krasieva TB, Scadeng M, Dvornikova AK, et al. A murine model of inflammation-induced cerebral microbleeds. J Neuroinflammation 2016; 13: 1–12.

Thej MJ, Kalyani R, Kiran J. Atherosclerosis in coronary artery and aorta in a semi-urban population by applying modified American Heart Association classification of atherosclerosis: An autopsy study. J Cardiovasc Dis Res 2012; 3: 265–271.

Thompson AJ, Baranzini SE, Geurts J, Hemmer B, Ciccarelli O. Multiple sclerosis. Lancet (London, England) 2018; 391: 1622–1636.

Tutuncu M, Tang J, Zeid NA, Kale N, Crusan DJ, Atkinson EJ, et al. Onset of progressive phase is an age-dependent clinical milestone in multiple sclerosis. Mult Scler 2013; 19: 188–98.

Vis JC, de Bruin-Bon RH, Bouma BJ, Backx AP, Huisman SA, Imschoot L, et al. 'The sedentary heart': Physical inactivity is associated with cardiac atrophy in adults with an intellectual disability. Int J Cardiol 2012; 158: 387–393.

Vukusic S, Confavreux C. Natural history of multiple sclerosis: risk factors and prognostic indicators. Curr Opin Neurol 2007; 20: 269–274.

Wakefield AJ, More LJ, Difford J, McLaughlin JE. Immunohistochemical study of vascular injury in acute multiple sclerosis. J Clin Pathol 1994; 47: 129–133.

Wardlaw J., Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. Lancet Neurol 2013; 12: 483–97.

Wardlaw J., Smith E., Biessels GJ, Cordonnier C, Fazekas F, Frayne R, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol 2013; 12: 822–838.

Wardlaw JM, Allerhand M, Doubal FN, Valdes Hernandez M, Morris Z, Gow AJ, et al. Vascular risk factors, large-artery atheroma, and brain white matter hyperintensities. Neurology 2014; 82: 1331–8.

Webber BJ, Seguin PG, Burnett DG, Clark LL, Otto JL. Prevalence of and risk factors for autopsy-determined atherosclerosis among US service members, 2001-2011. JAMA 2012; 308: 2577–83.

Welser J V., Halder SK, Kant R, Boroujerdi A, Milner R. Endothelial α6β4 integrin protects during experimental autoimmune encephalomyelitis-induced neuroinflammation by maintaining vascular integrity and tight junction protein expression. J Neuroinflammation 2017; 14: 217.

Wiseman SJ, Bastin ME, Jardine CL, Barclay G, Hamilton IF, Sandeman E, et al. Cerebral small vessel disease burden is increased in systemic lupus erythematosus. Stroke 2016; 47: 2722–2728.

Wuerfel J, Haertle M, Waiczies H, Tysiak E, Bechmann I, Wernecke KD, et al. Perivascular spaces--MRI marker of inflammatory activity in the brain? Brain 2008; 131: 2332–2340.

Yates RL, Esiri MM, Palace J, Mittal A, Deluca GC. The influence of HLA-DRB1*15 on motor cortical pathology in multiple sclerosis. Neuropathol Appl Neurobiol 2015; 41

Figure legends

Figure 1. **Cohort selection and Systemic Vascular Disease (SysVasc) score and Global cerebral Small Vessel Disease (cSVD) Index construction**

A human autopsy cohort of eighty-five pathologically confirmed multiple sclerosis cases from a total of 111 available cases and 68 out of the 90 selected age- and sex-matched controls older than 35 years with comprehensive, standardised full body autopsy reports and brain material were selected for study. Autopsies were performed between 1971 and 2007 at the Neuropathology department, Oxford University Hospitals, the majority for medical interest, regarding patients followed at the Oxford University Hospitals, 3 (1 MS and 2 controls) for medico-legal purposes and 3 (1 MS and 2 controls) as part of the Oxford Project to Investigate Memory and Ageing (OPTIMA) study. Multiple sclerosis cases and controls with severe brain damage were excluded, such as traumatic brain injury (TBI), herpes encephalitis, meningitis, CNS tumour, subarachnoid haemorrhage (SAH), cerebral venous thrombosis (CVT). Variables considered for matching were: age (1 year range), sex, autopsy report and tissue availability.

The spectrum of atheroma burden in the aorta, coronary, cervical and intracranial large arteries was assessed to devise a semi-quantitative score. Measures of vascular related end-organ damage included presence of myocardial infarction (MI), macroscopic ischemic stroke (IS), renal hypertensive disease (RH), and severity of left ventricular hypertrophy (LVH) (not present/mild to moderate/severe). As information on aorta and coronary atheroma, MI, IS and LVH, was consistently available (> 80% of the reports) and measured with strong inter-rater agreement (free marginal multi-rater kappa coefficient >0.6), these variables were included in the final vascular scores (i.e. **Atheroma score** = mean aorta and coronary atheroma scores, **End Organ score** = mean MI, IS and LVH scores, with **SysVasc Score** = mean of Atheroma and End Organ Scores). In a subset of cases, the extent of the presence and extent of cerebral small vessel disease features was assessed (0 – not present; 1 - present): 1. arteriolosclerosis, 2. fibrinoid necrosis and microaneurysms (not shown), 3. periarteriolar space dilatation, 4. periarteriolar hemosiderin leakage, 5. microinfarcts, lacunar infarcts, 6. large infarcts (not shown) and 7. Microhaemorrhages. Total scores for each of the pathological features were obtained by adding the scores derived from each brain region analysed with the sum of each of the total scores contributing to a global cerebral small vessel disease index (**Global cSVD Index**). Maximum total score possible was 28.

NA – not available, FWM – frontal WM, OWM – occipital WM, BG – basal ganglia.

Figure 2. SysVasc Score and Global SVD Index and causes of death and age at death.

A. SysVasc Score and **B.** Global SVD Index were higher in all cases with Vascular causes of death (i.e. MI, IS, pulmonary embolism and or deep venous thrombosis) compared to Other causes of death (excluding cancer, infection, multiple sclerosis). **C.** SysVasc and **D.** Global SVD Index increased with age at death**.**

Mann-Whitney test *p<0.01; **** p<0.0001

Figure 3. Periarteriolar changes in multiple sclerosis (outside plaque). Periarteriolar dilatation (**A-C**, arrowheads; box in Panel B magnified in Panel C), 'veils' (**D**, **E**, arrows), and hemosiderin deposition (**F**, dashed arrows) are typical vessel-related features seen in multiple sclerosis WM in excess of matched controls. Periarteriolar inflammatory cuffs (**G**) are observed in multiple sclerosis but rarely in controls. Asterisk (*) marks cerebral cortical grey matter. A, D, F, G – haematoxylin and eosin; B – proteolipid protein; C, E – smooth muscle actin. Measure bars: A, B – 500 μm; C – 200 μm; D, E, G – 100 μm; F – 50 μm

Figure 4. Relationship between SysVasc score and Global cSVD Index for multiple sclerosis and control cases. Global cSVD Index significantly increased with SysVasc Score in multiple sclerosis, but not in controls (**A**). Young at death multiple sclerosis cases showed an excess of Global SVD Index compared with controls with the same SysVasc score (**B** and **see Table 3 Model age ≤60 years**)). Global cSVD Index increased with SysVasc score in multiple sclerosis (**C**), but multiple sclerosis was not independently associated with Global cSVD in the older at death group (**see Table 3 Model >60 years**).

Supplemental material

Supplemental Table 1. Antibodies used for histological analysis (PLP, proteolipid protein; SMA, smooth muscle actin)

Antibody	Clone	Classification	Pre-treatment	Dilution	Supplier	Catalogue Number
PLP	plpc1	Monoclonal mouse	Microwave citrate pH 6.0	1:1000	AbD Serotec	MCA8399
SMA	1A4	Monoclonal mouse	Microwave TRIS-EDTA pH 9.0	1:50	Dako	M0851
CD68	PGM-1	Monoclonal mouse	Microwave citrate	1:100	Dako	M0876

Supplemental Table 2. Other causes of death in multiple sclerosis cases and controls

	Multiple Sclerosis	Controls
Infection N (%)		
Total	42	12
Pneumonia	34 (81.0)	8 (66.7)
Pyelonephritis	3 (7.0)	1 (8.3)
Septicaemia	0	1 (8.3)
Meningitis/encephalitis	2 (4.8)	2 (16.7)
Skin infection	2 (4.8)	0
Peritonitis	1 (2.4)	0
Cancer N (%)		
Total	5	16
Bladder	1 (20.0)	2 (12.5)
Lung	0	5 (31.3)
Gynaecological	1 (20.0)	2 (12.5)
Gastrointestinal	1 (20.0)	2 (12.5)
Haematological	0	3 (18.8)
CNS	1 (20.0)	2 (12.5)
Breast	1 (20.0)	0
Other causes of death		
Total	19	22
Metabolic	1 (5.3)	5 (22.7)
GI bleed/perforation	4 (21.1)	3 (13.6)
Suicide (intoxication/overdose/hanging)	4 (21.1)	6 (27.3)
Asthma/another lung disease	5 (26.6)	1 (1.5)
Non-atheromatous vascular disease	1 (13.6)	3 (13.6)
Trauma	2 (9.1)	1 (1.5)
Anaemia	1 (4.5)	1 (1.5)
CNS disorder	1 (4.5)	1 (1.5)

Supplemental Table 3. Atheroma scores according to pathologist

Coronary atheroma N (%)	**ME Controls(N=46)**	**All other pathologists Controls (N=25)**	**ME Multiple Sclerosis (N=42)**	**All other pathologists Multiple Sclerosis (N=43)**
Not present	15 (32.6)	7 (28.0)	18 (42.9)	12 (27.9)
Mild	11 (23.9)	10 (40.0)	7 (16.7)	19 (44.2)
Moderate	9 (19.6)	5 (20.0)	12 (28.6)	5 (11.6)
Severe	11 (23.9)	3(12.0)	5 (11.9)	7 (16.3)
Aorta Atheroma N (%)	**ME Controls(N=42)**	**All other pathologists Controls (N=25)**	**ME Multiple Sclerosis (N=40)**	**All other pathologists Multiple Sclerosis (N=35)**
Not present	9 (21.4)	5 (20.0)	12 (30.0)	8 (22.9)
Mild	13 (31.0)	8 (32.0)	14 (35.0)	16 (45.7)
Moderate	12 (28.6)	8 (32.0)	6 (15.0)	7 (20.0)
Severe	8 (19.0)	4 (16.0)	8 (20.0)	4 (11.4)

Supplemental Table 4. Correlation matrix for individual SysVasc features included in the SysVasc Score (whole cohort) *p<0.05, **p<0.01, LVH – left ventricular hypertrophy

	Aorta atheroma	Coronary atheroma	Myocardial Infarction	LVH	Ischemic Stroke
Aorta atheroma	1	0.571**	0.312**	0.193*	0.124
Coronary atheroma	0.571**	1	0.496**	0.258**	0.154
Myocardial Infarction	0.312**	0.496**	1	0.272**	0.157*
LVH	0.193*	0.258**	0.272**	1	0.265**
Ischemic Stroke	0.124	0.154	0.157**	0.265**	1

Supplemental Table 5. Numerical results of a simple linear model (Model 1) and with an interaction term (Model 2) to study the effect of Multiple Sclerosis (MS) diagnosis on SysVasc and Atheroma scores for all cases and excluding myocardial infarction (MI) and/or ischaemic stroke (IS) deaths.

		SysVasc Score			Atheroma score		
	Parameter	B	95%CI for B	p	B	95%CI for B	p
All cases							
Model 1	Intercept(b_0)	-0.130	-0.310,0.500	0.150	-0.230	-0.45,-0.020	0.05
	Age(b1)	0.080	0.005,0.010	<0.001**	0.010	0.008,0.020	<0.001**
	MS(b_2)	-0.090	-0.160,-0.020	0.010*	-0.090	-0.18,0.003	0.059
F-statistic: 17.13 on 2 and 152 DF, p<0.001					F-statistic: 19.8 on 2 and 152 DF, p<0.001		
Model 2	Intercept (b_0)	0.123	-0.132,0.378	0.341	0.063	-0.260,0.390	0.701
	Age(b1)	0.004	0.000-0.008	0.079	0.007	0.001,0.010	0.017 *
	MS(b_2)	-0.572	-0.923, -0.220	0.002**	-0.632	-1.080,-0.190	0.006 **
	Age*MS(b_3)	0.008	0.002,0.014	0.007**	0.009	0.002,0.016	0.002 **
F-statistic: 14.43 on 3 and 152 DF, p<0.001					F-statistic: 15.75 on 3 and 152 DF, p<0.001		
No MI/IS							
Model 1	Intercept(b_0)	-0.070	-0.32,0.020	0.080	-0.285	-0.510,-0.060	0.013*
	Age(b1)	0.008	0.005,0.010	<0.001**	0.012	0.008,0.016	<0.001**
	MS(b_2)	-0.075	-0.146,-0.005	0.036*	-0.080	-0.170,0.011	0.085
F-statistic:16.1 on 2 and 134 DF, p<0.001					F-statistic:21.3 on 134 and 2 DF,p <0.001		
Model 2	Intercept (b_0)	0.055	-0.195, 0.205	0.664	- 0.037	-0.363,0.290	0.825
	Age(b1)	0.004	0.000, 0.008	0.050	0.008	0.002,0.013	0.07 *
	MS(b_2)	-0.449	-0.787,-0.111	0.010*	-0.529	-0.972,-0.086	0.02 *
	Age*MS(b_3)	0.006	0.001, 0.120	0.027*	0.008	0.000,0.015	0.043 *
F- statistic: 12.74 on 3 and 134 DF, p<0.001					F-statistic: 15.9 on 3 and 134 DF, p<0.001		

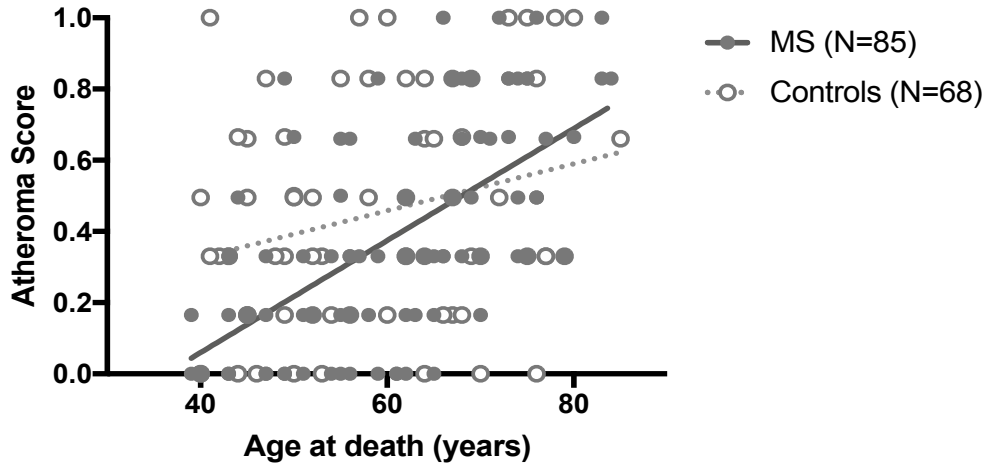
Note: The linear model with an interaction term was used to compare the two regression lines for the MS and control groups. The estimated equation of the model is y= $b_0 + b_1 x_1 + b_2 x_2 + b_3 x_1 x_2$ where y is the response variable (SysVasc or atheroma score), x_1 represents the variable age and x_2 is a binary variable with $x_2 = 1$ if the subject belongs to the MS group and $x_2 = 0$ if the subject belongs to the control group. If x_2=0 we have y=b_0+b_1x_1, and b_0 and b1 are the intercept and the slope of the regression line associated with the control group. If x_2=1 we have y= (b_0+b_2) + (b_1+b_3) x_1, b_0+b_1 and b_1+b_3 are, respectively, the intercept and the slope of the regression line of the MS group. The coefficient b_2, indicates how much higher (lower) the intercept of the line for the MS group is than the intercept of the line for the control group, while the coefficient of the interaction, b_3, measures the differential between the slopes of the two regression lines. *p<0.05, **p<0.01

Supplemental Table 6. Frequency of cerebral small vessel disease pathological features per brain region in controls and Multiple Sclerosis (outside plaque). Comparisons were made between controls and Multiple Sclerosis (outside plaque). Chi-squared test or Fisher's exact test**p<0.01, *p<0.05.

		Arteriolosclerosis	Periarteriolar space dilatation	Periarteriolar hemosiderin leakage	Microbleeds	Micro-infarcts	Large infarcts	Fibrinoid necrosis
% (N) in one or more areas	**Controls (N=39)**	**61.5 (24)**	**64.1 (25)**	**46.2 (18)****	**35.9 (14)**	**15.4 (6)**	**2.6 (1)**	**5.1(2)**
% (N) in one or more areas	**Multiple Sclerosis (N=42)**	**61.9 (26)**	**85.7 (36)**	**78.6 (33)****	**33.3 (14)**	**2.4 (1)**	**0 0**	**0 0**
Pons % (N)	**Controls (N=39)**	12.8 (5)	23.1(9)**	7.7 (3)	10.3 (4)	2.6 (1)	0	0
	Multiple Sclerosis (N=42)	11.9 (5)	52.4 (22)**	19.0 (8)	19.0 (8)	2.4 (1)	0	0
BG % (N)	**Controls (N=39)**	51.3 (20)	51.3 (20)	17.9 (7)	17.9 (7)	5.1 (2)	2.6 (1)	5.1 (2)
	Multiple Sclerosis (N=42)	45.2 (19)	35.7 (15)	30.9 (13)	14.3 (6)	2.4 (1)	0	0
FWM % (N)	**Controls (N=39)**	33.3 (13)	35.9(14)	41.0 (16)	2.6 (1)	2.6 (1)	0	0
	Multiple Sclerosis (N=42)	31.0 (13)	54.8 (23)	45.2 (19)	0	0	0	0
OWM % (N)	**Controls (N=39)**	20.5 (8)	35.9 (14)	35.9 (14)**	15.4 (6)*	5.1 (2)	0	0
	Multiple Sclerosis (N=42)	35.7 (15)	54.8 (23)	64.3 (27)**	2.4 (1)*	0	0	0

Supplemental Table 7. Demyelination outcomes per brain region in multiple sclerosis

Total N cases= 42	Pons	Basal Ganglia	Frontal White Matter	Occipital White Matter	Total
Number of cases with plaques (%)	31 (73.8)	28 (66.6)	29 (69.0)	24 (57.1)	38 (90.5)
Median total number of plaques (IQR)	1 (4)	1 (2.25)	1 (2.25)	1 (1)	5 (9.0)
Median percentage of plaque area (IQR)	1.75 (11.5)	2.11 (10.8)	0.72 (10.2)	1.56 (16.8)	2.75 (13.1)
Median total number of active plaques (IQR)	0 (3.25)	0 (1)	0.5 (2)	0 (1)	2.0(8.5)
- Active	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)
- Mixed active/inactive	0 (3)	0 (1)	0 (1)	0 (1)	1 (6)
% cases with 1 or more active plaques (N)	45.2 (19)	30.9 (13)	52.4 (22)	38.1 (16)	71.4 (30)
Median number of inactive plaques (IQR)	0 (1)	0.5 (1)	0 (1)	0 (0)	2.0 (1.0)
% cases with 1 or more inactive plaques	40.5 (17)	50.0 (21)	28.6 (12)	19.0 (8)	76.2 (32)



Supplemental Figure 1. The relationship between atheroma score and age at death in Multiple Sclerosis (MS) and controls

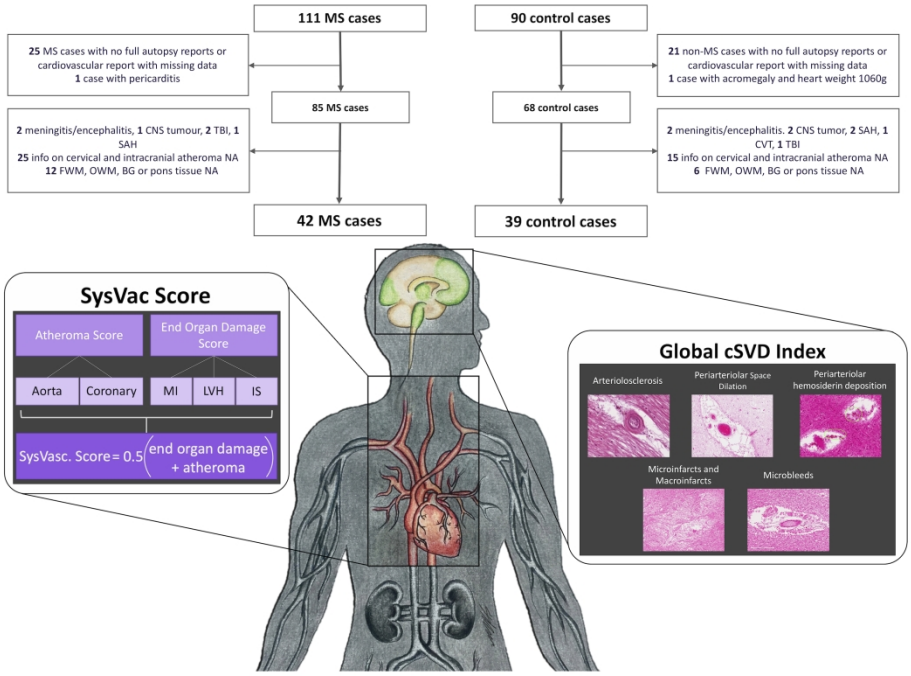


Figure1. Cohort selection and Systemic Vascular Disease (SysVasc) score and Global cerebral Small Vessel Disease (cSVD) Index construction

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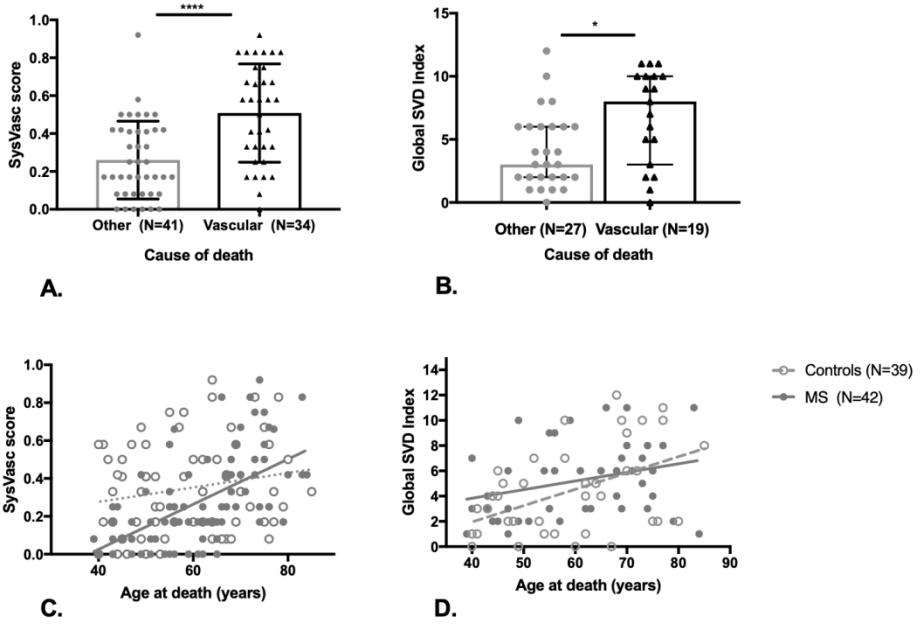


Figure 2. SysVasc Score and Global SVD Index and causes of death and age at death.

268x209mm (150 x 150 DPI)

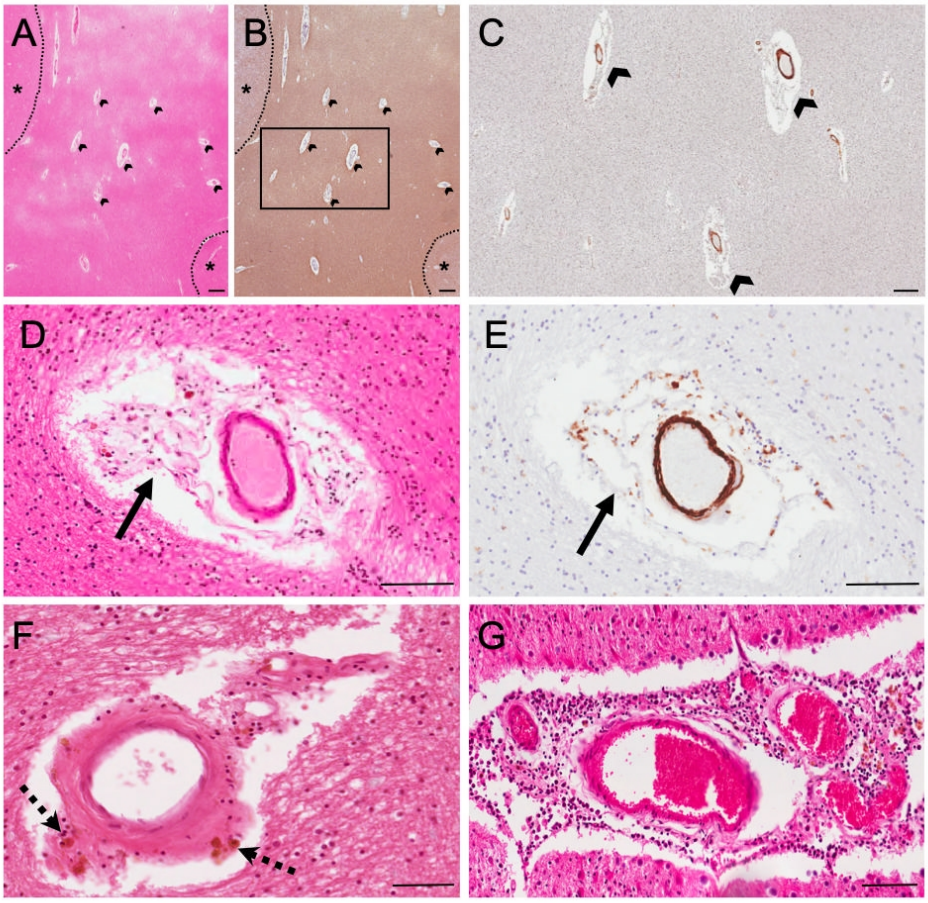


Figure 3. Periarteriolar changes in multiple sclerosis (outside plaque).

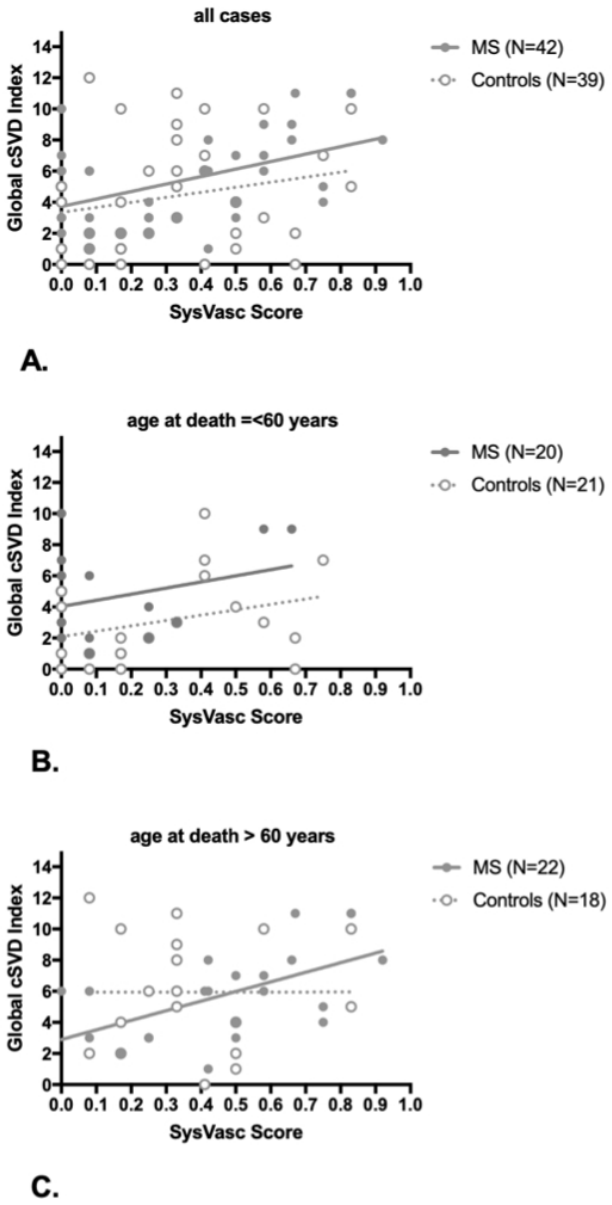


Figure 4. Relationship between SysVasc score and Global cSVD Index for multiple sclerosis and control cases.

115x170mm (150 x 150 DPI)

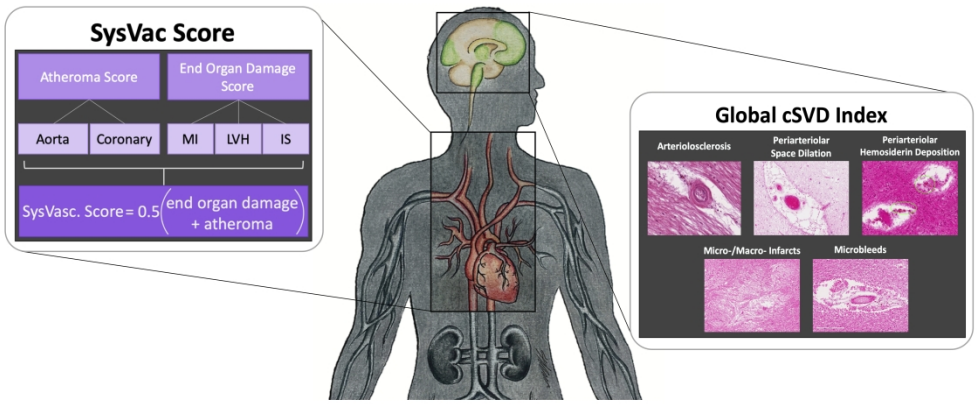
STROBE statement: Reporting guidelines checklist for cohort, case-control and cross-sectional studies

SECTION	ITEM NUMBER	CHECKLIST ITEM	REPORTED ON PAGE NUMBER:
TITLE AND ABSTRACT			
	1a	Indicate the study's design with a commonly used term in the title or the abstract	1
	1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
INTRODUCTION			
Background and objectives	2	Explain the scientific background and rationale for the investigation being reported	3, 4
	3	State specific objectives, including any pre-specified hypotheses	3
METHODS			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6a	Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants	5 and Figure 1
	6b	Cohort study—For matched studies, give matching criteria and number of exposed and unexposed Case-control study—For matched studies, give matching criteria and the number of controls per case Variables	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-8
Data sources/measurements	8*	For each variable of interest, give sources of data and details of methods of assessment	5-8

SECTION	ITEM NUMBER	CHECKLIST ITEM	REPORTED ON PAGE NUMBER:
		(measurement). Describe comparability of assessment methods if there is more than one group.	
Bias	9	Describe any efforts to address potential sources of bias.	8
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why.	7-9
Statistical methods	12a	Describe all statistical methods, including those used to control for confounding	7-9
	12b	Describe any methods used to examine subgroups and interactions	8, 9
	12c	Explain how missing data were addressed	8
	12d	Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	34 – Fig.1
	12e	Describe any sensitivity analyses	8-9
RESULTS			
Participants	13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10, Figure 5
	13b	Give reasons for non-participation at each stage	Figure 1 legend
	13c	Consider use of a flow diagram	Figure 1
Descriptive Data	14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	**10,11**
	14b	Indicate number of participants with missing data for each variable of interest	10, 34, Fig.1
	14c	Cohort study—Summarise follow-up time (eg, average and total amount)	10
Outcome Data	15*	Cohort study—Report numbers of outcome events or summary measures over time Case-control study—Report numbers in each exposure category, or summary measures of exposure Cross-sectional study—Report numbers of outcome events or summary measures	10-16

SECTION	ITEM NUMBER	CHECKLIST ITEM	REPORTED ON PAGE NUMBER:
Main Results	16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-17
	16b	Report category boundaries when continuous variables were categorized	9
	16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
	16d	Report results of any adjustments for multiple comparisons	8
Other Analyses	17a	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	11-15
	17b	If numerous genetic exposures (genetic variants) were examined, summarize results from all analyses undertaken	N/A
	17c	If detailed results are available elsewhere, state how they can be accessed	N/A
DISCUSSION			
Key Results	18	Summarise key results with reference to study objectives	21
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	25
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22,23, 25
Generalisability	21	Discuss the generalisability (external validity) of the study results Other information	25
FUNDING			
	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.



Systemic and cerebral vascular disease in multiple sclerosis

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