

Supplementary material

Supplementary 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

Supplementary 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)

Supplementary 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)

Supplementary 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

Supplementary 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(b_1)	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(b_1)	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(b_1)	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(b_1)	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		

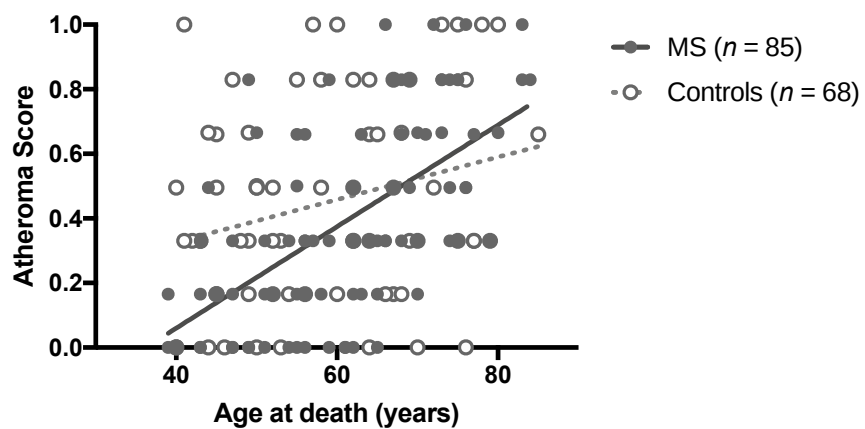
Note: The linear model with an interaction term was used to compare the two regression lines for the MS and control groups. The estimated equation of the model is $y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_1 x_2$ where y is the response variable (SysVasc or atheroma score), x_1 represents the variable age 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 b_1 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. * $p<0.05$, ** $p<0.01$

Supplementary 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**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
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

Supplementary 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	0 (3)	0 (1)	0 (1)	0 (1)	1 (6)
active/inactive					
% 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)



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