Supplementary Materials and Methods

The study population comprised of subjects recruited in a case–control study of cardiovascular, environmental and genetic risk factors for disease progression in patients with multiple sclerosis (MS) (CEG-MS study; IRB ID: MODCR00000352).1

Subjects with the following characteristics were included: having MS according to the revised McDonald criteria2 or being a healthy individual (HI), having a magnetic resonance imaging (MRI) scan at the 3T scanner using the standardized MRI protocol, age between 18 and 75 years and physical/neurologic examination within 30 days from the standardized MRI study protocol. The exclusion criteria consisted of presence of relapse and steroid treatment within the 30 days preceding study entry, pre-existing medical conditions known to be associated with brain pathology (e.g. neurodegenerative disorders, cerebrovascular disease, positive history of alcohol abuse, etc.) and pregnancy.

Subjects underwent neurological and MRI examinations and provided blood samples. The collected data included demographic and clinical information. The study protocol was approved by the local Institutional Review Board and all participants gave their written informed consent. MRI acquisition and image analysis have been previously described in detail.1

The cerebral microbleeds (CMBs) analysis was performed on susceptibility weighted imaging (SWI) minimum intensity projection images and susceptibility maps by two experienced neuroimagers, who were also blinded to MR images, were obtained with other sequences. CMBs were classified as focal, small, round to ovoid punctuate areas of signal hypo-intensity on SWI minimum intensity projection images, as previously reported.1 Signal voids caused by sulcal vessels, calcifications and signal averaging from bone were considered mimics of microbleeds. The presence and number of definite CMBs were determined on SWI minimum intensity projection images by using the Microbleed Anatomic Rating Scale.3 The CMB volume was calculated on susceptibility maps by using a semi-automated edge detection contouring and thresholding technique.4

Soluble vascular adhesion protein-1 (sVAP-1) levels were measured in ethylenediaminetetraacetic acid plasma samples obtained at the follow-up visit, using Luminex Screening Assays magnetic bead kits (R&D Systems Inc., Minneapolis, Minnesota, United States). Samples were processed following the manufacturer’s recommended protocols and read on a MAGPIX instrument equipped with the MILLIPLEX-Analyt Software 5.1 (Merk Millipore) using a five-parameter non-linear regression formula to compute sample concentrations from the standard curves.5 Concentrations were expressed as ng/mL. The calculated inter-assay coefficient of variations for sVAP-1 was 3.7%.

SPSS (IBM Corp., Armonk, New York, United States, version 24.0) statistical software was used for all statistical analyses and GraphPad (GraphPad Software, Inc., La Jolla, California, United States, prism version 6.01) for the figures. Fisher’s exact test was used to compare differences in categorical variables, Student’s t-test was used to compare age and analysis of covariance, with age and gender as covariates, was used to compare brain volume measurements between the total MS and HI groups. Spearman’s rank correlation was used to assess associations of sVAP-1 levels with demographic characteristics, Expanded Disability Status Scale and disease duration. Differences in VAP-1 levels between MS patients with or without CMBs were addressed using Mann Whitney U-test. Linear regression was used to detect association between VAP-1 and CMBs volume. The associations of MRI measures with sVAP-1 were assessed using multiple regression analysis. All regression analyses included the MRI measure of interest as the dependent variable; the predictor variables were age, gender, drug treatment and sVAP-1. The Kruskal–Wallis test, followed by Dunn’s multiple comparison test were used to analyse variations of sVAP-1 levels according to the disease-modifying treatments. p-Values of ≤ 0.05 were considered as statistically significant.

References