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Association of Brain Age With Physical Disability and Cognitive Impairment in People With Multiple Sclerosis of the Same Age

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Abstract

Background and Objectives

The brain-predicted age difference (brain-PAD) is a novel marker of neurodegeneration in multiple sclerosis (MS). Brain-PAD has been associated with clinical disability in heterogeneous MS patient cohorts of varying ages and disease durations. In this study, we investigate the relation between clinical disability and brain-PAD in a unique birth-year cohort of people with MS (pwMS) and healthy controls (HCs) of the same age all born in 1966, eliminating age as a confounding factor.

Methods

This was a cross-sectional cohort study conducted in the Netherlands. Disability was quantified using the expanded disability status scale (EDSS), 9-hole peg test (9HPT), and the timed 25-foot walk test (T25FWT). Cognition was assessed using the Minimal Assessment of Cognitive Function in MS battery. The brain-PAD was calculated by subtracting the person's chronological age from the predicted brain age derived from 3-dimensional T1-weighted brain MRI scans using machine learning (brainageR software). Brain-PAD for HCs and MS subtypes (relapsing remitting, secondary progressive, and primary progressive) were compared using a generalized linear model. The relation between brain-PAD and disease duration and disability and cognitive measures were tested using univariate linear regression. In addition, the clinical explanatory value added by brain-PAD to those of brain parenchymal fraction (BPF) and T2 lesion volume was investigated.

Results

The study included 116 HC (mean age 52.9 ± 1.1 years, 61% female) and 237 pwMS (mean age 52.9 ± 0.9 years, median disease duration 16.3 years [interquartile range 8.2–24.4] and a median EDSS of 3.5 [interquartile range 2.5–4.0]). Brain-PAD was higher in pwMS compared with HC by 9.7 years (SE = 0.82, $p < 0.0001$). Longer disease duration was associated with a higher brain-PAD ($\beta = 0.21$, $p < 0.001$). A higher brain-PAD was associated with worse performance on the T25FWT ($\beta = 0.0063$, $p < 0.05$), 9HPT ($\beta = 0.0074$, $p < 0.001$), and EDSS ($\beta = 0.028$, $p < 0.05$). Brain-PAD was higher for cognitively impaired people with MS, compared with cognitively preserved pwMS and HC ($p < 0.0001$). Brain-PAD had added explanatory value over BPF in clinical outcome measures.

Discussion

In a cohort unbiased by age differences, greater brain ageing was associated with worse performance on disability and cognitive tests, underscoring the potential of brain-PAD as a marker for neurodegeneration and disease severity in MS.

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Supplementary Material

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Glossary

3D = 3-dimensional; **9HPT** = 9-hole peg test; **ANOVA** = analysis of variance; **BPF** = brain parenchymal fraction; **brain-PAD** = brain-predicted age difference; **BVMT-R** = Brief Visuospatial Memory Test-Revised; **CI** = cognitively impaired; **COWAT** = Controlled Oral Word Association Test; **CP** = cognitively preserved; **D-KEFS** = Delis-Kaplan Executive Function System sorting test; **EDSS** = expanded disability status scale; **FLAIR** = fluid attenuated inversion recovery; **GLM** = general linear model; **GPR** = Gaussian Process Regression; **HC** = healthy control; **HSD** = honestly significant difference; **JLO** = Benton Judgment of Line Orientation Test; **MACFIMS** = Minimal Assessment of Cognitive Function in MS; **MS** = multiple sclerosis; **PCA** = principal component analysis; **PMS** = progressive MS; **PPMS** = primary progressive MS; **pwMS** = people with MS; **RRMS** = relapsing remitting MS; **SDMT** = Symbol Digits Modalities Test; **SPMS** = secondary progressive MS; **T1w** = T1-weighted; **T2LV** = T2 lesion volume; **T25FWT** = timed 25-foot walk test; **VLGT** = Verbale Leer-en Geheugentaak.

Introduction

Multiple sclerosis (MS) is a chronic neurodegenerative disease of the CNS that is characterized by neuroinflammation, demyelination, and neurodegeneration.[1] Next to physical disability, cognitive decline occurs in 40%–70% of patients with MS and severely affects daily functioning.[2,3] Age and neurodegeneration are associated with disease progression in MS, influencing both disability and cognitive decline.[4] Older individuals and those with more brain atrophy exhibit more disability and cognitive impairment regardless of disease duration.[5] Some of the changes to brain structure observed in healthy ageing appear similar to neurodegenerative brain changes because of MS.[6] While atrophy is commonly assessed by MRI-based brain volume quantification,[7,8] that approach presents several limitations: brain volume is strongly age-dependent, varies across different brain regions, and can be difficult to interpret clinically.[9]

Recent artificial intelligence techniques, trained on healthy ageing individuals, can estimate a person's age from conventional brain-MRI scans.[10-12] This is quantified using the brain-predicted age difference (brain-PAD), which is the difference between the individual's brain predicted age and chronological (true) age. In people with MS (pwMS), brain-PAD was found to be higher than in healthy controls (HCs).[13,14] Previous research shows that in pwMS, higher brain-PAD is related to more clinical disability as measured by the expanded disability status scale (EDSS),[13] as well as to more cognitive decline[15] as measured with the Symbol Digits Modalities Test (SDMT). However, these studies used single measures of disability and cognition in groups with varying ages, which means that age remains an important confounding factor.

As MS disease duration is correlated with age, disentangling their individual contributions to disease progression remains challenging. A clearer understanding of how neurodegenerative processes affect the brain in MS requires studying cohorts independent of chronological age. This study investigates the individual differences of brain-PAD for pwMS in a population based cross-sectional cohort of pwMS all born in the same year of 1966.[16] The goal of this study was to investigate the association of brain-PAD with (1) different MS disease subtypes, (2) clinical disability, and (3) cognitive impairment in a cohort of pwMS of the same age. In addition, we examine the added explanatory value of brain-PAD to the brain parenchymal fraction (BPF) and to T2 lesion volume (T2LV) on the clinical outcome measures.

Methods

Study Population

This retrospective study included participants who were part of Project Y from the MS Centre Amsterdam, as previously described,[16] which is a population based cross-sectional cohort of pwMS born in 1966 in the Netherlands. To be considered eligible for participation in Project Y, patients were required to meet all of the following criteria: (1) born in the Netherlands in 1966; (2) currently living in the Netherlands; (3) diagnosis of MS according to the 2010[17] or 2017[18] McDonald criteria. HC had to meet the following criteria (1) born in the Netherlands between 1965 and 1967; (2) currently living in the Netherlands; (3) no history of MS. After screening, a total of 367 pwMS were included, of which 271 pwMS and 125 HC visited our center for a full day of tests. Inclusion criteria of this analysis were pwMS and HC who had undergone MRI, cognitive assessments, and, for pwMS, disability evaluations. Participants were excluded if they had missing data on the clinical measures or if no MRI scan of sufficient quality was available.

Standard Protocol Approvals, Registrations, and Patient Consents

The Medical Ethical Committee of the Amsterdam UMC, location VUmc approved the Project Y protocol. Written informed consent was obtained from all participants at inclusion, according to the Declaration of Helsinki. The study is registered at the Netherlands Trial Register (NL6362).

Physical Disability

All examinations were performed during a 1-day study visit, between December 2017 and January 2021. To assess disability, the EDSS was used.[19] Upper limb functioning was quantified using the 9-hole peg test (9HPT),[20] where the average of the 2 dominant hand and 2 nondominant hand

trials was used. Lower limb functioning was quantified using the timed 25-foot walk test (T25FWT)[21] and was scored by averaging 2 trials.

Cognitive Measures

Objective cognitive functioning was measured using a test battery based on the Minimal Assessment of Cognitive Function in MS (MACFIMS) study.[21] The battery consists of multiple tests assessing: verbal fluency (Controlled Oral Word Association Test, COWAT), visuospatial perception (Benton Judgment of Line Orientation Test, JLO), visuospatial memory (Brief Visuospatial Memory Test-Revised, BVMT-R), verbal memory (Verbale Leer-en Geheugentaak, VLGT, the Dutch version of the California verbal learning test), information processing speed (SDMT), and executive functioning (Delis-Kaplan Executive Function System sorting test, D-KEFS).

MRI

All participants underwent 3T MRI of the brain, using the same scanner for all subjects (3T, Discovery MR750; GE, Milwaukee, WI). Brain age was determined using a 3-dimensional (3D) T1-weighted (T1w) fast spoiled gradient-echo sequence (repetition time 8.2 milliseconds, echo time 3.2 milliseconds, flip angle 12°, 1 × 1 × 1 mm voxel size, acquisition direction: sagittal). A 3D fluid attenuated inversion recovery (3D-FLAIR) image (repetition time 8,000 milliseconds, echo time 125 milliseconds, inversion time 2,350 milliseconds, 1.2 × 1 × 1 mm voxel size, acquisition direction: sagittal) was used for brain T2 lesion detection.

Brain Age Prediction

Brain age was predicted using a publicly available software (brainageR),[10,22-24] which uses unprocessed 3D T1w-MRI scans to predict brain age with Gaussian Process Regression (GPR).[25] brainageR was trained on data from 3,377 healthy individuals (mean age ± SD 40.6 ± 21.4 years; range 18–92 years) across 7 publicly available datasets and tested on an independent cohort of 857 healthy individuals (mean age ± SD 40.1 ± 21.8 years; range 18–90 years). All participants were confirmed healthy based on local study data.

Our input T1w scans were preprocessed with SPM12[26] for segmentation and normalization to a template, then converted into feature vectors representing grey matter, white matter and CSF, which were masked using a brainageR-specific template and reduced through principal component analysis (PCA) using PCA parameters derived from the original training set, as part of the standard pipeline. The input T1w scans were not lesion filled, based on previous work that showed lesion filling did not significantly influence brain age estimations.[27] Principal components were used as input to the GPR model to predict brain age. Brain-PAD was calculated as brain predicted age minus chronological age.

MRI Features

Raw 3D T1w-MRI scans were bias-field corrected using ANTS,[28] followed by skull-stripping for T1w and 3D-FLAIR images using HD-BET.[29] After linearly registering FLAIR to T1w, lesion segmentation was performed using nicMSlesions.[30] The resulting lesions masks were manually corrected and used for T1 lesion filling with Lesion Segmentation Tool[31] to avoid potential variation on tissue segmentation because of MS lesions.[32] The manually corrected masks were used to determine T2 lesion volume with fslstats, from the FMRIB Software Library.[33] The recon-all pipeline of FreeSurfer 7.1.1[34] was used to automatically perform whole-brain tissue-type segmentation on the lesion-filled 3D T1w images. The BPF was obtained by dividing the total brain volume excluding ventricles (BrainSegVolNotVent) by the estimated total intracranial volume. Volume of the ventricles, white matter volume, deep grey matter volume, and cortical thickness were also obtained. These volumes were normalized for estimated total intracranial volume.

Statistical Analyses

Statistical analyses were performed using R Statistical Software (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria). Variables were tested for normality using histogram and QQ-plot inspection. The 9HPT and T25FWT scores were log-transformed. A p value <0.05 was considered statistically significant.

Group Differences

Brain-PAD and BPF were compared between HC and pwMS, and the subtypes relapsing remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS), using a general linear model (GLM) corrected for sex. Explanatory analysis was done to investigate group differences between HC, RRMS, and progressive MS (PMS, SPMS, and PPMS grouped), given the recognition of MS as a disease continuum.[35] Post hoc comparisons were conducted using Tukey's honestly significant difference (HSD) test, to account for multiple comparisons.

To standardize the neuropsychological test scores, all scores were corrected for effects of age, sex, and years of education present in the HC group, using linear regression models. Corrected scores were converted to Z-scores for all participants based on the means and standard deviations of the HC group. Participants were considered cognitively preserved (CP) if no more than 1 of 6 test scores fell below $Z = -1.5$, and otherwise as cognitively impaired (CI). Brain-PAD was compared between controls, CP and CI using a generalized linear model, adjusting for sex. The Tukey HSD test was used to account for multiple comparisons.

Univariate Regression Models

To investigate the influence of disease duration on brain ageing, we assessed its association with brain-PAD using linear regression, corrected for sex and disease type. The analysis was repeated for the association of BPF and disease duration. Linear regression corrected for sex was performed to investigate the association between brain-PAD and BPF, ventricle volume, white matter volume, deep grey matter volume, and cortical thickness.

Univariate linear regression models were used to examine the relation between brain-PAD as a predictor and disability as the outcome measure, for EDSS, and the log-transformed 9HPT and T25FWT. Patients unable to perform the T25FWT (≥180 seconds) or 9HPT (≥300 seconds) were excluded. To assess whether brain-PAD provides additional explanatory value beyond BPF and T2LV in relation to EDSS, we conducted a model comparison analysis. We used analysis of variance (ANOVAs) to compare models with BPF or T2LV as the sole predictor to models that additionally included brain-PAD. We also compared the adjusted R^2 values of the models to quantify the additional variance explained by brain-PAD.

The association between brain-PAD and each neuropsychological test (*Z*-score) was assessed using univariate linear regression, with neuropsychological performance modelled as the outcome. To evaluate the additional explanatory value of brain-PAD beyond BPF and T2LV on cognition as captured by the SDMT, we used again ANOVAs and compared the adjusted R^2 for the models with and without brain-PAD. All aforementioned regression models were corrected for sex and not corrected for multiple testing.

Data Availability

Data may be shared (pseudonymized) at the request of any qualified investigator for purposes of replicating procedures and results.

Results

Descriptives and Group Differences

Demographic, clinical, and MRI characteristics are presented in Table 1. From the original Project Y cohort, we excluded 130 out of 367 pwMS and 9 out of 125 HC because of missing or insufficient quality data. The final cohort in this study included 116 HC (75% female) and 237 pwMS (72% female), comprising 150 RRMS, 54 SPMS, and 33 PPMS. Mean age was 52.9 (±1.14) years for HC and 52.8 (±0.90) years for pwMS. There were no differences in the distributions of age and sex between pwMS and HC. The median EDSS was 3.5 (interquartile range 2.5–4.0). The disease duration varied for all MS subtypes: 16.0 (±9.4) years for RRMS, 20.8 (±8.3) years for SPMS, and 10.3 (±7.2) years for PPMS. PwMS had a lower median educational level compared with HC (level 5 for pwMS vs level 6 for HC).

Group Differences

Brain-PAD was significantly lower than 0 for HC at −5.74 (±6.5) years ($p < 0.0001$). In our cohort, compared with HC, brain-PAD was higher for all pwMS (+9.7 years, $p < 0.0001$) and each separate disease type: RRMS (+8.7 years, $p < 0.0001$), SPMS (+12.7 years, $p < 0.0001$), and PPMS (+9.5 years, $p < 0.0001$). Brain-PAD was higher for SPMS vs RRMS (+4.0 years, $p < 0.01$) (Figure 1). Brain-PAD of PPMS did not differ from RRMS and SPMS. BPF was lower for all pwMS (BPF = 0.712 ± 0.035) compared with HC (BPF = 0.737 ± 0.028). When grouping SPMS and PPMS to PMS, brain-PAD was not significantly higher for PMS, compared with RRMS (2.49 years, $p = 0.062$). There were no differences in BPF between MS subtypes (Figure 2). BPF did not differ between RRMS and PMS (−0.00098, $p = 0.97$).

Classification based on 2 or more cognitive tests with a *Z*-score below −1.5 classified 149 pwMS as CP, and 86 pwMS as CI. Analyses for differences between HC, CP and CI using a GLM showed group differences in brain-PAD between all 3 cognitive groups (all $p < 0.0001$, Figure 3): mean brain-PAD was −5.74 (±6.5) years for HC, 2.23 (±7.9) years for CP, and 6.99 (±8.8) years for CI.

Univariate Regression Models

Brain-PAD was associated with longer disease duration ($\beta = 0.210$, $p < 0.001$) (Figure 4). The association indicates that for every 10 years of disease duration, brain-PAD gets higher by 2.10 years. BPF was associated with disease duration ($\beta = -8.28e\text{-}05$, $p = 0.00123$). This association was stronger for brain-PAD (std. $\beta = 0.261$) than for BPF (std. $\beta = 0.21$), when comparing standardized betas.

Brain-PAD was significantly correlated to EDSS ($\beta = 0.028$, $p < 0.05$). In addition, brain-PAD was associated with the 9-HPT ($\beta = 0.0074$, $p < 0.001$) and the T25FWT ($\beta = 0.0063$, $p < 0.05$) (Figure 5). In addition, we investigated whether brain-PAD adds explanatory value to the relation between BPF and EDSS. The model comparison using ANOVA revealed a significant improvement in model fit with the addition of brain-PAD ($p < 0.01$). The adjusted R^2 of the model of only BPF was 0.030, while the model including brain-PAD had an R^2 of 0.054, indicating that brain-PAD explains additional variance in EDSS. The adjusted R^2 of only T2LV on EDSS was 0.054, while the model including brain-PAD had an adjusted R^2 of 0.067. The model comparison using ANOVA showed no significant improvement with the addition of brain-PAD to T2LV ($p = 0.10$). Higher brain-PAD was associated with lower BPF, higher ventricle size, lower white matter volume, deep grey matter volume, and lower cortical thickness (eTable 1).

Linear regression with brain-PAD as predictor showed an association with the *Z*-scores of all cognitive tests: SDMT: $\beta = -0.056$, $p < 0.0001$; COWAT: $\beta = -0.023$, $p < 0.05$; JLO: $\beta = -0.021$, $p < 0.01$; DKEFS: $\beta = -0.021$, $p < 0.01$; VLGT: $\beta = -0.031$, $p < 0.01$; and BVMT: $\beta = -0.036$, $p < 0.001$ (eFigure 1). Additional analysis showed that adding brain-PAD to the model significantly improved the explanation of the relation between BPF and SDMT ($p < 0.001$). The adjusted R^2 of the model without brain-PAD was 0.160, while the model including brain-PAD had an adjusted R^2 of 0.191, indicating that brain-PAD explained additional variance for the SDMT beyond BPF. The adjusted R^2 of

Table 1 Demographics, Clinical, and MRI Characteristics of People With MS and HC

	HC (n = 116)	All patients (n = 237)	RRMS (n = 150)	SPMS (n = 54)	PPMS (n = 33)
Demographic features					
Age, y, mean (SD)	52.9 (1.14)	52.8 (0.90)	52.8 (0.93)	52.7 (0.78)	53.1 (0.94)
Sex (female), n (%)	87 (75)	171 (72)	122 (81)	33 (61)	16 (48)
Level of education, median (IQR)[a]	6 (5–6)[b]	5 (5–6)	6 (5–6)	5 (5–6)	6 (5–6)
Clinical features					
Disease duration since onset, y, median (IQR)		16.3 (8.2–24.4)	16.0 (8.1–24.4)	20.8 (15.7–27.9)	10.3 (5.0–15.1)
Time since last clinical MS relapse					
DMT ever (yes), n (%)		172 (69.9)	114 (73.1)	47 (83.9)	11 (32.4)
Brain age, y, mean (SD)	47.2 (6.7)[b]	56.8 (8.6)	55.8 (9.0)	59.8 (8.1)	56.8 (6.9)
Brain-PAD, y, mean (SD)	−5.7 (6.5)[b]	4.0 (8.6)	3.0 (9.0)	7.0 (8.0)	3.8 (6.7)
Brain parenchymal fraction, mean (SD)	0.737 (0.028)[b]	0.712 (0.035)	0.712 (0.030)	0.711 (0.037)	0.712 (0.048)
T2 lesion volume mL, median (IQR)	0.62 (0.0026–0.54)[b]	10.1 (2.9–13.2)	9.0 (2.8–11.7)	13.0 (3.7–18.3)	10.4 (2.7–12.9)
Clinical scores					
EDSS, median (IQR)		3.5 (2.5–4.0)	3.0 (2.3–4.0)	5.5 (3.75–6.25)	4.0 (3.5–6.0)
9HPT (left and right), s, median (IQR)[c]		21.7 (19.5–25.1)	4.0 (18.9–23.4)	24.2 (21.5–39.5)	23.1 (21.7–27.7)
T25FWT, s, median (IQR)[c]		4.9 (4.2–6.4)	4.5 (3.9–5.4)	7.2 (5.0–15.7)	5.9 (4.9–8.0)
SDMT, mean (SD)	58.8 (8.3)	50.6 (10.9)	52.7 (10.1)	46.5 (10.4)	48.4 (12.9)
COWAT, mean (SD)	13.8 (3.7)	12.0 (4.0)	12.6 (3.8)	10.8 (3.7)	11.3 (4.7)
JLO, mean (SD)	26.5 (3.2)	25.4 (4.1)	25.5 (3.8)	25.4 (5.0)	25.4 (3.1)
DKEFS, mean (SD)	10.9 (1.9)	9.9 (2.3)	10.1 (2.2)	9.7 (2.4)	9.3 (2.6)
VLGT-r, mean (SD)	10.9 (2.1)	9.7 (2.2)	10.0 (2.1)	9.2 (2.3)	9.3 (2.4)
BVMT-r, mean (SD)	8.9 (1.5)	7.8 (2.2)	8.0 (2.0)	7.5 (2.2)	7.7 (2.7)

Abbreviations: BVMT-r = Brief Visuospatial Memory Test-Revised; COWAT = Controlled Oral Word Association Test; DKEFS = Delis-Kaplan Executive Function System sorting test; DMT = disease-modifying therapy; IQR = interquartile range; JLO = Benton Judgment of Line Orientation Test; 9HPT = 9 hole peg test; PPMS = primary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SDMT = symbol digit modalities test; SPMS = secondary progressive multiple sclerosis; T25FWT = timed 25 foot walk test; VLGT-r = Verbale Leer-en Geheugentaak-Revised.
[a] The scale used to assess education ranged from 1 (did not finish primary school) to 7 (university or higher); 5 = secondary vocational education (MBO); 6 = higher professional education (HBO).
[b] Indicates a significant difference ($p < 0.05$) between patients with MS and HC.
[c] The average of the measurements is shown.

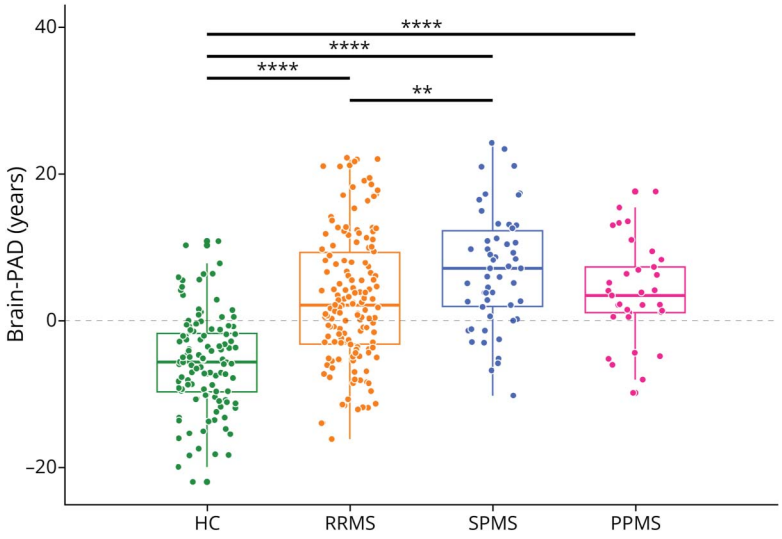
only T2LV on SDMT was 0.195, while the model including brain-PAD had an adjusted R^2 of 0.216. The model comparison using ANOVA showed significant improvement with the addition of brain-PAD to T2LV ($p < 0.001$).

Discussion

To better understand the relation of the brain-PAD with disability and cognitive function in multiple sclerosis, without the confounding effects of age, we investigated these associations in a large cohort of pwMS of the same age (52.8 years). In this same-age cohort, pwMS had higher brain-PAD than HC, and higher brain-PAD was significantly associated with worse physical and cognitive disability. In addition, brain-PAD had added value over BPF in explaining clinical disability and cognition outcome measures.

We have found that brain-PAD of HC was significantly below zero, indicating that their predicted brain age is consistently lower than their chronological age. A brain-PAD deviating from zero may reflect a systematic bias in the brain age model, possibly because of differences in MRI parameters or scanner effects,[36] emphasizing the need for harmonization or adjustment strategies. In addition, this could be due to different demographics in the study population and the population used to train the brain age model which should be validated with an additional control dataset. Such baseline offsets underscore the importance of considering model bias when interpreting group differences: Absolute brain-PAD values may be shifted, but relative comparisons between patient and

Figure 1 Boxplots of Brain-PAD for HC and MS Subtypes

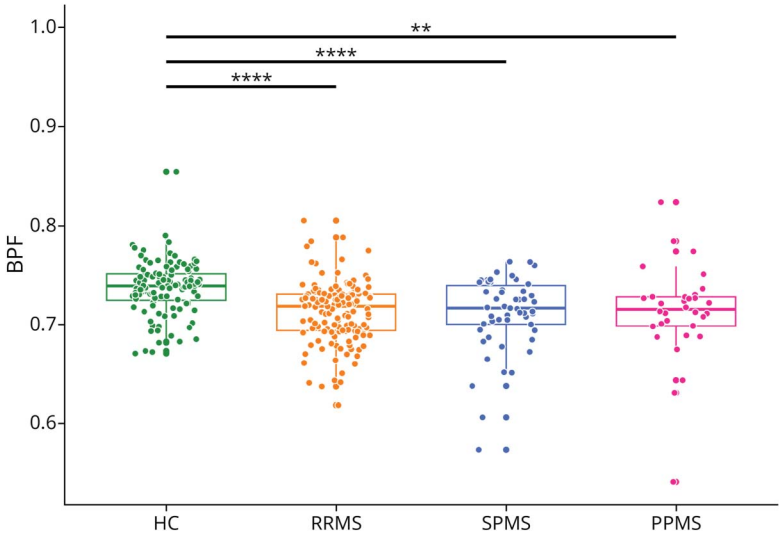


Boxplots depicting the distributions of brain-PAD in years for HC and the 3 subtypes of MS (RRMS, SPMS, and PPMS). There were no significant differences between PPMS and the other MS-subtypes. $^{**}p < 0.01$, $^{****}p < 0.0001$. Brain-PAD = brain-predicted age difference; HC = healthy control; MS = multiple sclerosis; PPMS = primary progressive MS; RRMS = relapsing remitting MS; SPMS = secondary progressive MS.

control groups remain informative if bias affects both groups similarly. Because this study examined only a single cohort, relative comparisons within the groups are valid despite any baseline offset in absolute brain-PAD values. Brain-PAD was higher for all subtypes of MS compared with HC, and between the MS subtypes, brain-PAD was found to be higher in individuals with SPMS compared with RRMS. This difference is likely driven by longer disease duration in SPMS, as we also found a positive relation between disease duration and brain-PAD. These findings support the concept that brain-PAD reflects cumulative neurodegenerative processes over time because of MS, which are independent of ageing.[27] We did not observe differences in BPF between any of the MS subtypes. This suggests that brain-PAD captures brain changes that are not fully reflected by global volumetric measures. Grouping SPMS and PPMS to PMS did not show differences between RRMS and PMS for both brain-PAD as BPF, indicating that brain-PAD is sensitive to disease stage (i.e., RRMS/SPMS) and not to disease subtype (RRMS vs PPMS/PMS). In a previous study on these data of differences in MRI measures of brain damage and disability and MS subtypes, the difference between subtypes was found to be significantly smaller than the difference between patients and controls.[37]

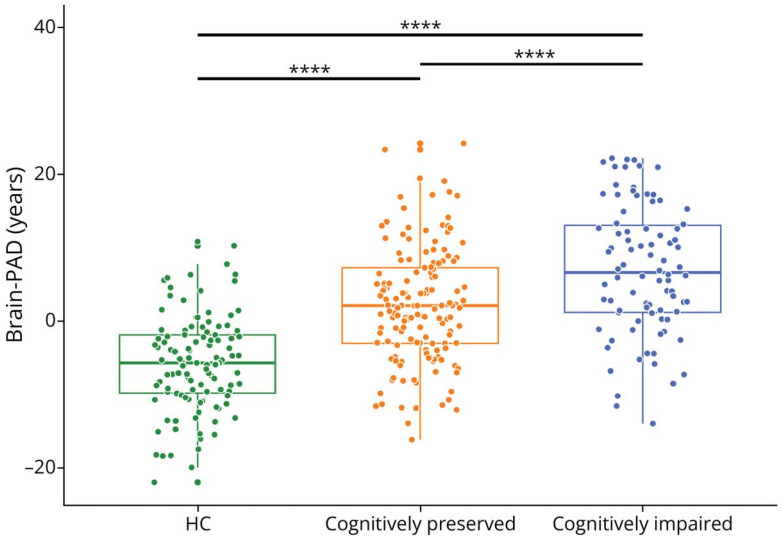
Our findings show that brain-PAD was associated with more severe disability in pwMS. Higher brain-PAD were related to

Figure 2 Boxplots of Brain Parenchymal Fraction for HC and MS Subtypes



Boxplots depicting the distributions of BPF for HC and the 3 subtypes of MS (RRMS, SPMS, and PPMS). There were no significant differences between the MS-subtypes. $^{**}p < 0.01$, $^{****}p < 0.0001$. BPF = brain parenchymal fraction; HC = healthy control; MS = multiple sclerosis; PPMS = primary progressive MS; RRMS = relapsing remitting MS; SPMS = secondary progressive MS.

Figure 3 Boxplots of Brain-PAD for HC and Cognitive Groups

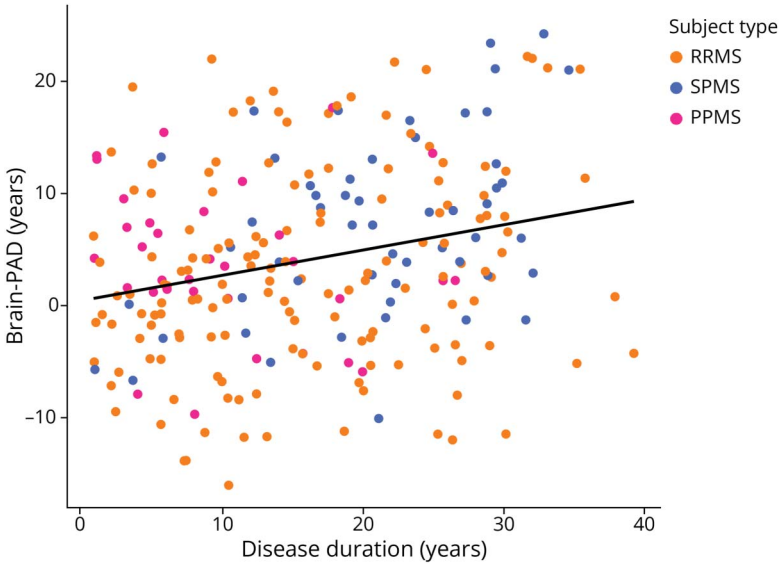


Boxplots depicting the distributions of brain-PAD in years for HC, cognitively preserved and cognitively impaired pwMS. There were differences between all 3 groups. ****$p < 0.0001$. Brain-PAD = brain-predicted age difference; HC = healthy control.

higher EDSS scores and worse performance on the 9HPT and T25FWT, indicating that greater brain ageing is related to higher global disability and reduced upper and lower limb functioning. These results are consistent with previous studies reporting associations between increased brain age and physical disability in MS.[27,38] Adding brain-PAD to the model improved the explained variance for EDSS beyond BPF, suggesting that brain-PAD captures much of the relevant neurodegenerative burden related to disability. This aligns with the concept that brain-PAD may reflect broader neurodegenerative changes associated with disability, highlighting its potential role as a marker of disease severity.[39] The modest adjusted R^2 values of our models align with the multifactorial nature of disability and cognitive measures in MS, which are influenced by a wide range of biological and clinical factors. This means that these findings should be interpreted cautiously, particularly with regard to their predictive value at the individual patient level. Brain-PAD did not statistically significant explain additional variance beyond T2LV for EDSS. Brain-PAD was associated with all MRI measures of structural brain damage, indicating that it reflects structural changes across the entire brain.

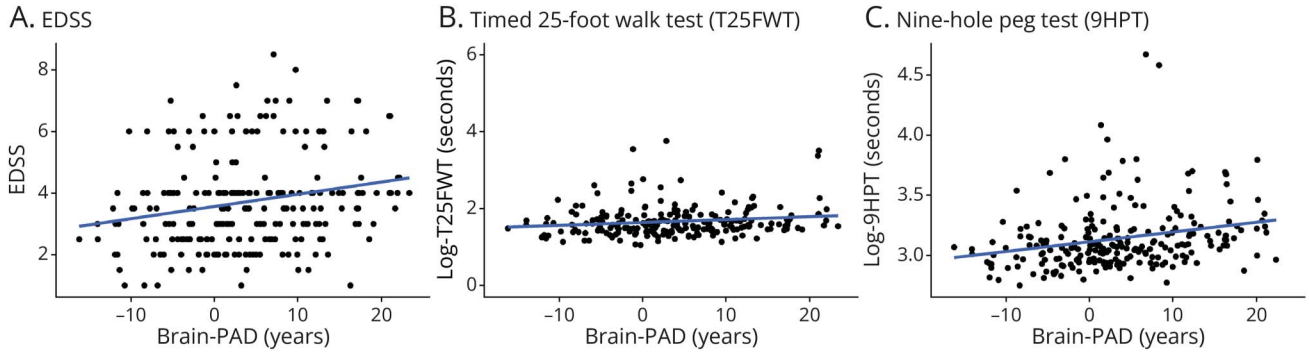
In addition, we examined the relation with cognitive performance using the MACFIMS battery, providing additional

Figure 4 Scatterplot of Brain-PAD Plotted Against Disease Duration



Scatterplot of the brain-PAD in years of the 3 MS-subtypes plotted against the disease duration in years and the corresponding correlation (β = 0.21, $p < 0.001$). Brain-PAD = brain-predicted age difference; MS = multiple sclerosis; PPMS = primary progressive MS; RRMS = relapsing remitting MS; SPMS = secondary progressive MS.

Figure 5 Scatterplot of Disability Measures Plotted Against Brain-PAD



Relation between brain-PAD and (A) EDSS ($p < 0.05$), (B) timed 25-foot walk test ($p < 0.05$), and (C) 9-hole peg test ($p < 0.001$). Brain-PAD = brain-predicted age difference; EDSS = expanded disability status scale.

insights into the cognitive domains affected in MS. We found that brain-PAD was higher for CI pwMS, compared to CP pwMS and HC. Interestingly, CP individuals with MS also showed higher brain-PAD compared with HC, despite no measurable cognitive impairment. This suggests that CP may already exhibit subtle brain ageing related changes, which is in line with the concept of cognitive reserve.[40] However, the extent of these changes may not yet be sufficient to cause clinical consequences. This may be explained by cognitive reserve, which can help maintain cognitive functioning despite underlying brain aging-related changes, thereby delaying the onset of observable symptoms. Greater brain aging may serve as an early marker of neurodegenerative burden for cognitive impairment. These findings are in line with previous research demonstrating associations between brain age and SDMT performance in MS. Extending this work, our study shows that advanced brain ageing is not only related to worse performance on SDMT but is also associated with broader cognitive impairment as assessed by the full MACFIMS battery.[15] Individual analysis using univariate linear regression of brain-PAD and the cognitive measures showed a relation between brain-PAD and each cognitive domain. The strongest relation was found for the SDMT and the BVMT, indicating that accelerated ageing of the brain affects the cognitive domains of information processing speed, and the visuospatial memory the most. Brain-PAD significantly improved the prediction of the SDMT-scores, beyond BPF and T2LV. The increase in explained variance suggests that next to brain volume measurements and lesion volume, brain-PAD carries additional, independent information relevant to cognitive performance.

The strength of this study is the unique cohort of pwMS and HC all of the same age used in this study, minimizing age-related variation. All participants were scanned on the same MRI scanner, eliminating the need to correct for different scanner of software differences. Building on earlier research, this study adds value by incorporating well-documented and comprehensive measures of disability and cognition to investigate brain age in MS.

Several limitations should be acknowledged. First, the cross-sectional design of the study prevents assessment of the evolution of brain-PAD and its effect on disability progression. Second, the small sample size of the PPMS group may have influenced the results, potentially explaining inconclusive findings for this subtype. In addition, including patients with a short disease duration would be of interest, although this is not feasible in the current cohort consisting of relatively older patients. Finally, only 1 brain age prediction method, brainageR, was used in this study. While brainageR is considered one of the widely used methods,[41] other models may yield different or potentially more accurate results, which could further enhance our understanding of brain aging in MS.

Future studies should examine the influence of disease modifying factors not related to MS on brain aging to provide further insight into how these variables relate to brain-PAD. We plan to investigate how the different brain structures and volumetrics relate to brain-PAD, to explain what the driver of an increase in brain age is in MS, in additional cohorts with different disease durations. Given its association with both disability progression and cognitive decline, brain-PAD could serve as a valuable biomarker for monitoring neurodegeneration in pwMS. Further validation in larger, longitudinal studies is necessary to confirm its clinical utility and establish standardized protocols for its use in routine clinical practice. These studies could clarify whether brain-PAD can complement existing clinical tools, such as quantitative MRI measures or clinical testing. Future studies should also address issues of feasibility, including computational demands, interpretability for clinicians, integration into existing diagnostic workflows, and potential legal considerations, such as those relating to ethics and governance. In addition, these longitudinal studies could stratify patients based on progression independent of relapse activity vs relapse-driven progression because progression independent of relapse activity reflects underlying neurodegeneration[42] and brain age

may serve as a useful marker of this process, which we were unable to do in this study.

To conclude, this study demonstrates that individuals with MS exhibit excessive brain aging compared with HCs, even within an age-matched, population-based cohort. Greater brain aging is associated with longer disease duration and greater disease severity. Older-appearing brains are related to more severe physical disability and reduced cognitive functioning. These findings underscore the potential of brain age as a marker for neurodegeneration and overall disease severity in MS.

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Author Contributions

L. Bos: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. A.M. Wink: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. J.H. Cole: drafting/revision of the manuscript for content, including medical writing for content. E.M.M. Strijbis: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. B. Moraal: drafting/revision of the manuscript for content, including medical writing for content; study concept or design. J. Killestein: drafting/revision of the manuscript for content, including medical writing for content. H. Vrenken: drafting/revision of the manuscript for content, including medical writing for content. B.M.J. Uitdehaag: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design. F. Barkhof: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design. M.M. Schoonheim: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. B. Jasperse: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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