

Early imaging predictors of long-term outcomes in relapse-onset multiple sclerosis

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ABSTRACT

The clinical course of relapse-onset multiple sclerosis is highly variable. Demographic factors, clinical features and global brain T2 lesion load have limited value in counselling individual patients. We investigated early MRI predictors of key long-term outcomes including secondary progressive multiple sclerosis, physical disability and cognitive performance, 15 years after a clinically isolated syndrome (CIS). A cohort of CIS patients (n=178) was prospectively recruited within 3 months of clinical disease onset and studied with MRI scans of the brain and spinal cord at study entry (baseline) and after 1- and 3-years. MRI measures at each time point included: supratentorial, infratentorial, spinal cord and gadolinium-enhancing lesion number, brain and spinal cord volumetric measures. The patients were followed-up clinically after ~15 years to determine disease course, and disability was assessed using the Expanded Disability Status Scale (EDSS), Paced Auditory Serial Addition Test (PASAT) and Symbol Digit Modalities Test (SDMT). Multivariable logistic regression and multivariable linear regression models identified independent MRI predictors of secondary progressive multiple sclerosis and EDSS, PASAT and SDMT respectively. After 15 years, 166 (93%) patients were assessed clinically: 119 (72%) had multiple sclerosis (94 [57%] relapsing-remitting, 25 [15%] secondary progressive), 45 (27%) remained CIS and 2 (1%) developed other disorders. Physical disability was overall low in the multiple sclerosis patients (median EDSS 2, range 0 – 10); 71% were untreated. Baseline gadolinium-enhancing (odds ratio [OR] 3.16, $p < 0.01$) and spinal cord lesions (OR 4.71, $p < 0.01$) were independently associated with secondary progressive multiple sclerosis at 15 years. When considering 1- and 3-year MRI variables, baseline gadolinium-enhancing lesions remained significant and new spinal cord lesions over time were associated with secondary progressive multiple sclerosis. Baseline gadolinium-enhancing ($\beta = 1.32$, $p < 0.01$) and spinal cord lesions ($\beta = 1.53$, $p < 0.01$) showed a consistent association with EDSS at 15 years. Baseline gadolinium-enhancing lesions was also associated with performance on

the PASAT ($\beta = -0.79, p < 0.01$) and SDMT ($\beta = -0.70, p = 0.02$) at 15 years. Our findings suggest that early focal inflammatory disease activity and spinal cord lesions are predictors of very long-term disease outcomes in relapse-onset multiple sclerosis. Established MRI measures, available in routine clinical practice, may be useful in counselling patients with early multiple sclerosis about long-term prognosis, and personalising treatment plans.

INTRODUCTION

The course of relapse-onset multiple sclerosis is highly variable with marked differences in the rate of disease progression, accrual of physical disability and cognitive impairment. Demographic and clinical variables have limited value of predicting the course of multiple sclerosis in individual patients and there is a need for robust prognostic markers in people with early multiple sclerosis to: (1) inform patients about how their condition might progress in the future; (2) to help select disease-modifying therapies and personalise treatment plans; and (3) to identify patients at high-risk for disease progression in order to target future neuroprotective treatments.

Magnetic resonance imaging (MRI) is an established prognostic marker in patients with clinically isolated syndromes (CIS) suggestive of multiple sclerosis. Asymptomatic T2-hyperintense brain lesions are helpful in identifying patients at high-risk of a second clinical attack (Fisniku *et al.*, 2008; Optic Neuritis Study Group, 2008; Tintore *et al.*, 2015), but the relationship between brain T2 lesion load and long-term disability is less robust. Previous studies with long-term follow-up (>10 years) have found only a modest relationship between global brain T2 lesion load at CIS onset and later physical disability (Fisniku *et al.*, 2008; Optic Neuritis Study Group, 2008). Change in global brain T2 lesion load, particularly in the first few years after disease onset, may have a stronger relationship with long-term physical disability, albeit still limited (Fisniku *et al.*, 2008). Lesion location and activity may be better predictors of future physical disability, at least in the short to medium term. Asymptomatic infratentorial (Minneboo *et al.*, 2004; Swanton *et al.*, 2009; Tintore *et al.*, 2010), spinal cord (Swanton *et al.*, 2009; Brownlee *et al.*, 2017; Arrambide *et al.*, 2018) and gadolinium-enhancing lesions (Swanton *et al.*, 2009; Di Filippo *et al.*, 2010) are associated with the

development of physical disability (measured using the Expanded Disability Status Scale [EDSS]) over the first 5 – 7 years after a CIS.

In the longer term, the onset of secondary progression is the major determinant of physical disability in relapse-onset multiple sclerosis. Natural history studies suggest that half of patients will develop secondary progressive multiple sclerosis in the first 15 years after disease onset (Scalfari *et al.*, 2014), although more recent observational cohorts suggest this risk may be lower than previously thought (Kerbrat *et al.*, 2015; Cree *et al.*, 2016). Identifying patients with a shorter latency to onset of secondary progression is a key priority in order to select appropriate treatments strategies. Whether lesional MRI abnormalities associated with short-term changes in physical disability (Swanton *et al.*, 2009; Tintore *et al.*, 2010; Brownlee *et al.*, 2017; Arrambide *et al.*, 2018) are also important in the development of secondary progressive multiple sclerosis and long-term disability is unclear.

Cognitive impairment is also an important source of disability in people with multiple sclerosis, impacting on physical functioning, quality of life and employment. Cognitive impairment can develop in the absence of significant physical disability and is not adequately captured by commonly used multiple sclerosis disability metrics, including the EDSS. Advanced MRI measures including whole brain and grey matter tissue damage (detected using magnetization transfer imaging) and short-term changes in brain volume have been associated with long-term cognitive impairment in established multiple sclerosis (Deloire *et al.*, 2011; Filippi *et al.*, 2013). However, the relationship between conventional MRI measures available in routine clinical practice, such as T2 lesion load, location and activity, and long-term cognitive performance is unresolved.

We aimed to identify early MRI predictors of long-term outcomes in relapse-onset multiple sclerosis, including (i) secondary progressive disease course; (ii) physical disability; and (iii) cognitive performance. We studied a cohort of patients with clinically isolated syndromes suggestive of multiple sclerosis who had MRI scans around the time of presentation and follow-up MRI scans after 1 and 3 years. We investigated the prognostic value of established MRI measures available clinically (including T2 lesion number, lesion location and gadolinium-enhancing lesions), plus early changes in brain and spinal cord volume, in the earliest stages of relapse-onset multiple sclerosis are associated with outcomes after 15 years.

MATERIALS AND METHODS

Patients

Between 1995 and 2004, patients seen at the National Hospital for Neurology and Neurosurgery and Moorfields Eye Hospital, London, United Kingdom with CIS suggestive of multiple sclerosis were invited to take part in a longitudinal clinical and imaging study. The inclusion criteria for the study: (1) age 16-50 years; (2) a “typical” syndrome suggestive of multiple sclerosis e.g. unilateral optic neuritis, partial myelitis, brainstem/cerebellar syndrome; and (3) no previous history of neurological symptoms. All of the patients were assessed clinically and with MRI within 3 months of presentation and invited to return for scheduled clinical and MRI follow-up after approximately 1, 3 and 5 years (Swanton *et al.*, 2009; Brownlee *et al.*, 2017). Between 2014 and 2016 (~15 years after disease onset) all patients were then invited to attend a long-term, clinical follow-up to ascertain clinical status and disability.

All patients provided written informed consent at the time of study entry and consent for continued participation in the study was obtained at each follow-up. The study was approved by the institutional research ethics committee and conducted in accordance with the Declaration of Helsinki.

Clinical assessments

Multiple sclerosis was diagnosed at 15 years using the McDonald 2010 criteria (Polman *et al.*, 2011) and disease course was classified as CIS, relapsing-remitting multiple sclerosis or secondary progressive multiple sclerosis using the Lublin and Reingold 1996 disease course definitions (Lublin *et al.*, 1996). At the last clinical visit, about 15 years after disease onset, physical disability was assessed using the Expanded Disability Status Scale (EDSS), the timed 25-foot walk test (TWT) and the 9-hole peg test (9-HPT) by one examiner (WJB). Cognition was assessed using the Paced Auditory Serial Addition Test (PASAT) (Cutter *et al.*, 1999) and Symbol Digit Modalities Test (SDMT) (Smith, 1982) by the same clinician (WJB). The PASAT and SDMT scorers were converted to *z*-scores using published age-matched norms, and patients with a *z*-score ≤ -1.5 were considered impaired. Premorbid cognitive performance was estimated using the National Adult Reading Test (NART) (Nelson, 1982). Self-reported fatigue was measured using the Fatigue Severity Scale (FSS) (Krupp *et al.*, 1989), with patients with scores of ≥ 5 classified as “fatigued” (Tellez *et al.*, 2006).

Physical disability was assessed using the telephone EDSS in people who were unable to attend in person for follow-up (Lechner-Scott *et al.*, 2003). Disease course and disability was corroborated wherever possible using hospital records in patients who were assessed by telephone interview.

MRI protocol and image-analysis

The patients underwent MRI scans of the brain and whole spinal cord within 3 months of CIS onset (“baseline”) and then after approximately 1 year and 3 years (Figure 1). Details of the MRI acquisition protocol are shown in Supplementary Table 1. All of the MRI scans were done on the same 1.5T Signa scanner (General Electric, Wisconsin, USA).

All MRI scans were reviewed by an experienced neuroradiologist who was blinded to the patient’s clinical status. The number of T2-hyperintense supratentorial, infratentorial and spinal cord lesions at baseline, and the number of new lesions after 1 year and 3 years, was recorded from proton density (PD)/T2-weighted scans of the brain and whole spinal cord. The number of gadolinium-enhancing lesions at baseline, 1 and 3 years was identified from post-contrast 2D T1-weighted scans. For brain volumetric measurements we used post-contrast 2D T1-weighted scans. T1-hypointense lesions were identified on the 2D T1-weighted scans and outlined using a semi-automated edge finding tool (JIM6, Xinapse systems, Aldwinckle, UK). Lesion masks used to fill T1-hypointense lesions (Prados *et al.*, 2016). The normalised brain volume (NBV) at baseline was calculated using SIENAX and the percentage brain volume change (PBVC) at 1- and 3-years (compared to baseline) was calculated using SIENA. The SIENAX and SIENA analyses used brain extraction tool (BET) for the skull only and the brain tissue mask was computed using Similarity and Truth Estimation for Propagated Segmentations (STEPS), to avoid bias due to cerebrospinal fluid (Cardoso *et al.*, 2013; Cawley *et al.*, 2018). The mean upper cervical cord cross-sectional area (UCCA) at the level of C2/C3 from reformatted sagittally-acquired images using an active surface model, as previously

described (Horsfield *et al.*, 2010; Brownlee *et al.*, 2017). The UCCA at baseline plus the absolute change and percentage change in UCCA after 1-year and 3-years were calculated.

Statistical analysis

Descriptive statistics are presented as median (interquartile range) unless otherwise stated. Patients were grouped by clinical status at 15 years (CIS, relapsing-remitting multiple sclerosis, secondary progressive multiple sclerosis). The Mann-Whitney U test was used to investigate differences in MRI measures at baseline and changes in MRI measures over time in different patient groups.

Multivariable logistic regression models were used to identify independent predictors of conversion to secondary progressive multiple sclerosis at 15 years. Three separate models were constructed using MRI data available at each of the three time points, and also using the following potential confounders: baseline EDSS, age at onset, gender, type of CIS presentation, the exact interval in years between onset and the 15-year assessment (i.e. disease duration). Use of disease-modifying treatments was not included, since the reverse causation involved in selecting patients for treatment (resulting in use disease-modifying treatments predicting greater odds of worse disease course) distorts the association between earlier variables and outcome. The following MRI data were included in each model:

- (i) Baseline model – MRI predictor variables were supratentorial, infratentorial, spinal cord and gadolinium-enhancing lesion number, using either binary (0/1+) or categorical (0/1/2+) alternatives); and NBV and UCCA as continuous variables.

- (ii) 1-year model – baseline MRI predictors (again using binary or categorical alternatives) plus the change in supratentorial, infratentorial and spinal cord lesion number at 1 year, the number of gadolinium-enhancing lesions at 1 year; and as continuous variables the PBVC from baseline to 1 year and the change in UCCA from baseline to 1 year.

- (iii) 3-year model – baseline and 1-year MRI predictors (again using binary or categorical alternatives) plus the change in supratentorial, infratentorial and spinal cord lesion number at 3 years, the number of gadolinium-enhancing lesions at 3 years; and as continuous variables the PBVC from baseline to 3 year and the change in UCCA from baseline to 3 year.

For these logistic regression models a manual forward step-wise process was used for the MRI predictors. The non-MRI baseline potential confounders were added singly to the best MRI models, and retained only if they materially affected either the MRI coefficients or their p-values, or were themselves significant. These potential confounders had a negligible impact on the MRI contributions, and were themselves non-significant, so were excluded from reported models. The binary form of MRI predictors was used unless the categorical alternative significantly improved model fit (assessed by log likelihood ratio comparison of nested models). Model predicted probabilities are reported for the various combinations of MRI predictor values. Overall model fit is reported using the model C-statistic (0.5, no predictive power, to 1, perfect prediction) and the model accuracy (using a 0.5 probability cut-off).

The same process was used to build multiple linear regression models for 15-year EDSS, the MSFC components, and SDMT. Baseline EDSS, age at onset, gender, type of CIS presentation and disease duration were investigated as possible confounders in the models, along with years of education and NART pre-morbid IQ (NART) for the PASAT and SDMT. Regression residuals showed no heteroscedasticity or important deviations from normality, but for EDSS as a precaution model inferences were checked using a non-parametric bias-corrected and accelerated bootstrap with 2000 replications; these bootstrap confidence intervals did not materially differ from the Normal-based estimates, so the latter are reported. Note, however, that for EDSS although the regression p-values, confidence intervals and R-squares are valid after the residual checks above, regression coefficients must be interpreted with caution: although they are meaningful relatively as comparisons of the strength of predictor associations, they are of limited value in absolute terms since the EDSS scale does not have a uniform linear interpretation.

Statistical analysis was performed in Stata 14.1. Statistical significance is reported as $p < 0.05$.

Data availability

Fully anonymised data is available on request to the corresponding author.

RESULTS

Out of 178 patients who were enrolled into the study at baseline, 166 (93%) were followed up after a mean of 15.1 years (range 11.2 – 19.7 years). Two patients were diagnosed with neuromyelitis optica spectrum disorder during follow-up (one each with aquaporin 4-IgG and myelin oligodendrocyte glycoprotein-IgG antibodies) and were excluded, leaving 164 patients. Disease course and physical disability at 15 years was assessed by telephone interview in 47 (29%) patients. The reasons for telephone interview were patient choice (n=34), living overseas (n=8), severe disability (n=3) and pregnancy (n=2). The baseline characteristics of the study cohort who were followed up after 15 years are shown in Table 1.

Disease course and disability after 15 years

During follow-up 119 (73%) patients developed multiple sclerosis and 45 (27%) patients remained CIS (i.e. a single clinical attack without MRI evidence of dissemination in space and time). At the time of CIS, 48 (29%) patients had MRI evidence of dissemination in space and time, so retrospectively would be classified as having multiple sclerosis. The proportion of patients who developed multiple sclerosis was similar in patients who presented with optic neuritis (74%), brainstem/cerebellar (65%) and spinal cord (64%) syndromes. Disease course was classified as relapsing-remitting multiple sclerosis in 94 (57% of the total cohort) patients and secondary progressive multiple sclerosis in 25 (15%) patients after 15 years. Thirty-four (21%) patients (all with multiple sclerosis) were receiving disease-modifying therapies at the time of follow-up, or had been exposed to one or more treatments in the past, including interferon- β (n=22), glatiramer acetate (n=11), natalizumab (n=5), fingolimod (n=4), dimethyl fumarate (n=1), teriflunomide (n=1) and alemtuzumab (n=1). The median time to starting disease-modifying therapy was 50 months (range 3 – 182 months) after CIS. Only 7 (4%) patients had been exposed to disease-modifying therapies for >6 months at the time of the 1 and/or 3 year follow-up MRI scans.

The median EDSS at follow-up was 0 (range 0 – 1) in patients who remained CIS and 2.0 (range 0 – 10) in patients with multiple sclerosis. A wide range of physical disability encompassing all levels of the EDSS was seen in the patients with multiple sclerosis at follow-up (Figure 2). Three patients had died from complications of severe multiple sclerosis and were assigned EDSS 10. The median EDSS at 15 years was similar in patients with different CIS presentations: optic neuritis 1.5 (range 0 – 10), brainstem syndrome 1.5 (range 0 – 10) and spinal cord syndrome 2.5 (range 0 – 8.5).

Cognition and self-reported fatigue were assessed in 104 (63%) patients at 15 years. Twenty-one (20%) patients had impaired performance on the PASAT and 27 (26%) patients on the SDMT, all of whom had multiple sclerosis. FSS scores in the fatigued range were reported in 31 (30%) patients, 29 of whom had multiple sclerosis.

Among the 12 (7%) patients who were lost to follow-up at 15 years, 75% were men. The other baseline demographic and clinical characteristics were similar. The mean follow-up for these patients was 3.8 years (range 0.6 – 8.7 years) and 9 (75%) were known to have developed multiple sclerosis with a median EDSS of 1 (range 0 – 2.5) at last follow-up.

Baseline and changes over the first 3 years predict secondary progressive course after 15 years

The baseline MRI scan was done a median of 44 days (range 4 – 90 days) after CIS onset. The baseline brain MRI showed one or more asymptomatic T2-hyperintense lesion(s) in 125 (76%) patients. Multiple sclerosis developed in 111 of 125 (89%) patients with an abnormal brain

MRI at baseline and 8 of 39 (21%) with a normal brain MRI. The baseline spinal cord MRI showed one or more asymptomatic T2-hyperintense lesion(s) in 58 (35%) patients. Multiple sclerosis developed in all 58 (100%) patients with an abnormal spinal cord MRI at baseline and in 62 of 106 (58%) patients with a normal spinal cord MRI. The baseline MRI showed one or more asymptomatic gadolinium-enhancing lesion(s) in 52 (32%) patients (49 brain MRI only, 3 spinal cord MRI only, 3 brain and spinal cord MRI). Multiple sclerosis developed in 51 (98%) patients with gadolinium-enhancing lesions. One patient with a single contrast-enhancing symptomatic brainstem lesion remained CIS.

At baseline, the patients who developed secondary progressive multiple sclerosis at 15 years had a higher median number of spinal cord lesions (1 vs 0, $p=0.022$) compared with patients who remained relapsing-remitting multiple sclerosis. The other baseline MRI findings were similar (Table 1).

Follow-up MRI scans of the brain and spinal cord were obtained after 1 ($n=135$) and 3 ($n=121$) years (Supplementary Table 2). Over the first year after CIS, the patients who developed secondary progressive multiple sclerosis showed a higher median number of new supratentorial (7 vs 5, $p=0.045$), infratentorial (1 vs 0, $p<0.001$) and spinal cord (1 vs 0, $p=0.001$) lesions compared with those with relapsing-remitting multiple sclerosis at 15 years (Supplementary Table 2). At 3 years, the patients who developed secondary progressive multiple sclerosis at 15 years also showed a higher median number of new infratentorial (2.5 vs 0, $p=0.015$) and spinal cord lesions (2.5 vs 0, $p<0.001$), and a higher number of gadolinium-enhancing lesions (1 vs 0, $p=0.030$) compared to the group with relapsing-remitting multiple sclerosis. A similar rate

of brain and spinal cord atrophy was observed in both groups over the first 3 years after disease onset.

Early MRI predictors of long-term secondary progressive multiple sclerosis course using regression models

Three multivariable logistic regression models, for potential predictors available at the three time points, were constructed to investigate the relationship between the development of secondary progressive multiple sclerosis at follow-up and early MRI abnormalities (Table 2). In the baseline MRI model gadolinium-enhancing lesions (≥ 2 lesions) and spinal cord lesions (≥ 1) were independently associated with a higher odds of conversion to secondary progressive at 15 years (C-statistic 0.76). In patients with no gadolinium-enhancing lesions and no spinal cord lesions at the time of CIS the estimated risk of secondary progressive multiple sclerosis at 15 years was 5.3% (95% CI 1.1, 9.5%), compared with 45.5% (95% CI 24.7, 66.4%) in those with at least one spinal cord lesion and two or more gadolinium-enhancing lesions.

In the model incorporating baseline and follow-up MRI data over 1 year, baseline gadolinium-enhancing lesions remained significant, and ≥ 1 new spinal cord and infratentorial lesions at 1 year were independently associated with a higher odds of secondary progressive multiple sclerosis at 15 years (C-statistic 0.86). In patients with no gadolinium-enhancing lesions at baseline and no new spinal or infratentorial lesions after 1 year the estimated risk of secondary progressive multiple sclerosis after 15 years was 3.0% (95% CI 0, 6.2%), compared with 85.2% (95% CI 67.7, 100%) in people with two or more gadolinium-enhancing lesions at baseline and new spinal and infratentorial lesions at 1 year. Finally, in the model incorporating all MRI data from baseline to 3 years, ≥ 1 new spinal cord lesions, and with borderline significance ≥ 1 new

infratentorial lesions, were independently associated with a higher odds of secondary progressive multiple sclerosis at 15 years (C-statistic 0.89). In patients with no new spinal or infratentorial lesions over the first 3 years after CIS the estimated risk of secondary progressive multiple sclerosis after 15 years was 0.9% (95%CI 0, 2.7%), compared with 53.1% (31.7, 74.6%) in patients with ≥ 1 new spinal and ≥ 1 new infratentorial lesions at 3 years.

Age, sex, CIS type, baseline EDSS and duration of follow-up had no material effect on any of the models, were not themselves significant, so were not retained.

Early MRI predictors of long-term physical disability

Multivariable linear regression models were used to examine the association of EDSS at 15 years and early MRI abnormalities at baseline, 1-year and 3-years (Table 3). In the model investigating baseline MRI predictors of long-term EDSS, ≥ 1 baseline supratentorial, gadolinium-enhancing and spinal cord lesions were independently associated with higher EDSS scores at 15 years ($R^2=0.31$). In the 1-year model, ≥ 1 baseline gadolinium-enhancing lesions and spinal cord lesions remained significant, and ≥ 1 new supratentorial, infratentorial and spinal cord lesions at 1-year were also associated with EDSS at 15 years ($R^2=0.48$). Finally, in the model considering all time points over the first 3 years after CIS, ≥ 1 baseline gadolinium-enhancing and spinal cord lesions remained significant, and ≥ 1 new spinal cord lesions and the PBVC at 3 years were also associated with higher EDSS ($R^2=0.58$).

TWT and 9HPT scores were available in a subgroup of patients at 15 years (n=112). One or more baseline gadolinium-enhancing and spinal cord lesions were independently associated

with slower walking speed, and ≥ 1 baseline gadolinium-enhancing lesions with worse 9HPT performance at 15 years.

Age, sex, CIS topography, baseline EDSS and duration of follow-up did not have any influence on the regression coefficients and were not retained in the final models examining physical disability.

Early MRI predictors of long-term cognitive performance

Cognitive testing was done in a subgroup of patients at 15 years (n=104). Multivariable linear regression models were used to examine the association of early MRI measures with long-term cognitive performance on the PASAT and SDMT (Table 4). One or more baseline gadolinium enhancing lesions were associated with reduced performance on the PASAT at 15 years. When 1-year MRI variables were included in the model ≥ 1 baseline gadolinium enhancing lesions remained significant and ≥ 1 new supratentorial lesions at 1 year were also associated with reduced performance on the PASAT at 15 years. The findings were similar for the SDMT (Table 4). The NART score, an estimate of premorbid intelligence, was significantly associated with PASAT (higher NART score associated with better PASAT performance) and was retained in all models.

Inclusion of MRI data from baseline to 3-years did not contribute additional information to the models, and baseline gadolinium enhancing lesions and new supratentorial lesions at 1 year remained the best predictors of PASAT and SDMT performance at 15 years.

DISCUSSION

We investigated early brain and spinal cord abnormalities over the first 3 years after a CIS to identify the most robust predictors of long-term outcomes after 15 years in people with relapse-onset multiple sclerosis. The major, novel findings of this work include: (1) a consistent association of spinal cord lesions, both at the time of presentation and new spinal cord lesions over the first 3 years of follow-up, with development of secondary progressive multiple sclerosis and physical disability after 15 years; (2) a consistent association of asymptomatic gadolinium-enhancing lesions seen at the time of CIS with the development of secondary progressive multiple sclerosis and later physical and cognitive performance; and (3) a stronger association of MRI measures of focal inflammatory disease activity (i.e. lesions) with long-term disease course, compared with brain and spinal cord atrophy, in the earliest stages of relapse-onset multiple sclerosis.

Previous studies have suggested that lesions in clinically-eloquent sites, such as the brainstem, cerebellum and spinal cord, may be associated with short-term changes in EDSS in CIS patients (Minneboo *et al.*, 2004; Swanton *et al.*, 2009; Tintore *et al.*, 2010; Brownlee *et al.*, 2017; Arrambide *et al.*, 2018). We evaluated the impact of lesion location on long-term disease outcomes in the most comprehensive analysis, considering not only global brain T2 lesion load but investigating separately the prognostic value of supratentorial, infratentorial and spinal cord lesions at the time of CIS, and early changes in lesion number at these sites. Spinal cord lesions seen at the time of CIS were associated with secondary progressive disease course and physical disability 15 years later. The importance of spinal cord lesions present at the time of CIS is emphasised in models incorporating changes in MRI measures over 1 and 3 years after CIS: baseline spinal cord lesions remained a significant predictor of EDSS at 15 years independent

of changes in brain and spinal cord lesions and atrophy at 1 and 3 years. New spinal cord lesions over time were also associated with secondary progressive multiple sclerosis at 15 years and later physical disability. . Collectively these findings suggest that early spinal cord damage is an important mechanism underlying both development of physical disability and secondary progression in early relapse-onset multiple sclerosis.

Pathological studies have found evidence of substantial axonal loss within chronic spinal cord lesions in multiple sclerosis (Lovas *et al.*, 2000) and this may result in more widespread neuroaxonal loss at distant sites due to the effects of Wallerian degeneration (Dziedzic *et al.*, 2010). Neuroaxonal loss within spinal cord pathways involved in locomotion, sensation and sphincter function may have important functional consequences, ultimately resulting in physical disability. In a recent study that quantified cervical cord lesion load on axial images with high in-plane resolution, patients with secondary progressive multiple sclerosis had a higher spinal cord lesion load compared relapsing-remitting multiple sclerosis patients, even after adjusting for differences in disease duration (Kearney *et al.*, 2015). These findings support the concept that focal spinal cord damage may be one of the factors involved in the development of secondary progression in relapse-onset multiple sclerosis.

Current guidelines highlight the utility of spinal MRI in patients with suspected multiple sclerosis presenting with a spinal cord syndrome and those with non-specific brain lesions, or brain lesions not satisfying criteria for dissemination in space (Rovira *et al.*, 2015). Spinal cord imaging is not done routinely because of the relatively modest additional diagnostic yield in unselected CIS patients (Arrambide *et al.*, 2018). Our findings suggest that spinal cord MRI findings have significant prognostic value in CIS patients and may be useful in identifying

those at high-risk of later disease progression and physical disability. The association of new spinal cord lesions over the first 3 years after a CIS over time, independent of brain MRI activity, might also suggest a role for spinal cord MRI when monitoring the course of multiple sclerosis. Incorporating spinal cord MRI into monitoring protocols is challenging not only because of increased cost and reduced resources associated with a longer scanning time, but also because of technical challenges that influence the sensitivity of lesion detection, including the MRI protocol used, pulsation artefacts from blood vessels and cerebrospinal fluid, and the experience of raters (Rovira *et al.*, 2015; Wattjes *et al.*, 2015). In this study spinal cord MRI was obtained on the same scanner with a standardised acquisition protocol, and all scans were reviewed by a single experienced Neuroradiologist, a situation that differs from routine clinical practice.

Like the spinal cord, the brainstem and cerebellum contain pathways critical for balance and locomotion. Two previous studies found that infratentorial lesions seen at the time of presentation with CIS are associated with physical disability after 5-7 years, although neither study included spinal cord MRI (Minneboo *et al.*, 2004; Tintore *et al.*, 2010). Infratentorial lesions seen at the time of CIS were not independently associated with long-term outcomes, possibly because the effect is eclipsed by the impact of spinal cord lesions. New infratentorial lesions at 1 year were associated with long-term physical disability and secondary progression, but overall, we found a more consistent association of spinal cord rather than infratentorial lesions with long-term disease outcomes.

Previous studies have found a relatively limited relationship between global brain T2 lesion load and long-term disease outcomes in relapse-onset multiple sclerosis (Fisniku *et al.*, 2008; Optic Neuritis Study Group). In our study, supratentorial lesions at baseline were

independently associated with EDSS and performance on tests of information processing speed after 15 years. Although supratentorial lesions showed a correlation with all long-term outcomes univariately (data not shown), their prognostic value was lost when other MRI measures were considered in the multivariable models. In contrast, asymptomatic gadolinium-enhancing lesions (found in approximately one third of patients within 3 months of CIS) showed a consistent association with disease course at 15 years, physical disability (EDSS, walking speed, upper limb dexterity) and cognitive performance. Importantly, the association of baseline gadolinium-enhancing lesions remained even after including changes in MRI measures at 1 and 3 years in the statistical models. Gadolinium-enhancing lesions reflect areas of acute inflammatory activity associated with breakdown of the blood-brain barrier (Filippi *et al.*, 2012), and potentially a greater severity of underlying inflammatory disease activity than T2 lesions.

The association of early gadolinium-enhancing lesions with long-term disability is consistent with findings from natural history studies showing that the number of early relapses in the first few years after disease onset (a clinical measure of inflammatory disease activity) is associated with long-term disability and development of secondary progressive disease course in relapse-onset multiple sclerosis (Scalfari *et al.*, 2014). The “baseline” MRI scan in our study was done within 3 months after CIS onset (median 44 days). In many healthcare settings a diagnostic MRI scan would be done much sooner in patients presenting with acute optic neuritis or transverse myelitis. Our findings suggest that very early follow-up MRI in this group of patients done 6-12 weeks after CIS onset may provide valuable prognostic information, with asymptomatic gadolinium-enhancing lesions indicating a worse long-term prognosis, and supporting the early initiation of disease-modifying therapies.

In this cohort of patients followed prospectively from CIS onset, lesional MRI measures, particularly lesion location (especially spinal cord) and lesion activity (i.e. gadolinium-enhancement), were more consistently associated with long-term outcomes than early brain or spinal cord atrophy. A similar rate of whole brain atrophy was observed over the first 3 years after CIS onset in patients who developed multiple sclerosis irrespective of disease course at 15 years. This is consistent with previous studies showing that whole brain atrophy begins early in the course of multiple sclerosis (Perez-Miralles *et al.*, 2013), and progresses at a similar rate throughout the course of the disease, irrespective of disease phenotype or disease duration (De Stefano *et al.*, 2010). Significant spinal cord atrophy was also observed over the first 3 years after CIS in this cohort, however, this was not independently associated with outcomes after 15 years, at least when considered in conjunction with other MRI measures.

A wide range of physical disability was observed at 15 years reflecting the variable prognosis of relapse-onset multiple sclerosis. In the patients with multiple sclerosis at 15 years physical disability was overall low: only 1 in 3 patients had an EDSS of ≥ 3 and only 1 in 5 developed secondary progressive multiple sclerosis. Although comprehensive neuropsychological testing wasn't undertaken, most multiple sclerosis patients performed well on tests of information processing speed (even using a liberal cut-off value to define impairment) and less than half reported significant fatigue. Our findings contrast with the much worse prognosis reported in natural history studies done in the 1970s and 1980s (Confavreux *et al.*, 2003; Scalfari *et al.*, 2014). There are a number of possible explanations. Firstly, we followed patients from the time of presentation with a CIS irrespective of disease status. The cohorts of patients followed up in natural history studies were enrolled at large hospital-based specialist multiple sclerosis clinics, potentially biased towards more severely affected patients. Secondly, patients with optic neuritis are the main subgroup in this cohort. Optic neuritis usually accounts for 30-40% of

people with CIS (Tintore *et al.*, 2015), compared with 80% in our cohort. Some studies have suggested that optic neuritis may be associated with a better prognosis compared with other CIS presentations, partly due to a lower rate of asymptomatic brain MRI abnormalities in optic neuritis patients (Tintore *et al.*, 2005; Tintore *et al.*, 2015). More than three quarters of the patients with optic neuritis in our cohort had an abnormal baseline MRI scan, indicating a group at high-risk for the development of multiple sclerosis and future physical disability (Fisniku *et al.*, 2008; Tintore *et al.*, 2015). Thirdly, during the course of the study disease-modifying therapies that reduce relapse rates and disease progression became available in the United Kingdom. However, less than a third of patients in the study received disease-modifying therapies, and most patients who were treated received first-line injectable therapies (beta interferon or glatiramer acetate). No adjustment was made for disease-modifying therapies in the multivariable models because criteria for treating patients in the United Kingdom at the time of the study required two clinically-significant attacks in two years in order to start treatment. More severely affected patients are likely to have received treatment leading to reverse causality in the prediction models. Finally, the diagnosis of multiple sclerosis was made using the McDonald criteria and includes people who in the past would have been labelled as having CIS rather than multiple sclerosis (Brownlee *et al.*, 2015). The so-called Will-Rogers phenomenon describes the apparent improvement in prognosis with changes to diagnostic criteria over time (Sormani *et al.*, 2008). Our findings of a more favourable long-term outcome than would be expected from natural history studies is consistent with other contemporary observational cohort studies (Kerbrat *et al.*, 2015; Tintore *et al.*, 2015; Cree *et al.*, 2016)

The strengths of this study include the prospective design, the uniquely long follow-up duration, the longitudinal MRI acquisition over the first 3 years after disease onset (including both brain and spinal cord MRI with gadolinium), and the very low number of patients lost to

follow-up. In addition to examining EDSS, we also investigated early imaging predictors of secondary progressive multiple sclerosis and long-term cognitive performance important clinical outcomes that have not been investigated in previous in long-term, observational clinical-MRI studies. Our cohort were predominantly untreated over the first few years after disease onset providing insights into the natural history of relapse-onset multiple sclerosis in the MRI era. Some limitations also need to be noted. Firstly, an inherent limitation to all longitudinal observational studies is drop-out of subjects over time. Not all patients initially recruited into the study had follow-up MRI scans at 1 year and 3 years (~90% had at least one follow-up MRI). Secondly, clinical status after 15 years was assessed in a significant number of patients by telephone interview because not all patients were able to return for a follow-up visit to be examined in person. The use of telephone EDSS has been validated previously for use in clinical trials (Lechner-Scott *et al.*, 2003) and has also been used in previous longitudinal clinical-MRI studies (Fisniku *et al.*, 2008). Thirdly, although EDSS was assessed at disease onset, cognitive performance was only assessed cross-sectionally at 15-years. However, we estimated premorbid intelligence (measured using the NART) was considered in all models. Fourthly our aim was to examine the prognostic value of early MRI measures in patients with CIS and early multiple sclerosis. In models evaluating long-term outcomes we didn't include the number of relapses or change in EDSS over the first 3 years after disease onset. Higher relapse number and progression of physical disability have already been established as markers of worse prognosis in natural history studies (Confavreux *et al.*, 2003; Scalfari *et al.*, 2014) , Fifthly, we estimated the rates of brain atrophy from 2-dimensional spin echo T1-weighted scans. While this acquisition can provide robust estimates of whole brain atrophy, we were unable to examine the prognostic impact of early tissue-specific brain atrophy. Recently, deep grey matter volume loss has been identified as a key mechanism responsible for disease progression in multiple sclerosis (Eshaghi *et al.*, 2018). We also did not acquire double-

inversion recovery or other advanced MRI sequences sensitive to the detection of focal grey matter lesions, which are known to be important in the disease progression in multiple sclerosis (Scalfari *et al.*, 2018). Finally, although the mean duration of follow-up was over 15 years the course of multiple sclerosis often unfolds over much longer and the number of people developing secondary progression and worsening disability in this cohort is likely to increase with time. However, the findings do identify early MRI predictors associated with a more aggressive course over the first 15 years after disease.

CONCLUSION

MRI abnormalities seen around the time of presentation with CIS and over the first few years after disease onset predict the development of long-term outcomes in relapse-onset multiple sclerosis. Spinal cord and gadolinium-enhancing lesions showed a consistent association with the development of secondary progression and physical disability, and gadolinium-enhancing lesions with cognitive performance 15 years after disease onset. These findings suggest that the accrual of focal lesions in clinically-eloquent sites and the extent of early inflammatory disease activity are important predictors of long-term disability and secondary progression in relapse-onset multiple sclerosis. Conventional MRI measures available in routine clinical practice may be useful in counselling patients with CIS and early multiple sclerosis about long-term disease course and might be helpful in personalising treatment plans.

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COMPETING INTERESTS

Dr Altmann, Dr Miszkiel and Dr Prados report no disclosures.

Dr Brownlee has received speaker honoraria for educational activities for Merck-Serono, Roche and Sanofi-Genzyme.

Dr Eshaghi has received speaker's honoraria from Biogen and Roche.

Prof Gandini Wheeler-Kingshott is editor of *Functional Neurology*.

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Prof Ciccarelli is a consultant for Novartis, Biogen-Idec, Genzyme and General Electric, and all the payments are made to the UCL Institute of Neurology. She is an associate editor of *Neurology*.

FIGURE LEGENDS

Figure 1. Representative images of patients classified as clinically isolated syndrome (CIS), relapsing-remitting multiple sclerosis (RRMS) and secondary progressive multiple sclerosis (SPMS) at 15 years. MRI scans obtained at baseline (the time of CIS), 1-year and 3-years.

Figure 2. EDSS scores after 15 years in the patients who developed multiple sclerosis (n=119).

SUPPLEMENTARY MATERIALS

Supplementary Table 1 MRI acquisition protocol.

Supplementary Table 2 Follow-up MRI findings at 1 and 3 years grouped by clinical status at 15 years.

Supplementary Table 3 Multivariable linear regression models investigating MRI predictors of timed 25-foot walk test (TWT) and 9-hole peg test (9HPT) at 15 years.

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