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The cortical damage, early relapses, and onset of the progressive phase in multiple sclerosis

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Neurology® 2018;90:e2107-e2118. doi:10.1212/WNL.0000000000005685

Abstract

Objective

To investigate the relationship among cortical radiologic changes, the number of early relapses (ERs), and the long-term course of multiple sclerosis (MS).

Methods

In this cohort study, we assessed the number of cortical lesions (CLs) and white matter (WM) lesions and the cortical thickness (Cth) at clinical onset and after 7.9 mean years among 219 patients with relapsing remitting (RR) MS with 1 (Low-ER), 2 (Mid-ER), and ≥3 (High-ER) ERs during the first 2 years. Kaplan-Meier and Cox regression analyses investigated early factors influencing the risk of secondary progressive (SP) MS.

Results

Fifty-nine patients (27%) converted to SPMS in 6.1 mean years. A larger number of CLs at onset predicted a higher risk of SPMS (hazard ratio [HR] 2.16, 4.79, and 12.3 for 2, 5, and 7 CLs, respectively, $p < 0.001$) and shorter latency to progression. The High-ER compared to the Low-ER and Mid-ER groups had a larger volume of WM lesions and CLs at onset, accrued more CLs, experienced more severe cortical atrophy over time, and entered the SP phase more rapidly. In the multivariate model, older age at onset (HR 1.97, $p < 0.001$), a larger baseline CL (HR 2.21, $p = 0.005$) and WM lesion (HR 1.32, $p = 0.03$) volume, early changes of global Cth (HR 1.36, $p = 0.03$), and ≥3 ERs (HR 6.08, $p < 0.001$) independently predicted a higher probability of SP.

Conclusions

Extensive cortical damage at onset is associated with florid inflammatory clinical activity and predisposes to a rapid occurrence of the progressive phase. Age at onset, the number of early attacks, and the extent of baseline focal cortical damage can identify groups at high risk of progression who may benefit from more active therapy.

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Glossary

CL = cortical lesion; **DIR** = double inversion recovery; **EDSS** = Expanded Disability Status Scale; **ER** = early relapse; **GM** = gray matter; **HR** = hazard ratio; **MP-RAGE** = magnetization-prepared rapid gradient-echo; **MS** = multiple sclerosis; **RR** = relapsing remitting; **SP** = secondary progressive; **TE** = echo time; **3D** = 3-dimensional; **TR** = repetition time; **WM** = white matter.

The evolution of relapsing remitting (RR) multiple sclerosis (RRMS) is largely unpredictable, and the prevention of the secondary progressive (SP) phase is a major unmet therapeutic need.[1,2] Pathologic processes that lead to the conversion to SPMS remain unclear.[3,4] In addition, the lack of surrogate biomarkers for late disability accumulation makes it difficult to identify, early in the disease course, patients requiring more aggressive therapies.[5]

Pathologic injury to the gray matter (GM) plays a major role in the accumulation of long-term disability.[6–8] Cortical lesions (CLs) and cortical atrophy can occur during the early stage of the RR phase,[9–14] increase over time,[15,16] accounting for the transition to SPMS,[17] and become diffuse during the late stage of the disease.[7,9,18]

Natural history studies consistently demonstrated that patients with a large number of inflammatory attacks during the first 2 or 5 years accumulate severe disability more rapidly.[1,19–21] This suggests that pathologic mechanisms occurring in the very early stage of the disease influence the severity of the individual outcomes.

In this context, we hypothesized that the early development of the cortical pathology might be associated with more rapid disability accumulation. Therefore, we set out to explore the prognostic value of early clinical and MRI features and to elucidate GM radiologic changes among patients with different early relapse (ER) frequencies.

Methods

Study population

In this longitudinal study, we assessed clinical and radiologic data from a cohort of patients currently attending the MS specialist center of Verona University Hospital. We selected 219 patients with RRMS with clinical onset between 2005 and 2008 for whom information on the number of ERs was available and who have been observed from the disease onset for at least 5 years. Patients were diagnosed according to McDonald criteria[22] and were clinically evaluated every 6 months or when experiencing acute attacks by a neurologist expert on MS (M.C.). Disability was scored with the Expanded Disability Status Scale (EDSS).[23] A complete MRI examination was performed at disease onset and on a yearly basis up to the end of the observation period. Clinical relapses were defined as acute development of new symptoms or worsening of existing symptoms lasting >24 hours. SPMS was defined by the occurrence of continuous disability accumulation that was not related to any relapse and was confirmed after 12 months. Although transitory plateaus in the progressive course were allowed, the steady progression was the rule.[24]

On the basis of the number of ERs that occurred during the first 2 years from disease onset, patients with RRMS were divided into 3 groups: patients with onset attack (Low-ER frequency) who experienced a second exacerbation after the second year, patients with 2 attacks within the first 2 years (Mid-ER frequency), and patients with at least 3 attacks (High-ER frequency) during the first 2 years. Patients were started on disease-modifying treatments after having experienced a second relapse within or after the first 2 years from onset (conversion to clinically defined MS). Patients who experienced a relapse while on treatment were escalated to a stronger therapy (second-line treatment).

Images acquisition protocol and analysis

Each patient had to be relapse and steroid free for at least 1 month before undergoing the MRI examination. All images were acquired with the same 1.5T scanner (Achieva, Philips Medical Systems, Best, the Netherlands), with a 33-mT/m power gradient and a 16-channel head coil. No major hardware upgrades of the scanner occurred during the study period, and bimonthly quality assurance sessions took place to guarantee measurement stability. At follow-up, participants were carefully repositioned according to published guidelines for serial MRI studies of MS.[25] The following images were acquired from each participant: (1) 3-dimensional (3D) double inversion recovery (DIR) (3D sequence without any interpolation techniques; repetition time [TR] 6.500 milliseconds, inversion time 2.800 milliseconds, delay 500 milliseconds, echo time [TE] 265 milliseconds, slice thickness 1.5 mm, number of averages 2, matrix 256 × 2562); (2) 3D fluid-attenuated inversion recovery (TR 10,000 milliseconds, TE 120 milliseconds, inversion time 2,500 milliseconds, echo train length 23, slice thickness 1.5 mm, matrix 172 × 288, and field of view 250 × 200 mm^2), and (3) 3D magnetization-prepared rapid gradient-echo (MP-RAGE) sequence (120 contiguous axial slices, TR 25 milliseconds, TE 4.6 milliseconds, flip angle 30°, slice thickness 1.0 mm, matrix 256 × 256, and field of view 250 ×250 mm^2).

In line with recent recommendations for CL scoring and cortical thickness measurement in patients with MS,[26,27] a neurologist (M.C.) and a neuroradiologist (A.M.), who both have a great deal of experience in MS and were blinded to patients' clinical details, evaluated all images. Because the

main objective of our study was to investigate the predictive effect of early radiologic changes on the long-term clinical evolution, we assessed MRI data at clinical onset (T0), at 2 years from onset (T2), and at the end of the observation period. The following MRI parameters were analyzed. First, cortical thickness/volume was determined. The mean cortical thickness was measured in each participant at T0, T2, and the end of the follow-up. The MP-RAGE dataset (including 3D MP-RAGE for each patient at each time point) was analyzed with the longitudinal stream of FreeSurfer image analysis suite (release version 5.3.0), which is available online (surfer.nmr.mgh.harvard.edu/). Topological defects in cortical surfaces due to white matter (WM) and leukocortical lesions were corrected with a semiautomated procedure that includes WM lesion segmentation and lesion filling. Second, CL number and volume at T0, T2, and the end of follow-up were assessed. The number of new and preexisting CLs was assessed on DIR images by consensus of the 2 observers (M.C. and A.M.). Owing to the suboptimal performance of the image-acquisition sequences on MRI in visualizing subpial lesions, the present analysis has taken into account mainly the intracortical and leukocortical lesions. Third, WM lesion number and volume at T0, T2, and the end of follow-up were examined. WM lesion volume was calculated on fluid-attenuated inversion recovery images with a semiautomatic threshold technique based on Fuzzy C mean algorithm, which is included in software developed at the NIH, Medical Images Processing, Analysis and Visualization (mipav.cit.nih.gov).

Statistical analyses

We used the χ^2 test, the binomial test, and the Wilcoxon signed-rank test to compare categorical data and analysis of variance and Kruskal-Wallis tests to compare means across patient groups for normal and nonnormal variables, respectively. The Shapiro-Wilks test was applied to test normality. Kaplan-Meier analysis estimated the time to the conversion to SPMS among patients stratified by number of attacks during the first 2 years or stratified by number of CLs at disease onset. The log-rank test investigated differences; survival was compared with groups with more relapses or with a larger number of CLs. The Cox regression univariate analysis calculated the risk of entering the SP phase on the basis of the number of baseline CLs; hazard ratios (HRs) were obtained through comparison with 0 CLs. Multivariate analysis allowed investigation of the risk of developing SPMS according to the concomitant effect of clinical, demographic, and radiologic features at disease onset and at 2 years after onset. Proportional hazards assumption was checked by visual inspection of Schoenfeld residual plots and corresponding statistical tests. We applied an automatic model selection strategy to identify the best predictive variables. These were used to develop a final model that calculated the relative risk of SP for every combination of variables values.

For consistency, 2 authors (A.S. and C.R.) carried out statistical analyses independently using IBM SPSS statistics version 22 and R software (r-project.org); results from the 2 analyses were reviewed, checked, and partially extended by 1 author (C.R.).

Standard protocol approvals, registrations, and patient consents

The local ethics committee approved the study. Informed consent was obtained from all patients.

Data availability

Data on disease-modifying treatments used during the observation period among patients participating to the study will be shared by request from any qualified investigator.

Results

Clinical and radiologic findings

We analyzed data from 219 patients with RRMS with a mean disease duration of 7.9 years (range 5.4–11.8 years). At disease onset, we detected 674 CLs in 76% (166 of 219) of patients; 60% (407 of 674) of the lesions were intracortical, and 40% (267 of 674) of the lesions were leukocortical. By the end of the observation period, 73% (160 of 219) of patients were still classified as RRMS and 27% (59 of 219) had converted to SPMS in 6.1 mean years. The subgroup who entered the SP phase, compared to those who remained in RR, had a larger proportion of men, older age at disease onset, and a higher number of ERs 2(table 1). In addition, the subgroup of patients whose disease became SPMS had at onset, after 2 years, and at the end of the observation period a significantly larger volume of WM lesions and CLs and a significantly lower global cortical thickness (table 2).

Association of baseline CLs with clinical outcome

The lack of CLs at disease onset was associated with a better clinical outcome. Among patients without radiologic evidence of focal cortical damage at the first attack (24% [53 of 219]), no one entered the SP phase (figure 1A), and only a few (n = 4) reached an EDSS score of 4 by the end of the observation period. In contrast, in the group with focal cortical damage at clinical onset, the probability of converting to SPMS increased proportionally with the number of CLs (figure 1B); patients with 2 (HR 2.16), 5 (HR 4.79), and 7 (HR 12.3) lesions had 2-, 4-, and 12-fold higher hazard of secondary progression, respectively. Accordingly, the group of patients with a larger number of baseline CLs entered the SP phase in larger proportions and in significantly ($p < 0.001$) shorter times (figure 1A).

The cortical pathology and ERs

Table 3 and figures 2 and 3 show the clinical and radiologic features of patients with RRMS with 1, 2, and ≥3 acute attacks during the first 2 years. At clinical onset, CLs were detected in 68% (79 of 116) of Low-ER (total CLs 239), in 81% (43 of 53) of Mid-ER (total CLs 182), and in 88% (44 of 50) of High-ER (total CLs 253) patients. Patients with High-ER compared to Mid-ER and Low-ER groups had a significantly higher mean number of CLs (figure 2A) and more prominent

Table 1 Clinical and demographic features of patients with RRMS

	RRMS (n = 219)	RRMS at the end of follow-up (n = 160)	SPMS at the end of follow-up (n = 59)	*p* Value
Female/male, n (%)	131 (60)/88 (40)	104 (65)/56 (35)	27 (45.7)/32 (54.3)	<0.001
Mean (SD) age at onset, y	32.4 (10.0)	31.5 (10.6)	34.2 (7.6)	0.02
Mean (SD) disease duration, y	7.9 (1.2)	7.8 (1.3)	8.2 (1.0)	0.01
Mean (SD) EDSS score at onset	1.4 (1.5)	1.3 (1.5)	1.7 (2.0)	<0.001
No. of ERs, n patients (%)				
1	116 (53)	100 (62.5)	16 (27.1)	<0.001
2	53 (24.2)	37 (23.1)	16 (27.1)	<0.001
≥3	50 (22.8)	23 (14.4)	27 (45.8)	<0.001
Clinical features at the end of follow-up period				
Mean (median) EDSS score		2.1 (2)	5.2 (5.5)	<0.001
Mean (SD) time from onset to SP, y		NA	6.1 (1.05)	
Mean (SD) age at onset of SP, y		NA	41.6 (7.4)	
Reached EDSS score of 4, n (%)		16 (10)	59 (100)	<0.001
Mean (SD) time to EDSS score of 4, y		5.8 (0.5)	5.0 (1.1)	<0.001

Abbreviations: EDSS = Expanded Disability Status Score; MS = multiple sclerosis; NA = not available; RR = relapse remitting; SP = secondary progressive. The *p* values were obtained by comparing the RR and the SP groups with binomial test for proportions (n [%] variables) and nonparametric Kruskal-Wallis test for nonnormal variables.

WM damage, although the 3 groups had similar cortical thickness (table 3). In addition, during the first 2 years of disease duration, the High-ER group had a significantly greater increase in WM lesions and CL volume and a more severe global cortical thinning (table 3). Finally, after 7 mean years from onset, patients with High-ER frequency entered the SP phase in a significantly shorter time and at a significantly younger age (table 3), accumulated a larger mean volume of CLs and WM lesions, and developed a significantly more severe global cortical atrophy compared to the Mid-ER and Low-ER groups (table 3 and figure 2, B and C).

Multivariate analysis: the risk of conversion to SPMS

Multivariate analysis demonstrated that at the first attack the risk of converting to SPMS was significantly higher among patients with older age at onset (HR calculated for year increase) and with a larger volume of GM and WM lesions (HR calculated for 1-mm^3 increase) (figure 4A). Two years after the clinical onset, older age at the first symptom and larger volume of baseline CLs still were significantly associated with a higher risk of entering the SP phase. In addition, the model demonstrated that changes of the global cortical thickness over the first 2 years significantly affected the probability of converting to SPMS, and High-ER frequency (≥3 attacks) exerted the largest predictive effect (HR 6.55, 95% confidence interval 2.6–16.0, $p < 0.001$), while the baseline WM damage had no effect on the clinical outcome (figure 4A).

We calculated the probability of experiencing the SP course on the basis of the combined effect of age at onset, baseline CL volume, and ER frequency (figure 4B). The model estimated that, in the groups with 1 and 2 ERs, patients with onset after the age of 40 and with a baseline CL volume between 600 and 900 mm^3 had a higher hazard of converting to SPMS (relative risk >1). In contrast, in the High-ER group, patients with a much younger age at onset (≥20 years) and with much smaller volume of baseline CLs (≥200 mm^3) had an increased risk of progression (relative risk >1).

Discussion

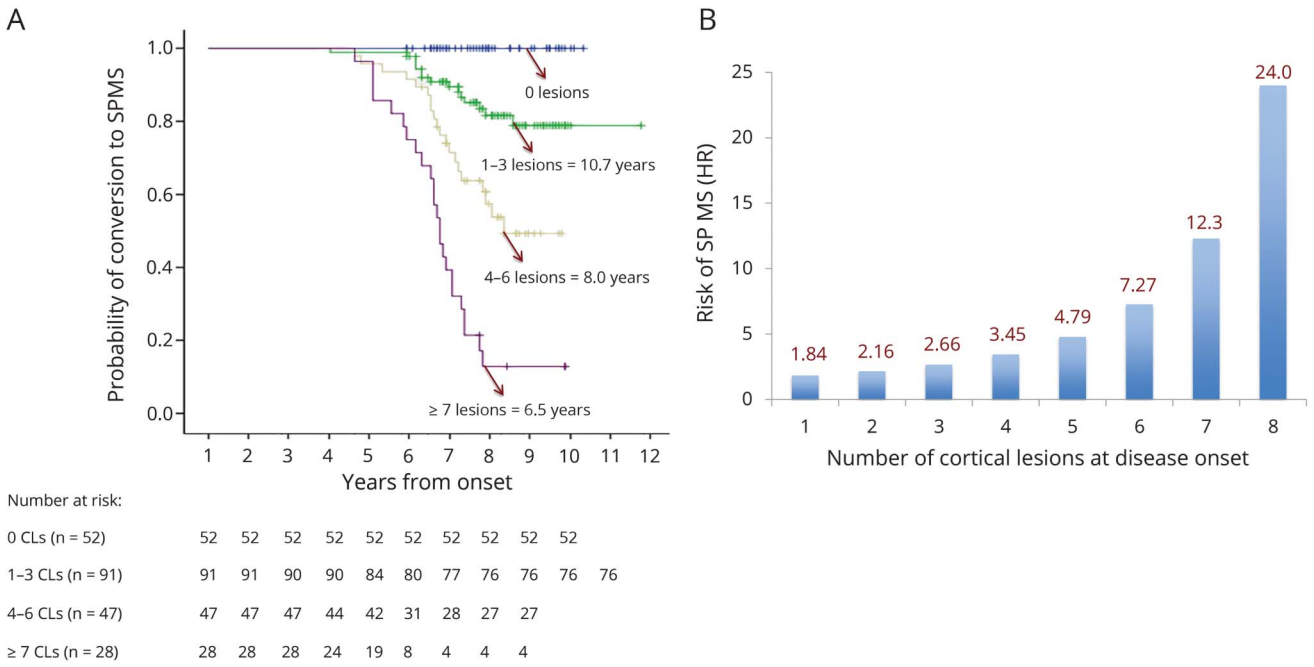
Among patients with RRMS, a higher frequency of ERs is associated with faster attainment of late disability,[1,19–21] suggesting that biological mechanisms occurring during the early phase of the disease influence the long-term disease evolution. On the basis of these observations, we hypothesized that the development of cortical damage early in the disease course might be associated with faster disability accumulation. Therefore, we specifically designed this study to investigate the relationship between the early cortical pathology and the probability of converting to SPMS and whether MRI-identified GM damage varies among patients with RRMS stratified by different frequencies of ERs. In a group of 219 patients with RRMS, we had the opportunity to assess GM radiologic changes at the disease onset, 2 years after onset, and

Table 2 Radiologic features of patients with RRMS

	RRMS (n = 219)	RRMS at the end of follow-up (n = 160)	SPMS at the end of follow-up (n = 59)	*p* Value
MRI parameters at disease onset				
Patients with CLs, n (%)	166 (76)	107 (67)	59 (100)	<0.001
Mean [95% CI] (SD) CLs, n	3.09 [2.64–3.54] (3.3)	1.80 [1.50–2.09] (1.8)	6.5 [5.54–7.61] (3.9)	<0.001
Mean [95% CI] (SD) volume of CLs, mm^3	313.7 [266.2–361.1] (355.5)	174.4 [141.7–207.0] (208.6)	689.1 [585.6–792.6] (397.1)	<0.001
Mean [95% CI] (SD) WM lesions, n	8.32 [7.77–8.86] (4.12)	7.64 [7.01–8.26] (4.00)	10.1 [9.13–11.17] (3.92)	<0.001
Mean [95% CI] (SD) volume of WM lesions, mm^3	3,661.4 [3,330.3–3,992.4] (2,480.0)	3,314.2 [2,936.0–3,692.5] (2,414.7)	4,596.9 [3,963.5–5,230.2] (2,430.3)	<0.001
Mean [95% CI] (SD) global cortical thickness, mm	2.56 [2.54–2.58] (0.16)	2.58 [2.55–2.61] (0.17)	2.51 [2.47–2.55] (0.14)	0.004
MRI parameters 2 y after onset				
Mean [95% CI] (SD) CLs, n	3.35 [2.86–3.84] (3.6)	2.01 [1.66–2.35] (2.2)	7.09 [5.96–8.21] (4.2)	<0.001
Mean [95% CI] (SD) volume of CLs, mm^3	341.7 [290.4–393.0] (384.9)	191.3 [154.8–227.7] (233.3)	749.7 [640.4–859.0] (419.5)	<0.001
Mean [95% CI] (SD) increase of CL number	0.24 [0.17–0.32] (0.5)	0.14 [0.07–0.22] (0.50)	0.52 [0.34–0.69] (0.6)	<0.001
Mean [95% CI] (SD) increase of CL volume, mm^3	29.4 [23.0–35.8] (48.1)	17.9 [11.7–24.2] (40.2)	60.5 [46.3–74.7] (54.3)	<0.001
Mean [95% CI] (SD) WM lesions, n	8.77 [8.21–9.33] (4.1)	7.99 [7.37–8.62] (4.0)	10.9 [9.90–11.96] (3.9)	<0.001
Mean [95% CI] (SD) volume of WM lesions, mm^3	4,117.8 [3,769.4–4,466.2] (2,616.0)	3,674.7 [3,278.5–4,070.8] (2,537.0)	5,319.4 [4,677.5–5,961.4] (2,463.3)	<0.001
Mean [95% CI] (SD) increase of WM lesion number	0.46 [0.33–0.59] (0.9)	0.39 [0.23–0.55] (1.0)	0.64 [0.41–0.87] (0.8)	<0.001
Mean [95% CI] (SD) increase of WM lesion volume, mm^3	458.3 [361.8–554.7] (724.3)	360.8 [254.0–467.6] (684.0)	722.5 [522.0–923.0] (769.4)	<0.001
Mean [95% CI] (SD) global cortical thickness, mm	2.44 [2.42–2.47] (0.16)	2.47 [2.45–2.50] (0.16)	2.37 [2.33–2.40] (0.14)	<0.001
Mean [95% CI] (SD) loss of global cortical thickness,[a] %	4.6 [4.8–4.3] (2.03)	4.2 [4.4–3.9] (1.69)	5.6 [6.2–5.0] (2.47)	<0.001
MRI parameters at end of the study				
Mean [95% CI] (SD) volume of CLs, mm^3	591.6 [496.7–686.6] (712.9)	258.1 [211.1–304.5] (297.6)	1,496.2 [1,306.7–1,685.6] (726.8)	<0.001
Mean [95% CI] (SD) increase of CL volume, mm^3	277.6 [214.5–340.7] (472.5)	84.7 [60.7–108.8] (153.7)	809.7 [645.1–974.2] (625.7)	<0.001
Mean [95% CI] (SD) volume of WM lesions, mm^3	4,777.0 [4,381.2–5,172.8] (2,971.9)	4,174.8 [3,738.5–4,611.1] (2,794.1)	6,410.1 [5,669.3–7,151.0] (2,842.8)	<0.001
Mean [95% CI] (SD) increase of WM lesion volume, mm^3	1,117.5 [953.6–1,281.4] (1,230.5)	861.0 [690.5–1,031.5] (1,091.9)	1813.2 [1,468.6–2,157.8] (1,322.3)	<0.001
Mean [95% CI] (SD) global cortical thickness, mm	2.35 [2.33–2.37] (0.17)	2.39 [2.37–2.42] (0.16)	2.24 [2.20–2.28] (0.14)	<0.001
Mean [95% CI] (SD) % loss of global cortical thickness[a]	8.2 [7.7–8.6] (3.3)	7.2 [6.8–7.7] (2.8)	10.7 [9.9–11.6] (3.2)	<0.001

Abbreviations: CI = confidence interval; CL = cortical lesion; ER = early relapse; MS = multiple sclerosis; RR = relapse remitting; SP = secondary progressive; WM = white matter.
The *p* values were obtained by comparing the RR and SP groups with analysis of variance test.
[a] Compared to baseline.

Figure 1 CLs at onset, time to SPMS, and probability of becoming progressive



Number at risk:										
0 CLs (n = 52)	52	52	52	52	52	52	52	52	52	52
1–3 CLs (n = 91)	91	91	90	90	84	80	77	76	76	76	76
4–6 CLs (n = 47)	47	47	47	44	42	31	28	27	27	
≥ 7 CLs (n = 28)	28	28	28	24	19	8	4	4	4	

(A) Kaplan-Meier analysis: estimated mean time from disease onset to the conversion to secondary progressive multiple sclerosis (SPMS) among patients with 0, low (1–3), intermediate (4–6), and high (≥7) number of cortical lesions (CLs) at onset. (B) Cox regression analysis: risk (hazard ratio [HR] on y-axis) of conversion to SPMS according to the number of CLs (x-axis) at clinical onset. Number of CLs at disease onset significantly predicts the probability of experiencing a progressive course. A larger number of baseline CLs is associated with a proportionally higher risk of conversion to SPMS and with a shorter latency to progression.

the end of the observation period after 7 mean years from the first clinical presentation.

As shown by previous studies,[28,29] we confirmed that a higher WM lesion load early in the disease course predicts a worse clinical outcome, but we also demonstrated that the extent of the focal cortical damage detected at clinical onset significantly influences the risk of becoming progressive and the latency to the SP phase. Patients who converted to SPMS within the follow-up period were distinguished at disease onset not only by more prominent WM damage but also by a more serious cortical pathology, which greatly increased over time (table 2). The survival analysis showed that a larger number of CLs at onset was associated with a proportionally higher risk of becoming progressive (figure 1B). The group with high CL load (≥7 lesions) exhibited an aggressive disease course and rapidly converted to SPMS (6.5 mean years, 95% confidence interval 5.9–7.0), on average 4 years earlier than those with 1 to 3 lesions (figure 1A). Therefore, the presence at clinical onset of focal inflammation of the GM is an early marker of worse outcome, and the severity of the cortical damage dictates the tempo of the latency to progression. This is line with previous radiologic studies demonstrating that more severe GM pathology correlates with worse clinical outcome[7,8,17,30–32]

As we hypothesized, our analysis also showed a correlation between the GM pathology and frequency of ERs. The cortical damage worsened dramatically over time in the High-ER group, while it changed minimally in the Mid-ER and Low-ER groups. During the observation period, patients with a high number of ERs had a greater increase not only of WM lesion volume (table 3) but also of CL volume and a more prominent global cortical volume loss (figure 2B), which accounted for their more rapid conversion to SPMS. The unexpected result of our study was the identification among the 3 groups with different ER frequencies of a significantly different focal cortical burden even at clinical onset. After the first demyelinating attack, patients who would experience a higher number of relapses within the next 2 years had radiologic evidence not only of a larger volume of WM lesions but also of a greater volume of CLs (figure 2A), suggesting an association between the early development of focal cortical damage and mechanisms underlying early clinical attacks.

The question of whether the cortical tissue damage is secondary to the WM pathology or is an independent process remains unresolved.[33] Although neuropathologic studies demonstrated a correlation between the severity of cortical damage and WM inflammation,[18,34] it has also been shown that the development of CLs is driven mostly by meningeal inflammation[18,35,36] and by higher intrathecal inflammation.[37] More prominent WM inflammatory activity does not necessarily entail more serious cortical pathology, which might be driven by independent mechanisms, at least to some extent.[17,32] Notably, in our cohort, among High-ER (≥3 ERs) patients, some (12%, 6 of 50) did not have any CLs at disease

Table 3 Demographic, clinical, and radiologic features of patients with RRMS with 1, 2, and ≥3 relapses during the first 2 years

	Low-ER (n = 116)	Mid-ER (n = 53)	High-ER (n = 50)	*p* Value
Female/male, n (%)	78 (67.2)/38 (32.8)	29 (54.7)/24 (45.3)	24 (48.0)/26 (52.0)	0.04
Mean (SD) age at onset, y	35 (10.0)	31.9 (10.1)	27 (7.5)	<0.001
Mean (SD) disease duration, y	7.7 (1.2)	8.0 (1.3)	8.2 (1.0)	0.06
Mean (median) EDSS score at onset	1.4 (1.5)	1.4 (1.5)	1.5 (1.5)	0.36
Clinical features at the end of the study				
Patients who remained RRMS, n (%)	100 (86.2)	37 (69.8)	23 (46.0)	<0.001
Patients who converted to SPMS	16 (13.8)	16 (30.2)	27 (54)	
Mean (median) EDSS score	2.5 (2.0)	3.3 (2.5)	3.8 (4)	<0.001
Mean (SD) EDSS score change	1.14 (1.30)	1.54 (1.89)	2.27 (1.62)	<0.001
Survival analysis				
Mean (95% CI) time from onset to SP, y	9.3 (9.1–9.6)	10.0 (9.2–10.7)	7.4 (6.7–8.0)	<0.001
Mean (95% CI) age at onset of SP, y	58.3 (55.8–60.7)	50.2 (46.7–53.7)	38.2 (36.1–40.2)	<0.001
MRI parameters at T0				
Mean [95% CI] (SD) CLs, n	2.07 [1.60–2.55] (2.58)	3.43 [2.58–4.28] (3.09)	5.06 [3.85–6.26] (4.23)	<0.001
Mean [95% CI] (SD) volume of CLs, mm^3	181.6 [139.1–224.1] (230.9)	386.8 [282.9–490.7] (373.1)	544.0 [420.6–667.5] (434.3)	<0.001
Mean [95% CI] (SD) WM lesions, n	7.97 [7.24–8.69] (3.95)	8.09 [6.85–9.33] (4.49)	9.36 [8.21–10.51] (4.02)	<0.001
Mean [95% CI] (SD) volume of WM lesions, mm^3	3,462.5 [3,023.9–3,901.1] (2,384.7)	3,115.6 [2,496.2–3,735.0] (2,247.2)	4,692.9 [3,939.1–5,446.7] (2,652.3)	<0.001
Mean [95% CI] (SD) global cortical thickness, mm	2.55 [2.51–2.58] (0.19)	2.59 [2.56–2.61] (0.10)	2.57 [2.53–2.61] (0.15)	0.27
MRI parameters at T2				
Mean [95% CI] (SD) CLs, n	2.31 [1.78–2.84] (2.8)	3.72 [2.80–4.63] (3.3)	5.38 [4.07–6.69] (4.6)	<0.001
Mean [95% CI] (SD) volume of CLs, mm^3	204.2 [155.3–253.1] (265.8)	415.5 [305.7–525.3] (398.2)	582.4 [450.6–714.3] (463.9)	<0.001
Mean [95% CI] (SD) increase of CL number	0.19 [0.09–0.29] (0.5)	0.28 [0.11–0.46] (0.63)	0.32 [0.16–0.48] (0.5)	<0.001
Mean [95% CI] (SD) increase of CL volume, mm^3	22.6 [13.1–32.0] (51.4)	35.9 [21.9–49.9] (50.8)	38.4 [28.7–48.0] (33.8)	<0.001
Mean [95% CI] (SD) T2 lesions, n	8.2 [7.5–8.9] (4.0)	8.6 [7.4–9.8] (4.2)	10.1 [8.9–11.3] (4.3)	<0.001
Mean [95% CI] (SD) volume of T2 lesions, mm^3	3,750.6 [3,298.6–4,202.6] (2,457.6)	3.500 [2,871.0–4,129.3] (2,282.5)	5,624.4 [4,836.1–6,412.7] (2.773.9)	<0.001
Mean [95% CI] (SD) increase of T2 lesion number	0.39 [0.25–0.52] (0.7)	0.30 [0.06–0.54] (0.8)	0.78 [0.38–1.18] (1.4)	<0.001
Mean [95% CI] (SD) increase of T2 lesion volume, mm^3	288.0 [209.1–367.0] (429.3)	384.5 [189.4–579.6] (707.8)	931.5 [638.0–1,225.0] (1,032.7)	0.001
Mean [95% CI] (SD) global cortical thickness, mm	2.45 [2.41–2.48] (0.19)	2.47 [2.44–2.50] (0.11)	2.40 [2.36–2.44] (0.15)	0.20
Mean [95% CI] (SD) % loss of global cortical thickness[a]	3.8 [4.1–3.6] (1.41)	4.2 [4.8–3.7] (2.11)	6.6 [7.1–6.0] (1.88)	<0.001

Continued

Table 3 Demographic, clinical, and radiologic features of patients with RRMS with 1, 2, and ≥3 relapses during the first 2 years *(continued)*

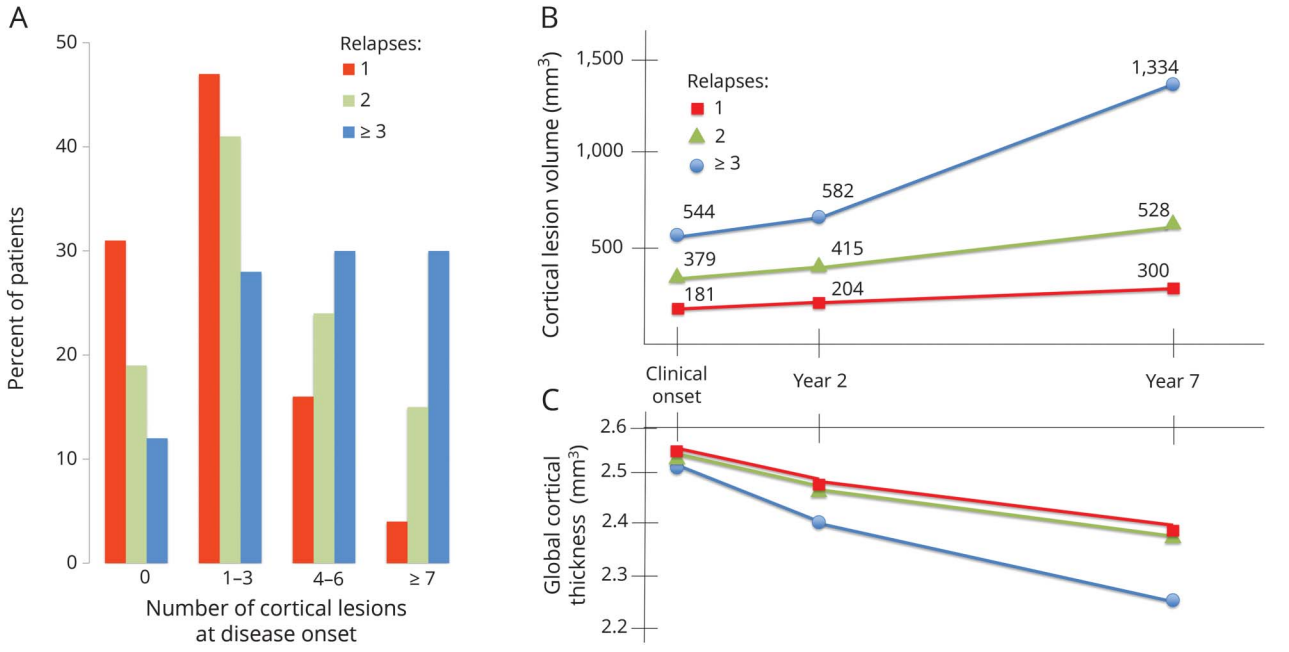
	Low-ER (n = 116)	Mid-ER (n = 53)	High-ER (n = 50)	*p* Value
MRI parameters at the end of the study				
Mean [95% CI] (SD) volume of cortical lesions, mm^3	300.4 [228.3–372.5] (391.8)	528.0 [386.8–669.2] (512.1)	1,334.6 [1,071.9–1,597.2] (924.2)	<0.001
Mean [95% CI] (SD) increase of CL volume, mm^3	118.8 [74.7–162.8] (239.5)	138.8 [83.4–194.1] (198.8)	790.5 [596.8–984.1] (681.4)	0.006
Mean [95% CI] (SD) volume of WM lesions, mm^3	4,289.8 [3,771.8–4,807.8] (2,816.4)	3,845.2 [3,176.1–4,514.4] (2,427.6)	6,895.1 [6,074.1–7,716.1] (2,888.9)	<0.001
Mean [95% CI] (SD) increase of WM lesion volume, mm^3	827.3 [647.9–1,006.6] (975.2)	729.6 [487.8–971.3] (877.1)	2,202.2 [1787.8–2,616.5] (1,457.8)	<0.001
Mean [95% CI] (SD) global cortical thickness, mm	2.38 [2.35–2.42] (0.19)	2.36 [2.32–2.39] (0.12)	2.26 [2.22–2.31] (0.14)	<0.001
Mean [95% CI] (SD) loss of global cortical thickness,[a] %	6.3 [5.9–6.7] (2.12)	8.7 [7.9–9.6] (3.13)	11.9 [11.2–12.6] (2.38)	<0.001

Abbreviations: CI = confidence interval; CL = cortical lesion; ER = early relapse; High-ER ≥3 early relapses; Low-ER =1 early relapse; Mid-ER = 2 early relapses; MS = multiple sclerosis; RR = relapse remitting; SP = secondary progressive; T2 = 2 years after onset; T0 = disease onset; WM = white matter.
The *p* values were obtained with analysis of variance test.
[a] Compared to baseline.

onset and did not become progressive despite the florid early clinical inflammatory activity. Overall, none of the patients without CLs at onset converted to SPMS by the end of the observation period (figure 1A).

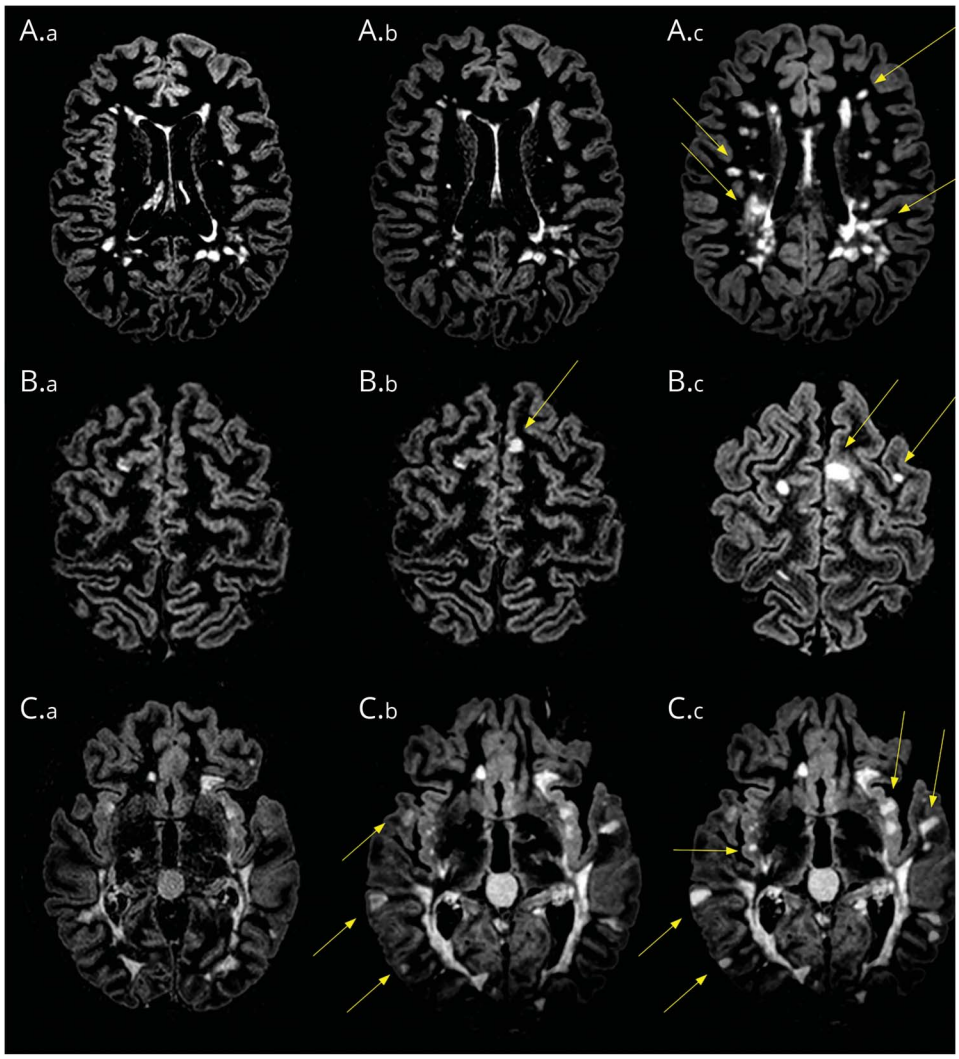
The multivariate model further emphasizes the prognostic relevance of the early cortical pathology by demonstrating that the extent of the focal cortical damage at disease onset affects the probability of developing a progressive course

Figure 2 Relationship between the cortical pathology and number of ERs



(A) Number of CLs at clinical onset. In each category with a different number of cortical lesions (CLs) at onset, the proportion of patients from each group with 1 (Low), 2 (Medium), and ≥3 (High) early relapses (ERs) during the first 2 years is indicated. (B) Mean CL volume and (C) mean global cortical thickness at clinical onset, 2 years from onset, and 7 mean years after onset among patients with 1, 2, and ≥3 relapses during the first 2 years. The number of CLs at onset increases proportionally with the number of relapses during the first 2 years. Patients who are destined to experience a high frequency of early attacks have greater focal gray matter (GM) damage at disease onset. The GM pathology worsened dramatically over time in the High-ER group, while it changed minimally in the Mid-ER and Low-ER groups. During the observation period, High-ER patients had a greater increase of CLs volume and a more prominent global cortical volume loss.

Figure 3 MRI images at T0 (A.a, B.a, C.a), T2 (A.b, B.b, C.b), and end of follow-up (A.c, B.c, C.c) of 3 representative patients with 1 to 3 ERs

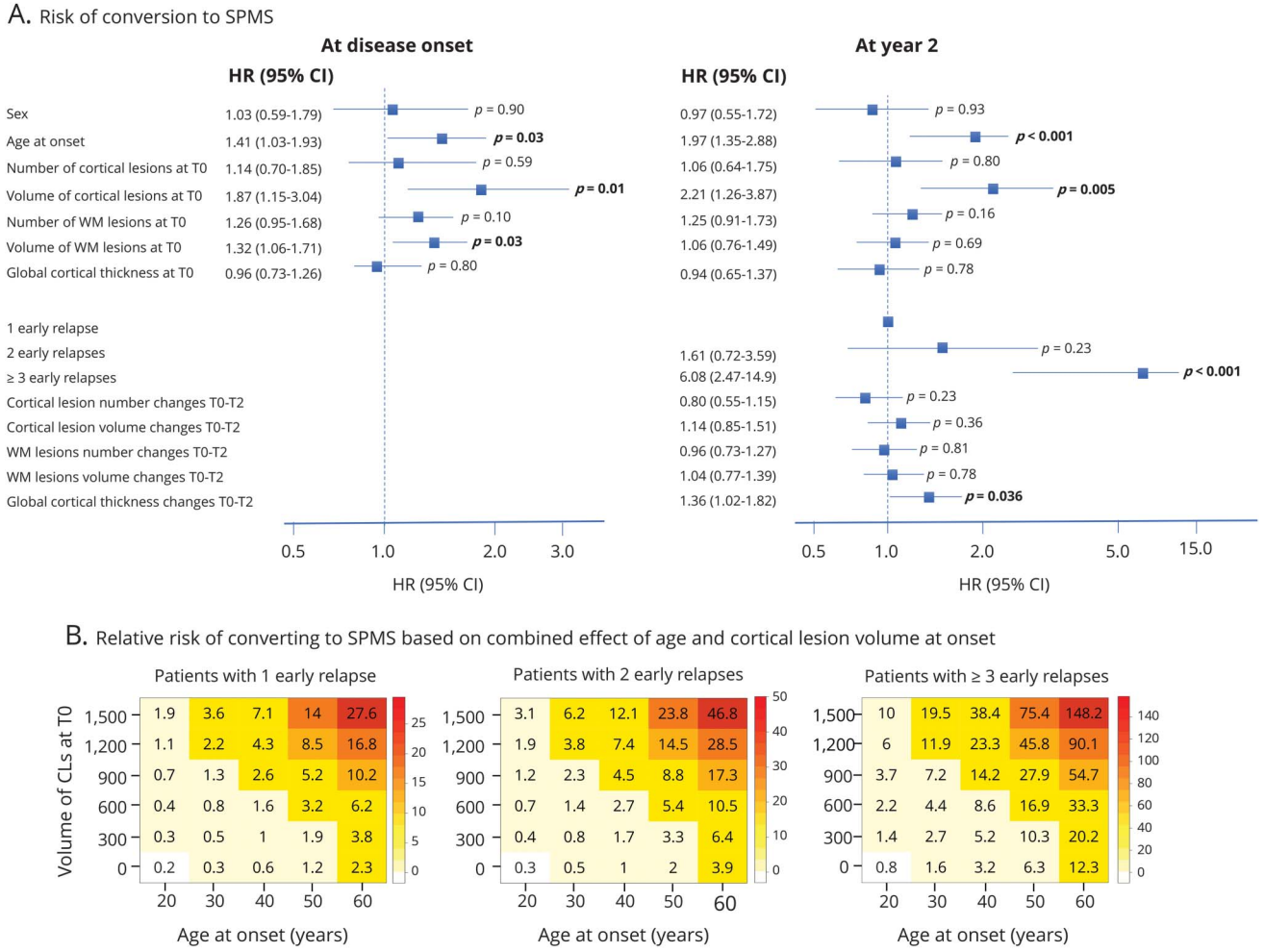


(A.a–A.c) MRI images of a patient with 1 early relapse (ER) who did not covert to secondary progressive multiple sclerosis (SPMS) and had an Expanded Disability Status Scale (EDSS) score of 2.5 at the end of the observation period. The patient had no cortical lesions (CLs) at onset and moderate white matter lesion load and accumulated little cortical damage over time. (B.a–B.c) MRI images of a patient with 2 ERs who did not convert to SPMS and had an EDSS score of 4 at the end of the observation period. The patient had a small number of CLs at onset, which increased moderately over time. (C.a–C.c) MRI of a patient with 3 ERs who rapidly converted to SPMS and had an EDSS score of 6 at the end of the observation period. The patient had high number of CLs at onset, which greatly increased over time.

independently of the WM pathology. The independent predictors of a significantly higher risk of converting to SPMS in a short time were old age at onset, a larger volume of WM lesions, and a high number of ERs, which are line with previous studies,[1,19–21,28,29,38,39] but also a large volume of CLs at onset and changes in global cortical thickness over the first 2 years (figure 4A). This indicates that the development of early white and GM damage affects the long-term outcome by determining an early accelerated cortical thinning.

Taken together, the findings of our analyses indicate that extensive focal cortical damage at the disease onset distinguishes patients destined to have a florid early clinical inflammatory activity and a rapid occurrence of the progressive phase, which is driven by the worsening cortical pathology over time. This supports the notion that the early disease stage is the crucial time when pathologic processes leading to SPMS are already active. Although early treatment initiation was shown to reduce the probability of disability accumulation,[40,41] it is still uncertain whether an aggressive therapeutic suppression of the early inflammatory activity can prevent the onset of progression.[2] In line with observations from alemtuzumab[42] and autologous hematopoietic stem cell transplantation studies,[43] our data suggest that there might be an early window of opportunity for treatment to achieve better disease control by targeting the early inflammation and preventing the development of the cortical pathology early, before irreversible changes take place. Notably, the majority of High-ER patients received a more aggressive therapeutic approach but experienced much faster disease progression compared to Mid-ER and Low-ER patients, who were treated predominantly with first-line treatments. However, the aim of this study was not to address the effect of early aggressive treatment, which requires larger samples and appropriate randomization.

Figure 4 Cox regression multivariate analysis: risk of developing SPMS.



(A) Clinical, demographic, and radiologic variables at clinical onset (T0) and 2 years after onset (T2) affecting the risk of conversion to secondary progressive multiple sclerosis (SPMS). (B) Relative risk of converting to SPMS based on varying age and extent of cortical lesion (CL) volume at onset in each group of patients with 1 (Low), 2 (Mid), and ≥3 (High) early relapses (ERs). After 2 years from onset, older age at onset, a larger volume of CLs at onset, more severe global cortical thinning, and a high number of ERs were independently and significantly associated with a higher probability of entering the SP phase. In the High-ER group, higher hazard of SP (relative risk >1) is estimated to occur when at clinical onset patients are >20 years old and have a CL volume ≥200 mm^3. In contrast, in the Mid-ER and Low-ER groups, an increased risk of progression occurs when onset is after the age of 40 and with a baseline CL volume between 600 and 900 mm^3. CI = confidence interval; HR = hazard ratio; WM = white matter.

Our predictive model provides proof of principle for the early stratification of patients based on their risk of becoming progressive. This can be used to optimize therapy and to improve randomization in trials in which the extent of the focal cortical pathology could be tested as a marker of late disability accumulation. Older age and more severe cortical damage at disease onset greatly increase the probability of becoming progressive, especially in the group with High-ER, for whom the maximum estimated hazard (relative risk 148.2) was 5 and 3 times higher compared to the Mid-ER (relative risk 27.6) and Low-ER (relative risk 46.8) groups, respectively (figure 4B).

Our study has some limitations. First, much of the analysis results are based on the definition of the clinical onset of the progressive phase, which has an inevitable degree of subjectivity. However, it is reassuring that our data on the clinical course are very much in line with results from natural history studies. In addition, the model investigating clinical and radiologic predictors of the probability of reaching EDSS 4 (data not shown) showed results remarkably similar to those of the multivariate analysis assessing the risk of SP. This indicates a low risk of bias affecting our results. Second, the DIR is known to have low sensitivity in detecting CLs, especially the subpial ones.[44] Despite this, it has been demonstrated that the number of radiologically detectable lesions correlates well with the overall size of the histologic cortical damage.[45] In addition, the present analysis has taken into account mainly the intracortical and leukocortical lesions; the imaging acquisition was carried out at a single center and was based on a highly homogenous set of images; and the identification of CLs was carried out by 2 experienced observers (M.C. and A.M.). Thus, we believe that our findings accurately reflect the extent of the cortical pathology among groups. Finally, the lack of data on gadolinium-enhancing

lesions might represent a potential limitation. However, we evaluated all patients with the same MRI scanner, which guarantees high consistency in follow-up imaging when detecting new or expanding WM lesions.

Our study demonstrates that, among patients with RRMS, the development of GM damage early in the disease course is associated with more frequent ERs and with a higher risk of experiencing rapid disability accumulation. The results provide a basis for patient stratification aimed at therapeutic optimization. From a biological perspective, these results highlight the importance of elucidating mechanisms involved in the early cortical pathology, which can be a potential target for future neuroprotective therapies.

Author contributions

Antonio Scalfari: study concept and design, analysis and interpretation of data, drafting/revising the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, study supervision. Chiara Romualdi: critical revision of the manuscript for important intellectual content, analysis and interpretation of data, statistical analysis. Richard Nicholas: study concept and design, analysis and interpretation of data, drafting/revising the manuscript, critical revision of the manuscript for important intellectual content. Miriam Mattoscio and Roberta Magliozzi: drafting/revising the manuscript, critical revision of the manuscript for important intellectual content. Aldo Morra: acquisition of MRI data. Salvatore Monaco: drafting/revising the manuscript, critical revision of the manuscript for important intellectual content. Paolo Muraro: study concept and design, analysis and interpretation of data, drafting/revising the manuscript, critical revision of the manuscript for important intellectual content. Massimiliano Calabrese: study concept and design, analysis and interpretation of data, drafting/revising the manuscript, critical revision of the manuscript for important intellectual content, acquisition of clinical and MRI data, obtained funding, study supervision.

Study funding

This study has been supported by the UK MS Society (grant 975 to Dr. Scalfari) and by the Progressive MS Alliance (grant PA 0124 to Dr. Calabrese).

Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

Received October 6, 2017. Accepted in final form April 2, 2018.

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The cortical damage, early relapses, and onset of the progressive phase in multiple sclerosis

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Neurology 2018;90;e2107-e2118 Published Online before print May 16, 2018
DOI 10.1212/WNL.0000000000005685