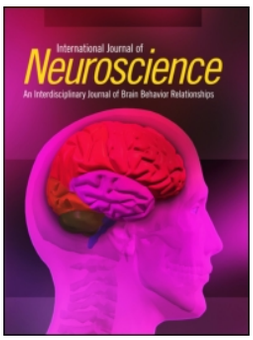

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ORIGINAL ARTICLE

A longitudinal uncontrolled study of cerebral gray matter volume in patients receiving natalizumab for multiple sclerosis

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Objective: Brain atrophy in multiple sclerosis (MS) selectively affects gray matter (GM), which is highly relevant to disability and cognitive impairment. We assessed cerebral GM volume (GMV) during one year of natalizumab therapy. *Design/methods*: Patients with relapsing–remitting ($n = 18$) or progressive ($n = 2$) MS had MRI ~1 year apart during natalizumab treatment. At baseline, patients were on natalizumab for (mean ± SD) 16.6 ± 10.9 months with age 38.5 ± 7.4 and disease duration 9.7 ± 4.3 years. *Results*: At baseline, GMV was 664.0 ± 56.4 ml, Expanded Disability Status Scale (EDSS) score was 2.3 ± 2.0, timed 25-foot walk (T25FW) was 6.1±3.4 s; two patients (10%) had gadolinium (Gd)-enhancing lesions. At follow-up, GMV was 663.9 ± 60.2 mL; EDSS was 2.6 ± 2.1 and T25FW was 5.9 ± 2.9 s. One patient had a mild clinical relapse during the observation period (0.052 annualized relapse rate for the entire cohort). No patients had Gd-enhancing lesions at follow-up. Linear mixed-effect models showed no significant change in annualized GMV [estimated mean change per year 0.338 mL, 95% confidence interval −9.66, 10.34, $p = 0.94$)], GM fraction ($p = 0.92$), whole brain parenchymal fraction ($p = 0.64$), T2 lesion load ($p = 0.64$), EDSS ($p = 0.26$) or T25FW ($p = 0.79$). *Conclusions*: This pilot study shows no GM atrophy during one year of natalizumab MS therapy. We also did not detect any loss of whole brain volume or progression of cerebral T2 hyperintense lesion volume during the observation period. These MRI results paralleled the lack of clinical worsening.

KEYWORDS: multiple sclerosis, MRI, natalizumab, gray matter, brain atrophy

Introduction

Progressive brain atrophy begins early in the disease course of multiple sclerosis (MS) and is commonly used as a secondary outcome measure in therapeutic trials [1,2]. Originally, white matter (WM) volume (WMV) loss was presumed to be the main contribution to brain atrophy in MS. However, it is now accepted that the major contribution is from gray matter (GM) volume (GMV) loss [2]. GM atrophy is also of high interest due to its strong relationship to clinical status including cognitive impairment [2–4] and physical disability [2–5].

Natalizumab, a humanized monoclonal antibody, is an alpha-4 integrin antagonist that limits T-lymphocyte entry into the central nervous system (CNS) [6]. As reviewed recently [6], this medication has proven effective in limiting relapses, progression of physical disability, and both the initial formation [7,8], and secondary evolution [9] of MRI-defined brain lesions in patients with relapsing–remitting (RR) MS. Natalizumab also showed a partial effect in limiting the rate of whole brain atrophy in a large randomized placebo-controlled phase III trial [8]. Given the potential interest in using GM atrophy to track therapeutic response [10,11] and the growing recognition of the importance of GM involvement in the pathophysiology of the disease [12,13], recent investigations have identified an

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Table 1. Baseline patient characteristics.

Patients with MS on natalizumab	$n = 20$
Time on natalizumab prior to study entry (months)	16.6 ± 10.9 (2.8–41.3)
Age (years)	38.5 ± 7.4 (22.9–55.1)
Women	$n = 14$ (70%)
Clinical course	
Relapsing–remitting MS	$n = 18$ (90%)
Progressive forms of MS*	$n = 2$ (10%)
Brain gadolinium-enhancing lesions present	$n = 2$ (10%)
Disease duration (years)†	9.7 ± 4.3 (1.9–20.2)
Expanded Disability Status Scale score	2.3 ± 2.0 (0–6.5)
Timed 25-foot walk (s)	6.1 ± 3.4 (3.9–17.0)

Note: Data are expressed as mean ± standard deviation (range); MS = multiple sclerosis.
*Secondary progressive MS ($n = 1$) and progressive-relapsing MS ($n = 1$).
†Time since first symptoms.

effect of natalizumab on limiting MRI-defined GM involvement [14–17]. The goal of our study was to assess the change in cerebral GMV in patients with MS during one year of treatment with natalizumab.

Methods

Subjects

Demographic and baseline clinical characteristics of the patients are summarized in Table 1. We retrospectively identified 20 consecutive patients by electronic chart review, followed at our institution. Inclusion criteria were as follows: (1) diagnosed with MS by the international panel criteria [18]; (2) on treatment with natalizumab for at least one year; (3) baseline and follow-up brain MRI performed ~one year apart; (4) age 18–60 years; (5) remained on natalizumab monotherapy during the observation period; (6) no corticosteroids within four weeks prior to MRI; (7) evaluated by a neurologist specializing in MS within three months of MRI at baseline and follow-up to assess the occurrence of relapses, and perform Expanded Disability Status Scale (EDSS) scoring [19] and timed 25-foot walk (T25FW) [20]. Due to the study design, informed consent was not required; the study protocol was approved by our institute's committee on human research and followed the Declaration of Helsinki regarding ethical principles for medical research involving humans.

MRI acquisition

All patients underwent brain MRI on the Signa 1.5T (General Electric, Milwaukee, WI) family scanners at our hospital. The scan protocols included axial conventional spin-echo dual-echo images covering the whole head (54 slices) using a repetition time (TR) = 2550–3000 ms, echo times (TE1/TE2) 30/80 ms, slice thickness 3 mm (no inter-slice gaps), with resulting pixel sizes of 0.7812–0.9375 mm^2. Thirty-one (77.5%) of the 40 scans were performed on the same scanner using the same protocol (TR 3000 ms, TE 30/80 ms, voxel size 0.9375 × 0.9375 × 3 mm). Nine of the scans had a deviation of some kind from the primary protocol, such as slightly smaller pixel sizes or slightly lower repetition times. Six of the scans on a different protocol were from the first time point, and the remaining three scans were from the second time point. Patients also underwent 3 mm axial T1-weighted spin-echo imaging both before and 5–7 min after single-dose intravenous gadolinium (Gd).

MRI analysis

Whole brain and GM atrophy: The dual echo images were processed by an automated template-driven segmentation [21] to calculate GMV, WMV and intracranial volume (ICV). Whole brain atrophy was estimated by an intra-subject normalized measure, the brain parenchymal fraction [BPF = (cerebral GMV + WMV/ICV)]. The automatically generated maps had misclassifications between GM and WM compartments. Thus, an experienced observer manually corrected the automated maps to create ground truth maps of GMV using 3D Slicer version 2.4 (http://www.slicer.org). For completeness of data analysis, the change in GMV was explored using both the raw GMV and an intra-subject normalized measure: GM fraction (GMF = cerebral GMV/ICV). The intra-rater reliability for the measurement of GMV (from five randomly chosen cases) was high, showing a mean coefficient of variation of 0.25%.

T2 hyperintense lesion volume: Quantification of total cerebral T2 hyperintense lesion volume (T2LV) was performed from the dual-echo images by first marking all lesions by the consensus of a trained observer and an experienced observer. The trained observer then traced each lesion using a semi-automated edge-finding tool in Jim software version 7.0 (Xinapse Systems Ltd., West Bergholt, UK, http://www.xinapse.com).

Statistical analysis

The longitudinal changes in MRI data between baseline and follow-up were assessed using a mixed-effects linear regression model to assess if there was an impact of time. T2LV was log-transformed prior to all statistical analysis. For the clinical measures (EDSS and T25FW), a mixed-effect linear regression model was also used. When a mixed-effect ordinal logistic regression model

Table 2. On-study MRI changes.

	Baseline	Follow-up	p Value
Cerebral gray matter volume (ml)	664.0 ± 56.4 (606.0–781.6)	663.9 ± 60.2 (590.3–820.3)	0.94
Cerebral gray matter fraction	0.481 ± 0.022 (0.437–0.523)	0.481 ± 0.023 (0.445–0.533)	0.92
Brain parenchymal fraction	0.846 ± 0.04 (0.727–0.895)	0.844 ± 0.037 (0.740–0.886)	0.64
Cerebral T2 hyperintense lesion volume (ml)*	11.5 ± 9.3 (1.4–33.9)	10.6 ± 9.0 (1.7–31.4)	0.10

Note: Data are mean ± standard deviation (range); cerebral gray matter fraction = gray matter volume/intracranial volume; brain parenchymal fraction = (gray matter + white matter volume)/intracranial volume; p values are from mixed-effects models.
*Log-transformed for analysis.

was used to account for the ordinal nature of the EDSS, the results were unchanged, but we reported the mixed-effect linear regression results due to the easier interpretation. The annualized relapse rate in this cohort was calculated as the total number of relapses divided by the total duration of follow-up. Differences were considered significant at $p < 0.05$. All statistical analyses were completed in Stata/IC version 14.0 (StataCorp LP).

Results

On-study MRI changes

Changes in MRI parameters during the study period are shown in Table 2 and Figures 1–8. Over one year of treatment with natalizumab, no significant longitudinal change was observed in GMV (estimated mean change = 0.338 mL per year, 95% confidence interval (CI) −9.66, 10.34, $p = 0.94$), GMF (estimated mean change = 0.0004 per year, 95% CI −0.0067, 0.0075, $p = 0.92$), BPF (estimated mean change = −0.0016 per year, 95% CI −0.0085, 0.0054, $p = 0.64$) or T2LV (estimated mean change = −0.092 mL per year, 95% CI −0.205, 0.020, $p = 0.10$) (Table 2). Figures 1–4 show the cohort mean and standard deviation and Figures 5–8 show the individual patient data on brain atrophy and T2LV MRI measures at baseline and follow-up. While two patients (10%) had cerebral Gd-enhancing lesions at baseline, none had Gd-enhancing lesions at follow-up.

On-study clinical changes

The clinical changes are shown in Tables 3 and 4. EDSS score was 2.3 ± 2.0 and T25FW was 6.1 ± 3.4 s at baseline. At follow-up, EDSS was 2.6 ± 2.1 and T25FW was 5.9 ± 2.9 s. There were no significant longitudinal changes in the cohort during the observation period in EDSS (estimated mean change =0.265 per year, 95% CI −0.21, 0.75, $p = 0.26$) or T25FW (estimated mean change = −0.124 s per year, 95% CI −1.09, 0.85, $p = 0.79$). None of the patients received high dose corticos-

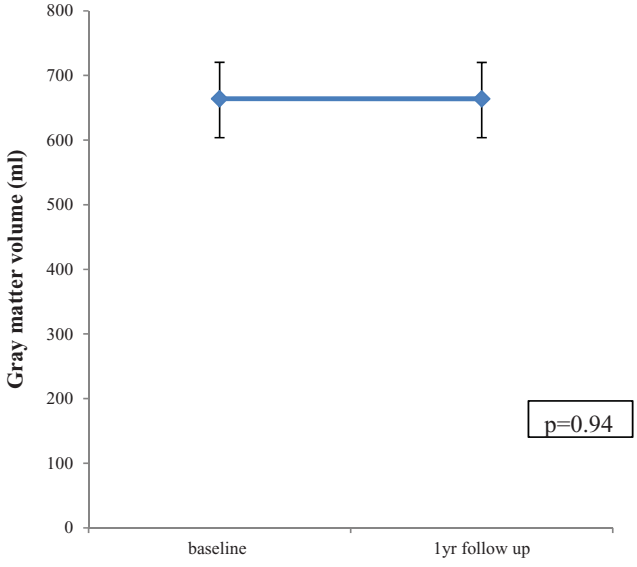

Figure 1. Cerebral gray matter volume. Mean and standard deviation at baseline and follow-up.

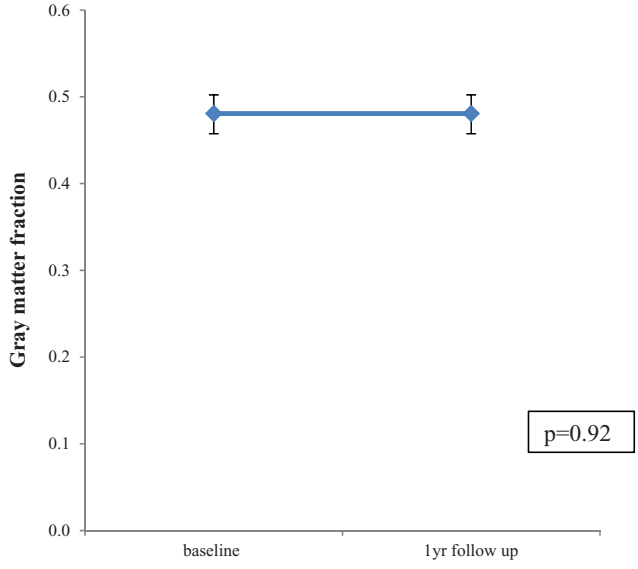

Figure 2. Cerebral gray matter fraction. Mean and standard deviation at baseline and follow-up.

Table 3. On-study change in physical disability (EDSS).

Category	Expanded disability status scale score	# of patients	(%)
Improved	Improvement of at least 1 point	$n = 2$	10
No change	Change of 0 or 0.5 points with baseline ≥ 1 or change of 1 point or less for baseline EDSS = 0	$n = 13$	65
Worsened	Increase of at least 1 point with baseline ≥ 1 or increase of at least 1.5 points for baseline EDSS = 0	$n = 5$	25

Note: EDSS = Expanded Disability Status Scale score.

Table 4. On-study change in ambulatory function (T25FW).

Category	Timed 25-foot walk (s)	# of patients	(%)
Improved	≥20% decrease	$n = 3$	15
No change	Change between −20% and +20%	$n = 12$	60
Worsened	≥20% increase	$n = 5$	25

Note: T25FW = Timed 25-foot walk.

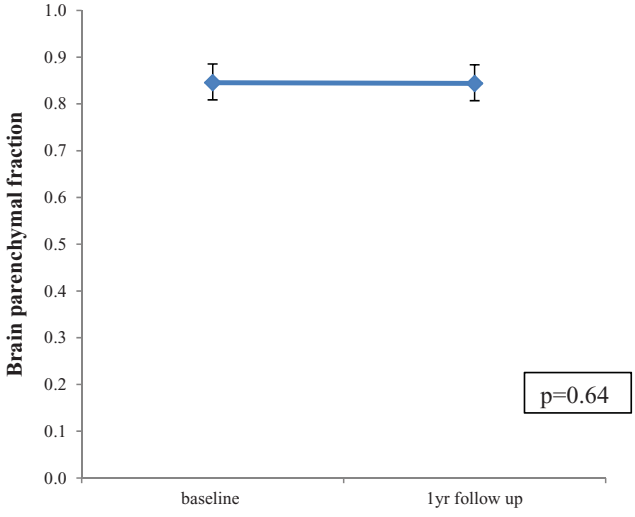

Figure 3. Brain parenchymal fraction. Mean and standard deviation at baseline and follow-up.

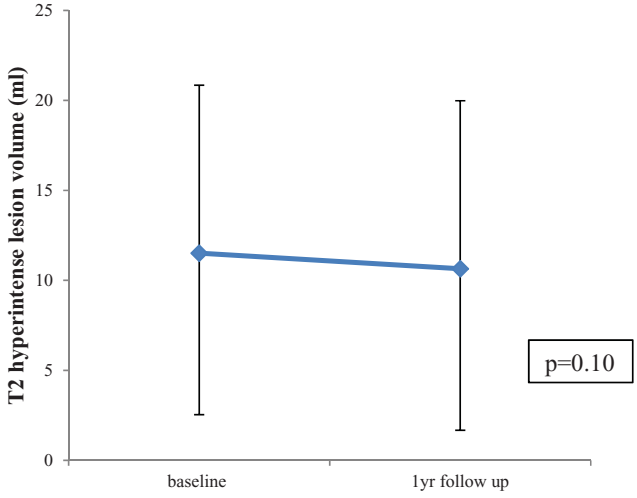

Figure 4. Cerebral T2 hyperintense lesion volume. Mean and standard deviation at baseline and follow-up.

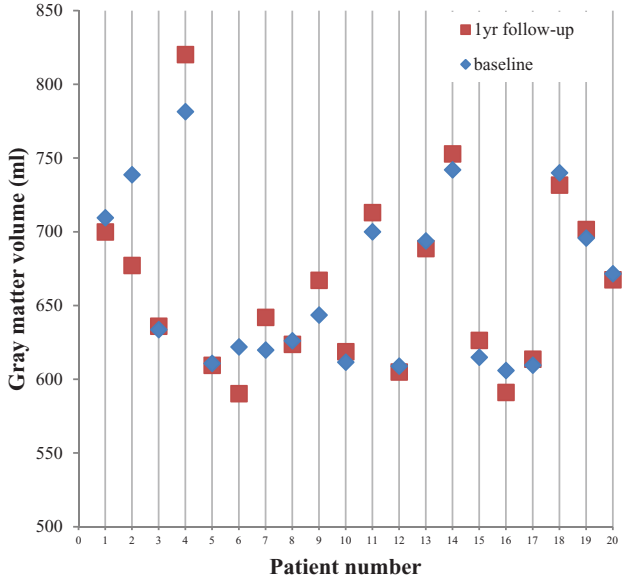

Figure 5. Gray matter volume.
Note: Individual patient data showing baseline and follow-up. MRI parameters at baseline (◆) and follow-up (■).

teroids during the observation period. One (5%) patient had a mild relapse in the setting of urinary tract infection and was successfully treated with antibiotics. Thus, the annualized relapse rate for the entire cohort was 0.052.

Potential confounding variables

To assess if our results were confounded by changes in scan protocol, we completed all of the analyses in only the subjects who were scanned with the same platform and protocol at both time points ($n = 11$). These patients had similar characteristics to the whole cohort, being on natalizumab for 15.4 ± 10.6 months prior to study entry, with baseline age 35.9 ± 6.9 years, disease

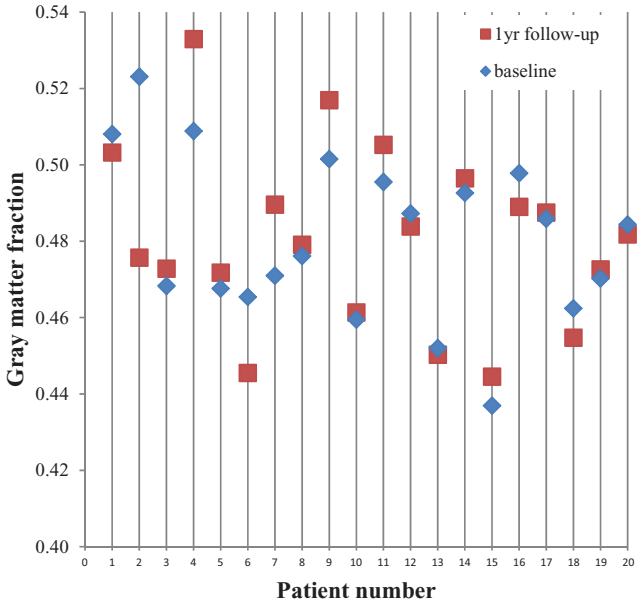

Figure 6. Gray matter fraction.
Note: Individual patient data showing baseline and follow-up. MRI parameters at baseline (◆) and follow-up (■).

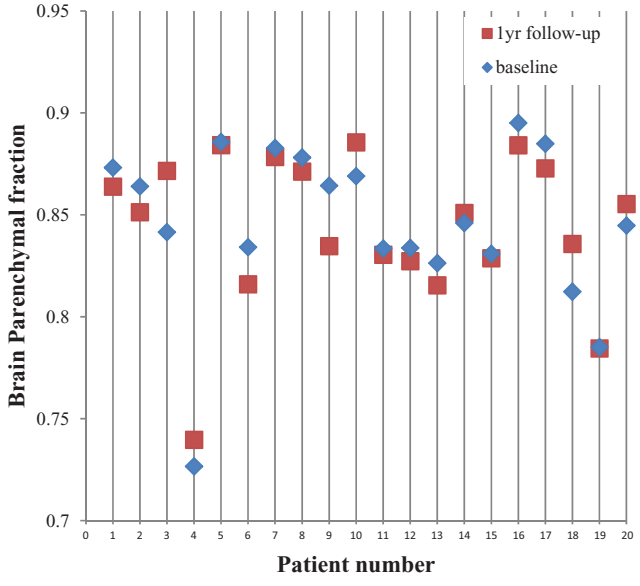

Figure 7. Brain parenchymal fraction.
Note: Individual patient data showing baseline and follow-up. MRI parameters at baseline (◆) and follow-up (■).

duration 10.5 ± 4.8 years, EDSS score 2.0 ± 1.7 and T25FW 6.1 ± 3.9 s; 8 (73%) were women. Furthermore, the p values for on-study MRI changes in GMV (0.45), GMF (0.25), BPF (0.52) and T2LV (0.08), and the direction of these changes was similar to the results reported in Table 2. Finally, the proportions of patients improved (18%), unchanged (45%) or worsened (36%) on EDSS score, and improved (18%), unchanged (55%) or worsened (27%) on T25FW during the study period were similar to the results reported in Tables 3 and 4.

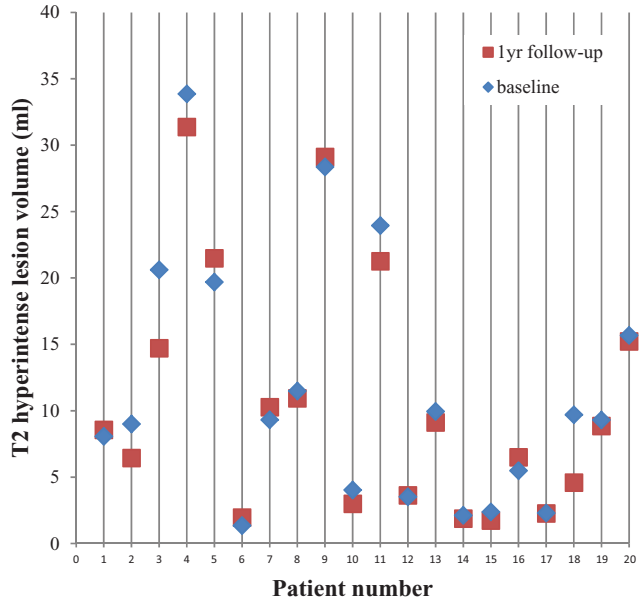

Figure 8. T2 hyperintense lesion volume.
Note: Individual patient data showing baseline and follow-up. MRI parameters at baseline (◆) and follow-up (■).

We assessed if there was an association between age and the change in any of the MRI metrics. For the BPF and T2LV, there was no significant association between either measure and age (BPF: $r = -0.13$, $p = 0.60$; T2LV: $r = 0.04$; $p = 0.87$). For the two GM measures, there was a statistically significant negative association between the change in the MRI measure and baseline age (GMV: $r = -0.49$, $p = 0.028$; GMF $= -0.45$, $p = 0.045$). This significant correlation shows that older subjects experienced a greater decline in the GMV and GMF.

Results were also re-analyzed without the two patients with progressive MS to remove any confounding effects of different disease types. All of the results in the remaining 18 patients were similar to the results in the full 20 patient sample. For example, the p values for on-study MRI changes in GMV (0.70), GMF (0.74), BPF (0.58) and T2LV (0.10), and the direction of these changes were similar to the results reported in Table 2. Furthermore, the proportion of patients improved (11%), unchanged (61%), or worsened (28%) on EDSS score, and improved (11%), unchanged (67%) or worsened (22%) on T25FW during the study period were similar to the results reported in Tables 3 and 4.

Discussion

We report longitudinal brain MRI data from a "real world" cohort of patients treated with natalizumab for

one year. The major finding in this study is the lack of any cerebral GM atrophy during the observation period. This paralleled the lack of any whole brain atrophy or progression of conventional WM lesion load. Furthermore, the patients had relative clinical quiescence and stability during this time as shown by a low relapse rate and no worsening of overall physical disability or walking speed.

Our findings add to a relatively small but growing body of evidence documenting the effect of MS disease-modifying therapies (DMTs) on GM pathology. Despite the emergence of more than 10 established DMTs in the past two decades from the successful completion of numerous multi-center randomized double-blind phase III RRMS studies, there are only two reports of GM outcome measures related to these studies, with mixed results. In the pivotal trial of intramuscular interferon beta-1a, a *post hoc* analysis showed a slowed rate of cerebral GM atrophy in the second year after the start of therapy compared to placebo [22]. However, in the trial of teriflunomide that included MRI assessments, a treatment effect was shown on cerebral WM atrophy but not GM atrophy [23]. Several small unblinded phase IV RRMS studies have also shown mixed results on the GM atrophy treatment effects of interferon beta-1a, interferon beta-1b and glatiramer acetate. This includes positive results with interferon beta-1a [24] and glatiramer acetate [25], negative results with interferon beta-1a and interferon beta-1b [25] and positive results when collectively considering interferon beta-1a and glatiramer acetate [26]. In addition, laquinimod, a therapy in development for MS, appears to limit cerebral GM atrophy [27].

Why is it important to study whether treatments known to benefit clinical and conventional MRI-based WM measures of disease progression have the same degree of effects on GM pathology? This arises from a major unmet need in the MS scientific field – to understand the pathophysiologic and mechanistic differences, if any, between WM and GM involvement [12,13]. Both the cortical GM and deep subcortical GM nuclei are characterized by atrophy and the presence of demyelinating lesions [13]. GM lesions show potentially important differences vs. those in WM, such as the paucity of lymphocytic inflammatory changes, abundance of microglial activation and the possible association with B-cell meningeal pathology of the former [13]. Several studies have suggested that GM and WM features of the disease may progress somewhat independently [13]. Thus, it remains to be proven whether GM involvement represents an unmet need from a therapeutic standpoint, given that the current DMTs target immune processes known to be associated with the development and progression of WM lesions, which may not necessarily translate to an effect on GM.

Arguing for the extension of a treatment effect of current DMTs to GM pathology, a few studies have shown the ability of interferon beta-1a [26–28], glatiramer acetate [26] and natalizumab [14] to limit the development of cortical lesions. Adding to evidence extending natalizumab's effect to GM pathology, magnetization transfer imaging has shown restoration of structural integrity after onset of therapy [17]. These parallel observations in WM known to follow the start of natalizumab [16]. Furthermore, the start of natalizumab leads to a restoration of T2 signal intensity in GM, a possible result of reducing iron-associated neurodegeneration [15].

Given the treatment effects of natalizumab on GM pathology suggested by these emerging studies, it is perhaps not surprising that one previous RRMS study showed that the new start of natalizumab therapy led to a two year reduction in the rate of cortical atrophy compared to the initiation of interferon beta-1a, glatiramer acetate or no DMT [14]. Because natalizumab-associated pseudoatrophy is thought to selectively affect WM [29], GM volume monitoring presents a more attractive option than whole brain volume to monitor overall destructive effects of the disease. Indeed, in a phase III trial, an increased rate of whole brain volume loss in the first year after initiation of natalizumab compared to placebo treatment, most likely represented pseudoatrophy and would have offset any benefit on limiting atrophy [8]. This pseudoatrophy effect may continue into the second year of such natalizumab [11,30]. In this study we did not detect any loss of whole brain volume, perhaps due to the fact that our study did not focus on new starts on natalizumab. In summary, our findings provide further evidence of a stabilizing effect of this medication on GM destructive effects and show the feasibility of using cerebral GMV metrics to track MS therapy response. However, our conclusions should be tempered by recent consensus guidelines on MS disease monitoring [31] highlighting the controversy of the role of atrophy monitoring of individual patient changes.

The limitations of our study include the study design and short observation period. This was an uncontrolled, single arm retrospective study of a small sample of patients. The sample was not enriched on the basis of active disease at baseline. Studies that include a high number of patients with Gd-enhancing lesions at baseline would have likely recorded a higher rate of GM atrophy during the observation period [30]. Nonetheless, ours was a consecutive sample from a comprehensive care MS clinic and would have relevance to an unselected population. Lack of a comparison group limits the generalizability of the findings. More definitive results on a GM atrophy treatment effect of natalizumab will likely come from *post hoc* analyses of large randomized placebo-controlled multi-center clinical trial data sets of a longer duration than this study.

Conclusions

This pilot study shows no GM atrophy during one year of natalizumab MS therapy. We also did not detect any loss of whole brain volume or progression of cerebral T2LV during the observation period. These MRI results paralleled the lack of clinical worsening.

Acknowledgements

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Declaration of Interest

Dr Healy received research support from Merck-Serono, Genzyme and Novartis. Dr Bakshi received consulting fees from AbbVie, Alkermes, Biogen, Novartis and Questcor and research support from Biogen, Merck-Serono, Novartis, Genzyme and Teva. The other authors have nothing to disclose. This work was presented in preliminary form at the 2015 annual meeting of the American Academy of Neurology, Washington, DC.

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References

1. Vidal-Jordana A, Sastre-Garriga J, Rovira A, et al. Treating relapsing-remitting multiple sclerosis: therapy effects on brain atrophy. J Neurol 2015;262:2617–26.
2. Bermel RA, Bakshi R. The measurement and clinical relevance of brain atrophy in multiple sclerosis. Lancet Neurol 2006;5:158–70.
3. Sanfilipo MP, Benedict RH, Weinstock-Guttman B, et al. Gray and white matter brain atrophy and neuropsychological impairment in multiple sclerosis. Neurology 2006;66:685–92.
4. Schoonheim MM, Popescu V, Rueda Lopes FC, et al. Subcortical atrophy and cognition: sex effects in multiple sclerosis. Neurology 2012;79:1754–61.
5. Fisniku LK, Chard DT, Jackson JS, et al. Gray matter atrophy is related to long-term disability in multiple sclerosis. Ann Neurol 2008;64:247–54.
6. Chataway J, Miller DH. Natalizumab therapy for multiple sclerosis. Neurotherapeutics 2013;10:19–28.
7. Miller DH, Khan OA, Sheremata WA, et al. A controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 2003;348:15–23.
8. Miller DH, Soon D, Fernando KT, et al. MRI outcomes in a placebo-controlled trial of natalizumab in relapsing MS. Neurology 2007;68:1390–401.
9. Dalton CM, Miszkiel KA, Barker GJ, et al. Effect of natalizumab on conversion of gadolinium enhancing lesions to T1 hypointense lesions in relapsing multiple sclerosis. J Neurol 2004;251:407–13.
10. Khoury S, Bakshi R. Cerebral pseudoatrophy or real atrophy after therapy in multiple sclerosis. Ann Neurol 2010;68:778–9.
11. De Stefano N, Arnold DL. Towards a better understanding of pseudoatrophy in the brain of multiple sclerosis patients. Mult Scler 2015;21:675–6. (See comment in PubMed Commons below).
12. Pirko I, Lucchinetti CF, Sriram S, et al. Gray matter involvement in multiple sclerosis. Neurology 2007;68:634–42.
13. Kutzelnigg A, Lassmann H. Pathology of multiple sclerosis and related inflammatory demyelinating diseases. Handb Clin Neurol 2014;122:15–58.
14. Rinaldi F, Calabrese M, Seppi D, et al. Natalizumab strongly suppresses cortical pathology in relapsing-remitting multiple sclerosis. Mult Scler 2012;18:1760–7.
15. Pawate S, Wang L, Song Y, et al. Analysis of T2 intensity by magnetic resonance imaging of deep gray matter nuclei in multiple sclerosis patients: effect of immunomodulatory therapies. J Neuroimaging 2012;22:137–44.
16. Zivadinov R, Dwyer MG, Hussein S, et al. Voxel-wise magnetization transfer imaging study of effects of natalizumab and IFNβ-1a in multiple sclerosis. Mult Scler 2012;18:1125–34.
17. Romme Christensen J, Ratzer R, Börnsen L, et al. Natalizumab in progressive MS: results of an open-label, phase 2A, proof-of-concept trial. Neurology 2014;82:1499–507.
18. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 2005;58:840–6.
19. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444–52.
20. Goldman M, Motl RW, Scagnelli J, et al. Clinically meaningful performance benchmarks in MS timed 25-foot walk and the real world. Neurology 2013;81:1856–63.
21. Wei X, Warfield SK, Zou KH, et al. Quantitative analysis of MRI signal abnormalities of brain white matter with high reproducibility and accuracy. J Magn Reson Imaging 2002;15:203–9.
22. Fisher E, Nakamura K, Lee JC, et al. Effect of intramuscular interferon beta-1a on gray matter atrophy in relapsing-remitting multiple sclerosis: a retrospective analysis. Mult Scler 2016;22:668–76.
23. Wolinsky JS, Narayana PA, Nelson F, et al. Magnetic resonance imaging outcomes from a phase III trial of teriflunomide. Mult Scler 2013;19:1310–19.
24. Zivadinov R, Locatelli L, Cookfair D, et al. Interferon beta-1a slows progression of brain atrophy in relapsing-remitting multiple sclerosis predominantly by reducing gray matter atrophy. Mult Scler 2007;13:490–501.
25. Bendfeldt K, Egger H, Nichols TE, et al. Effect of immunomodulatory medication on regional gray matter loss in relapsing-remitting multiple sclerosis–a longitudinal MRI study. Brain Res 2010;1325:174–82.
26. Calabrese M, Bernardi V, Atzori M, et al. Effect of disease-modifying drugs on cortical lesions and atrophy in relapsing-remitting multiple sclerosis. Mult Scler 2012;18:418–24.
27. Filippi M, Rocca MA, Pagani E, et al. Placebo-controlled trial of oral laquinimod in multiple sclerosis: MRI evidence of an effect on brain tissue damage. J Neurol Neurosurg Psychiatry 2014;85:851–8.
28. Rinaldi F, Perini P, Atzori M, et al. Disease-modifying drugs reduce cortical lesion accumulation and atrophy progression in relapsing-remitting multiple sclerosis: results from a 48-month extension study. Mult Scler Int 2015;2015:369348.

29. Vidal-Jordana A, Sastre-Garriga J, Pérez-Miralles F, et al. Early brain pseudoatrophy while on natalizumab therapy is due to white matter volume changes. Mult Scler 2013;19:1175–8.
30. Sastre-Garriga J, Tur C, Pareto D, et al. Brain atrophy in natalizumab-treated patients: a 3-year follow-up. Mult Scler 2015;21:749–56.
31. Wattjes MP, Rovira À, Miller D, et al. Evidence-based guidelines: MAGNIMS consensus guidelines on the use of MRI in multiple sclerosis-establishing disease prognosis and monitoring patients. Nat Rev Neurol 2015;11: 597–606.