

## SHORT REPORT

# Brain tissue loss occurs after suppression of enhancement in patients with multiple sclerosis treated with autologous haematopoietic stem cell transplantation

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To assess whether brain tissue loss occurs after profound and sustained suppression of magnetic resonance imaging (MRI) enhancement, we measured brain volume changes from 10 patients with rapidly evolving secondary progressive multiple sclerosis (MS) treated with autologous haematopoietic stem cell transplantation and followed up for 24 months. An average yearly decrease of brain volume of about 1.9% was observed despite only five enhancing lesions seen on triple dose follow up scans of two patients. This indicates that, in MS, progressive loss of tissue can occur independently of concomitant MRI-visible inflammation.

## INTRODUCTION

In multiple sclerosis (MS), the relationship between inflammatory demyelination and development of brain atrophy has not been clarified. Although histopathological studies have shown many more transected axons in lesions with large inflammatory infiltrates than in those without,<sup>1</sup> the magnitude of the correlation between brain tissue loss and enhancement was found to be either absent or weak in several *in vivo* magnetic resonance imaging (MRI) studies.<sup>2</sup> In addition, previous trials of various agents failed to show a treatment effect on progression of brain atrophy, despite a significant effect on enhancements.<sup>2</sup> However, in all these studies, a standard dose of gadolinium (Gd) was used and, in addition, complete suppression of MRI-detected inflammation was never achieved. Since many enhancing lesions go undetected when using standard dose MRI,<sup>3</sup> the discrepant effect of treatment on frequency of enhancement and development of brain atrophy might be a consequence of previous disease activity and persistent “low grade” inflammation.

A recent trial of patients with secondary progressive (SP) MS has shown that autologous haematopoietic stem cell transplantation (AHSCT) has a dramatic effect in reducing (~100%) the number of active lesions seen on triple dose enhanced scans (391 enhancing lesions were seen during the pretreatment period and only five after AHSCT) and on the formation of T2 lesions (162 new T2 lesions were seen during the pretreatment period and only three after AHSCT).<sup>4</sup> The purpose of this study was to assess whether brain tissue loss continues to occur even when profound and sustained suppression of MRI-visible activity is achieved.

## PATIENTS AND METHODS

Ten patients with rapidly evolving SPMS were enrolled in a phase I/II study to assess feasibility and safety of AHSCT.

Peripheral blood progenitor cells were mobilised with cyclophosphamide (CY) (4 g/m<sup>2</sup>), followed by granulocyte-colony stimulating factor (G-CSF) 5 µg/kg/day until the completion of the cell harvests. The conditioning regimen consisted of carmustine 300 mg/m<sup>2</sup> on day -7, cytosine-arabinoside 200 mg/m<sup>2</sup> and etoposide 200 mg/m<sup>2</sup> from day -6 to day -3, and melphalan 140 mg/m<sup>2</sup> on day -2. Rabbit antithymocyte globulin (5 mg/kg/day) was administered on day +1 and +2 as *in vivo* T-depletion. Intravenous ciclosporin A (1 mg/kg) was given during the conditioning regimen to prevent exacerbations due to cytokine release. Further details about the study population and treatment regimen have been reported previously.<sup>4</sup>

At study entry and at months 0 (30 days after mobilisation), 1 (30 days after conditioning regimen), and monthly for the following 5 months and every 3 months until month 24, dual-echo, pre-contrast and triple dose enhanced T1-weighted scans (5–8 min after the injection of 0.3 mmol/kg Gd-diethylenetriamine penta-acetic acid (Gd-DTPA) were obtained from all patients. Slices were axial, contiguous, 3 mm thick, with an in-plane resolution of approximately 1×1 mm. Additional information about image acquisition, lesion identification and lesion counting are reported elsewhere.<sup>4</sup>

Using pre-contrast T1-weighted images, percentage normalised brain volume change (PBVC) and cross-sectional normalised brain volume (NBV) were estimated, using SIENA (Structural Image Evaluation, using Normalisation, of Atrophy; University of Oxford, Oxford, UK) and SIENAX.<sup>5</sup> These are automated segmentation techniques, which require only minimal manual input from human observers and, as a consequence, provide highly reproducible results.<sup>5</sup> Technical details about the software are provided elsewhere.<sup>5</sup> The following metrics were obtained from each patient: the NBV at month 1 (this was considered the “baseline” scan, because at this timepoint enhancement was already profoundly suppressed,<sup>4</sup> and we tried to minimise the risk of measuring “pseudoatrophy” on follow up scans due to resolution of oedema), and the PBVC between baseline and month 12, and between months 12 and 24.

The correlations between the cumulative number of enhancing lesions in the pretreatment period and the PBVC during treatment were assessed using Spearman's rank correlation coefficient.

**Abbreviations:** AHSCT, autologous haematopoietic stem cell transplantation; DTPA, diethylenetriamine penta-acetic acid; G-CSF, granulocyte-colony stimulating factor; Gd, gadolinium; MRI, magnetic resonance imaging; MS, multiple sclerosis; NBV, normalised brain volume; PBVC, percentage normalised brain volume change; SPMS, secondary progressive multiple sclerosis

## RESULTS

The demographic and baseline characteristics of the patients studied and the effect of AHST on clinical metrics have been reported previously.<sup>4</sup> Only five enhancing lesions were seen in two patients during the first three months following AHST over a 24 month follow up period. As previously reported,<sup>4</sup> only three new T2 lesions were seen in one patient on the first three scans; no additional T2 lesions formed during the rest of the follow up. The mean PBVC between baseline and month 12 was  $-1.87\%$  (standard deviation (SD)  $2.19\%$ , range  $-5.24\%$  to  $0.53\%$ ) and between months 12 and 24 was  $-1.88\%$  (SD  $0.78\%$ , range  $-3.53\%$  to  $-0.84\%$ ). There was no correlation between disease activity before AHST and PBVCs between baseline and month 12 (PBVC  $\nu$  cumulative number of Gd enhancing lesions pre-AHST:  $r = 0.22$ ,  $p = 0.053$  and  $\nu$  new T2 lesions:  $r = 0.07$ ,  $p = 0.85$ ) and between months 12 and 24 (PBVC  $\nu$  cumulative number of Gd enhancing lesions pre-AHST:  $r = 0.07$ ,  $p = 0.85$  and  $\nu$  new T2 lesions:  $r = 0.10$ ,  $p = 0.78$ ). No correlation was also found between NBV at "baseline" and subsequent PBVCs.

## DISCUSSION

We investigated whether progressive tissue loss in MS patients can occur when MRI-visible inflammation has been almost completely suppressed for a relatively long period of time. The novelty of this study lies in the fact that we assessed MRI activity by means of serial scans after the injection of a triple dose of Gd, which is known to increase the sensitivity of MRI for the detection of enhancements by about  $75\%$ .<sup>3</sup> Whereas AHST had a profound and sustained effect on enhancement and T2 lesion formation, we observed a mean yearly decrease of brain volume of about  $1.9\%$  over the two year duration of the study, a figure which is approximately two-fold higher than that reported in previous MS "atrophy" studies.<sup>6</sup> Clearly, since we did not know the pretreatment rate of atrophy of these patients, we cannot comment whether or not AHST had any effect on brain atrophy progression. However, these findings supports the notion that tissue loss in MS is likely to be conditioned by a high inflammatory load, but proceeds even when inflammation has subsequently been suppressed.

Several factors might explain this somewhat unexpected finding. First, the patients enrolled in the trial had an extremely severe and rapidly progressive form of MS, which was unresponsive to "conventional" treatment. As a consequence, these patients might represent a subgroup of the most injured MS patients, in whom brain atrophy might be accumulated at a more rapid pace than in those with a more "classic" disease course. Secondly, during the pretreatment period, the patients experienced a very active phase of the disease.<sup>4</sup> Therefore, it is conceivable that the reported figure of brain tissue loss reflects the long-term consequences of previous disease activity on tissue integrity. Nevertheless, we cannot exclude that longer MRI-monitored pretreatment periods and a different patient population with less skewed disease activity might disclose a correlation. Thirdly, the dramatic suppression of MRI activity achieved with AHST and the consequent resolution of oedema might have resulted in some degree of pseudoatrophy at least during the first year of follow up. Finally, we cannot exclude that AHST itself might contribute to brain tissue loss. Brain atrophy was indeed observed in patients affected by chronic myeloid leukaemia, who underwent allogenic bone marrow transplantation with total body irradiation.<sup>7</sup>

There are, however, several other disease-related mechanisms that might explain progressive tissue loss in MS in the absence of concomitant inflammation. Previous repeated inflammatory insults might result in a reduced effectiveness

of remyelination. In persistently demyelinated lesions, axons might degenerate due to loss of trophic factors<sup>8</sup> or to altered electrical conduction.<sup>9</sup> The loss of secreted or contact glial factors might also contribute to axonal damage,<sup>10</sup> and hence to tissue loss. Additional studies are now warranted to establish the role of all these mechanisms in causing MS-related irreversible tissue loss, not directly linked to the inflammatory component of the disease.

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## Appendix

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