ORIGINAL RESEARCH

A Three-Year Study of Brain Atrophy after Autologous Hematopoietic Stem Cell Transplantation in Rapidly Evolving Secondary Progressive Multiple Sclerosis

M.A. Rocca
T. Mondria
P. Valsasina
M.P. Sormani
Z.H. Flach
P.A. Te Boekhorst
G. Comi
R.Q. Hintzen
M. Filippi

BACKGROUND AND PURPOSE: In multiple sclerosis (MS), autologous hematopoietic stem cell transplantation (AHSCT) induces a profound suppression of clinical activity and MR imaging-detectable inflammation, but it may be associated with a rapid brain volume loss in the months subsequent to treatment. The aim of this study was to assess how AHSCT affects medium-term evolution of brain atrophy in MS.

MATERIALS AND METHODS: MR imaging scans of the brain from 14 patients with rapidly evolving secondary-progressive MS obtained 3 months before and every year after AHSCT for 3 years were analyzed. Baseline normalized brain volumes and longitudinal percentage of brain volume changes (PBVCs) were assessed using the Structural Image Evaluation of Normalized Atrophy software.

RESULTS: The median decrease of brain volume was 1.92% over the first year after AHSCT and then declined to 1.35% at the second year and to 0.69% at the third year. The number of enhancing lesions seen on the pretreatment scans was significantly correlated with the PBVCs between baseline and month 12 ($r = -0.62$; $P = .02$); no correlation was found with the PBVCs measured over the second and third years.

CONCLUSIONS: After AHSCT, the rate of brain tissue loss in patients with MS declines dramatically after the first 2 years. The initial rapid development of brain atrophy may be a late consequence of the pretransplant disease activity and/or a transient result of the intense immunoablative conditioning procedure.

In the last few years, autologous hematopoietic stem cell transplantation (AHSCT) has been used to treat severe and progressive multiple sclerosis (MS), which was refractory to conventional treatment.[1] The rationale for AHSCT treatment in MS, which is basically an autoimmune disease, is to eradicate the abnormal immune system and establish a new and more tolerant one.[1] Four studies have shown that AHSCT has a dramatic effect in reducing the number of "active" lesions as seen on T2-weighted and T1-weighted enhanced MR images in patients with secondary-progressive (SP) MS.[1-4] This latter effect seems to persist more than 3 years after the transplantation.[3,5] In contrast, brain tissue loss was shown to occur at a very rapid pace, soon after[4] and during the first 2 years after AHCST.[5] Clearly such a rapid brain tissue loss is troublesome, because it may precede the accrual of disability in MS,[6] and AHCST is a potentially lethal treatment associated with severe adverse effects.[1,7]

The mechanisms that have been proposed to contribute to brain tissue loss after AHSCT (ie, "pseudoatrophy" because of resolution of inflammatory edema, axonal injury secondary to the florid pretreatment disease activity, and the chemotoxicity of the drugs used for immunoablation)[1-4] are all likely to be transient. Therefore, such a detrimental effect of AHSCT on the integrity of the brain tissue may be limited in time. However, the temporal profile of brain tissue loss in patients with MS after the first 2 years following AHSCT remains unknown.

In this 3-year follow-up MR imaging study of 14 patients treated with a maximum immunoablative conditioning scheme for rapidly evolving SPMS,[3] we investigated the medium-term dynamics of brain tissue loss and obtained additional information on the temporal relationship between suppression of MR imaging-visible inflammation and the rate of atrophy after this treatment.

Methods

Fourteen patients with rapidly evolving SPMS were enrolled in a study of stem cell transplantation using a protocol that aimed at maximum T-cell suppression.[3] Patients were considered as having a rapidly evolving disease if they had an Expanded Disability Status Scale (EDSS) score between 5.0 and 7.0 (7.0 excluded), an EDSS score of at least 3.0 at 2 years after diagnosis, an EDSS increase of at least 1.0 in the previous 2 years if the EDSS score was at least 5.5 or at least 1.5 if the EDSS score was 5.0.[3] Bone marrow was aspirated from the posterior iliac crest and $CD34^+$ stem cells were selected using affinity columns. The conditioning regimen consisted of anti-thymocyte globulin (lymphoglobulin or horse serum) at a dose of 15 mg/kg intravenously from days −7 to −3. On days −4 and −3, cyclophosphamide was added at a dose of 60 mg/kg intravenously, combined with mesnum (15 mg/kg; 4 times each day). Total body irradiation was given in 2 fractions of 5 Gy, at days −2 and −1. On day 0, the autologous graft was thawed and reinfused using a central venous catheter. Prednisolone (1 mg/kg twice daily) was given concurrently

Received December 4, 2006; accepted after revision March 2, 2007.

From the Neuroimaging Research Unit (M.A.R., P.V., M.P.S., M.F.), Department of Neurology (G.C., M.F.), Scientific Institute and University San Raffaele, Milan, Italy; Departments of Neurology (T.M., R.Q.H.), Radiology (Z.H.F.), and Haematology (P.A.T.B.), Erasmus Medical Center, Rotterdam, the Netherlands; Biostatistics Unit (M.P.S.), Department of Health Sciences, University of Genoa, Genoa, Italy.

R.Q.H. and M.F. contributed equally to this study.

Please address correspondence to Massimo Filippi, Neuroimaging Research Unit, Department of Neurology, San Raffaele Scientific Institute, via Olgettina 60, 20132 Milan, Italy; e-mail: filippi.massimo@hsr.it

DOI 10.3174/ajnr.A0644

and tapered after stem cell reinfusion. Further details about the study population, inclusion/exclusion criteria, study design, and treatment regimen are reported elsewhere.[3]

Using a 1.5T scanner, brain dual-echo, precontrast and postcontrast T1-weighted scans were obtained 3 months before stem cell transplantation and then at every scheduled visit. For all of the scans, sections were axial with a thickness of 5 mm and an in-plane resolution of approximately 0.9×0.9 mm. The detailed MR imaging acquisition protocol was presented in the original publication.[3]

Percentage of brain volume changes (PBVCs) and cross-sectional normalized brain volumes (NBVs) were measured on precontrast T1-weighted images, using Structural Image Evaluation of Normalized Atrophy (SIENA) and SIENAX.[8] These are highly automated segmentation techniques, which require only minimal manual input from human observers and, as a consequence, provide highly reproducible results. In both methods, the first stage is the extraction of the brain from each input MR image(s) using the Brain Extraction Tool. The original images are then registered to a canonical image in a standardized space. In the longitudinal method, to estimate changes between the images, SIENA finds all of the brain surface edge points by using tissue-type segmentation and then correlates differentiated 1D perpendicular profiles taken around the position of these points in both images. Taking the mean perpendicular edge motion over all of the edge points and converting this into PBVCs quantifies brain atrophy. The normalizing factor in the conversion is found by a self-calibration step, which involves finding estimated atrophy on an artificially scaled version of one image with respect to itself. In SIENAX, a similar registration process is applied, but instead of a second time point image, standard space average brain and skull images are used. The estimate of brain tissue volume for a subject is then multiplied by the normalization factor to yield the NBV. The accuracy of SIENA has been shown to be independent of section thickness and to range from 1 to 6 mm.[8] The following metrics were obtained from each patient: the NBV at month 1 (as suggested previously,[4] this was considered the "baseline" scan, because at this time point enhancement was already profoundly suppressed,[3] and we tried to minimize the risk of measuring "pseudoatrophy" because of resolution of edema on follow-up scans) and the PBVCs between baseline and month 12, months 12 and 24, and month 24 and the last follow-up scan (month 36 or, for one patient, month 48). The correlations between the number of enhancing lesions in the pretreatment period and the PBVCs during treatment were assessed using Spearman rank correlation coefficient.

Results

Eight women and 6 men with a mean age of 38 years (range, 23–50 years), a median baseline EDSS score of 6.0 (range, 5.0–6.5), and a median disease duration of 5 years (range, 2–12 years) underwent AHCST. The demographic and baseline characteristics of the patients studied and the effect of AHCST on disability progression have been reported previously.[3] A complete suppression of Gd enhancement during the follow-up was observed.[3] All of the 14 enrolled patients had an MR imaging scan usable for volumetry assessment at baseline and at month 12, 11 at month 24, and 9 at month 36 or 48 (1 patient). The mean NBV at baseline was 1490 mL (SD, 21 mL).

The median PBVCs between baseline and month 12 were −2.33% (range, −3.66% to 1.54%) in the whole cohort of patients and −1.35% (range, −4.27% to 0.21%) between months 12 and 24 in the 11 patients with MR imaging at this time point. When the 9 patients with a follow-up of at least 36 months were considered, the median PBVCs were −1.92% (range, −3.66% to 1.54%) at month 12, −1.35% (range, −4.27% to 0.41%) at month 24, and −0.69% (range, −0.99% to 0.33%) at final follow-up.

The number of enhancing lesions seen on the pretreatment scans was significantly correlated with PBVCs between baseline and month 12 ($r = -0.62$; $P = .02$), whereas no correlations were found with PBVCs measured over the second and third years after AHSCT. No correlation was found between NBV at baseline and subsequent PBVCs. At baseline, NBV was significantly correlated with EDSS score ($r = -0.67$; $P = .01$), whereas there was no correlation between PBVCs and EDSS change over time.

Discussion

We explored the dynamics of brain volume changes over 3 years in a group of patients with rapidly evolving SPMS treated with an AHSCT protocol that was aimed at maximum T-cell suppression and included total body irradiation (5 Gy) and high-dose cyclophosphamide.[3] This treatment protocol resulted in a complete suppression of enhancements, which lasted for the entire trial duration.[3] This fits with previous AHSCT trials of MS based on less aggressive immunoablative treatments,[1,2,4,5] which, however, showed that the effect of AHSCT, despite being marked on formation of enhancing and new T2 lesions, was negligible, if not detrimental, on the progression of brain atrophy over the first 2 years after treatment.[4,5] In this study, we assessed how aggressive AHSCT affected the temporal evolution of brain atrophy over a 3-year follow-up period in patients with SPMS. The duration of the trial is an important aspect of this study, because the observed mismatch between the effect of a given treatment on MR-detectable inflammation versus neurodegeneration in MS is not a unique finding of AHSCT,[9] and the relatively short durations (typically 1–2 years) of these studies have been suggested as one of the possible explanations for such a mismatch,[9,10] that is, the time needed to detect the complete effect of inflammatory activity on irreversible brain damage might be longer than that of available studies.

In the present cohort, the median brain volume decreases at months 12 and 24 agree with data from another cohort of patients with SPMS who were treated with AHSCT,[5] thus confirming that brain volume loss proceeds at a rather sustained pace in the first 2 years after such a treatment, a pace that is faster than that expected from natural history studies of patients with SPMS.[10] This may be secondary to the fact that patients of both studies had a severe and rapidly progressive form of MS, which was in a very active phase at the time of enrollment. The most intriguing finding of this study, however, stems from the analysis of the third-year scans, which shows that the rate of brain volume loss is reduced by approximately 50% in comparison with that observed during the first 2 years, thus becoming similar to the one commonly observed in patients with less aggressive forms of the disease.[10] This fits with the observation of another AHCST trial where corpus callosum area was measured over 3 years.[11] Clearly, because we do not know the pretreatment rate of atrophy of these patients, we cannot comment on whether AHSCT has any effect on brain atrophy progression. However, the third-year finding of a significant reduction of the rate of brain atrophy

development in these patients is extremely reassuring in terms of treatment safety.

With this in mind, it is important to understand which factors are most likely responsible for a rate of brain atrophy after AHSCT that is not constant over time but rather declines dramatically after the first 2 years following treatment in patients with MS. One plausible explanation for our observations is to interpret the rapid brain volume reduction seen in the first 2 years after AHSCT as a sort of "carry-over" effect conditioned by the high inflammatory load, which characterized the disease before treatment institution. This notion is supported by the correlation found in these patients between the rate of brain atrophy during the first year of follow-up and the number of enhancing lesions seen on the pre-AHSCT scans. Histopathologic analysis has indeed shown a large number of transected axons in MS lesions with inflammatory infiltrates,[12] as well as marked axonal injury and cortical demyelination in the presence of diffuse brain inflammation,[13] which was found to be typical of patients with a progressive form of the disease, like those enrolled in the present trial. Because brain white matter bulk consists predominantly of axons (46%) and myelin (24%),[10] the florid pretreatment inflammatory activity might have caused a persistent tissue loss during the first 2 years of the study, despite the absence of a concomitant MR imaging-detectable inflammation. Although part of the atrophy observed during the first year may be related to a loss of water without a loss of tissue (ie, pseudoatrophy), secondary to the resolution of focal and diffuse inflammatory edema, this probably played only a marginal role here, because, as suggested,[4] we used as the reference scans for PBVC measurements those that were obtained at month 1, when enhancement was already profoundly suppressed.[3] The subsequent decline of the brain atrophy rate during the third year of the study might then be the reflection that the impact of previous inflammatory demyelination on axon survival was over, at least at a magnitude that is measurable using MR imaging. Admittedly, we cannot exclude that an increased glial activity during the third year might have partially masked the effect of tissue loss, thus suggesting that reparative mechanisms are still operative 3 years after AHSCT. Future studies using proton MR spectroscopy might improve our insights into this issue, thanks to the ability of this technique to quantify markers of neuroaxonal injury (such as *N*-acetylaspartate) and glial activity (such as myo-inositol).[14]

Another factor that can plausibly explain (at least part of) the initial rapid decline of brain volume may be the neurotoxicity of the aggressive conditioning regimen used here.[15,16] Brain tissue, already injured by repeated and persistent inflammatory insults, may have indeed been further damaged by the neurotoxicity of both chemotherapy and irradiation. This hypothesis is strengthened by the results of another recent AHSCT study of patients with MS, where a short-term dramatic increase in brain atrophy was suggested to be secondary to the neurotoxic effects of the agents used for immunoablation.[4] Concerns have also been raised on the use of total body irradiation in the treatment of MS.[7] That the conditioning scheme used in the present trial might have a significant neurotoxic component is also suggested by the fact that most of our patients deteriorated immediately after conditioning in a gradual fashion (ie, without superimposed relapses).[3] In this context, the analysis of the third-year scans is again of importance, because it shows that the neurotoxic effects of our AHSCT scheme have a limited time window and fade off with time. Albeit still to be proved, it is conceivable that the time window of neurotoxicity associated with immunoablation and conditioning might be even shorter and milder with less aggressive AHSCT treatments.

Although these observations need to be replicated by assessing MR imaging scans from other AHSCT trials of MS and need to be confirmed by longer periods of observation, it is plausible to suggest that the suppression of disease activity immediately after AHSCT might also beneficially affect the medium-term evolution of brain atrophy in aggressive phenotypes of MS. On the other hand, this study also sheds light into the complex temporal relationship between inflammation and neurodegeneration in MS by suggesting that the time needed to see the irreversible consequences of inflammatory demyelination on axon survival might take longer than 2 years.

References

1. Blanco Y, Saiz A, Carreras E, et al. **Autologous haematopoietic stem-cell transplantation for multiple sclerosis.** *Lancet Neurol* 2005;4:54–63
2. Mancardi GL, Saccardi R, Filippi M, et al. **Autologous hematopoietic stem cell transplantation suppresses gadolinium-enhanced MRI activity in multiple sclerosis.** *Neurology* 2001;57:62–68
3. Samijn JPA, Te Boekhorst PAW, Mondria T, et al. **Intense T cell depletion followed by autologous bone marrow transplantation for severe multiple sclerosis.** *J Neurol Neurosurg Psychiatry* 2006;77:46–50
4. Chen JT, Collins DL, Atkins HL, et al. **Brain atrophy after immunoablation and stem cell transplantation in multiple sclerosis.** *Neurology* 2006;66:1935–37
5. Inglese M, Mancardi GL, Pagani E, et al. **Brain tissue loss occurs after suppression of transplantation treated with autologous haematopoietic stem cell enhancement in patients with multiple sclerosis.** *J Neurol Neurosurg Psychiatry* 2004;75:643–44
6. Fisher E, Rudick RA, Simon JH, et al. **Eight-year follow-up study of brain atrophy in patients with MS.** *Neurology* 2002;59:1412–20
7. Burt RK, Cohen B, Rose J, et al. **Hematopoietic stem cell transplantation for multiple sclerosis.** *Arch Neurol* 2005;62:860–64
8. Smith SM, Zhang Y, Jenkinson M, et al. **Accurate, robust and automated longitudinal and cross-sectional brain change analysis.** *NeuroImage* 2002;17:479–89
9. Inglese M, Benedetti B, Filippi M. **The relation between MRI measures of inflammation and neurodegeneration in multiple sclerosis.** *J Neurol Sci* 2005;233:15–19
10. Miller DH, Barkhof F, Frank JA, et al. **Measurement of atrophy in multiple sclerosis: pathological basis, methodological aspects and clinical relevance.** *Brain* 2002;125:1676–95
11. Saiz A, Blanco Y, Carreras E, et al. **Clinical and MRI outcome after autologous hematopoietic stem cell transplantation in MS.** *Neurology* 2004;62:282–84
12. Trapp BD, Peterson J, Ransohoff RM, et al. **Axonal transection in the lesions of multiple sclerosis.** *N Engl J Med* 1998;338:278–85
13. Kutzelnigg A, Lucchinetti CF, Stadelman C, et al. **Cortical demyelination and diffuse white matter injury in multiple sclerosis.** *Brain* 2005;128:2705–12
14. Narayana PA. **Magnetic resonance spectroscopy in the monitoring of multiple sclerosis [review].** *J Neuroimaging* 2005;15:46S–57S
15. Jager HR, Williams EJ, Savage DG, et al. **Assessment of brain changes with registered MR before and after bone marrow transplantation for chronic myeloid leukemia.** *AJNR Am J Neuroradiol* 1996;17:1275–82
16. Peper M, Steinvorth S, Schraube P, et al. **Neurobehavioral toxicity of total body irradiation: a follow-up in long-term survivors.** *Int J Radiat Oncol Biol Phys* 2000;46:303–11