Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study

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Summary

Background **Autologous non-myeloablative haemopoietic stem cell transplantation is a method to deliver intense immune suppression. We evaluated the safety and clinical outcome of autologous non-myeloablative haemopoietic stem cell transplantation in patients with relapsing-remitting multiple sclerosis (MS) who had not responded to treatment with interferon beta.**

Methods **Eligible patients had relapsing-remitting MS, attended Northwestern Memorial Hospital, and despite treatment with interferon beta had had two corticosteroid-treated relapses within the previous 12 months, or one relapse and gadolinium-enhancing lesions seen on MRI and separate from the relapse. Peripheral blood haemopoietic stem cells were mobilised with 2 g per m² cyclophosphamide and 10 μg per kg per day filgrastim. The conditioning regimen for the haemopoietic stem cells was 200 mg per kg cyclophosphamide and either 20 mg alemtuzumab or 6 mg per kg rabbit antithymocyte globulin. Primary outcomes were progression-free survival and reversal of neurological disability at 3 years post-transplantation. We also sought to investigate the safety and tolerability of autologous non-myeloablative haemopoietic stem cell transplantation.**

Findings **Between January, 2003, and February, 2005, 21 patients were treated. Engraftment of white blood cells and platelets was on median day 9 (range day 8–11) and patients were discharged from hospital on mean day 11 (range day 8–13). One patient had diarrhoea due to *Clostridium difficile* and two patients had dermatomal zoster. Two of the 17 patients receiving alemtuzumab developed late immune thrombocytopenic purpura that remitted with standard therapy. 17 of 21 patients (81%) improved by at least 1 point on the Kurtzke expanded disability status scale (EDSS), and five patients (24%) relapsed but achieved remission after further immunosuppression. After a mean of 37 months (range 24–48 months), all patients were free from progression (no deterioration in EDSS score), and 16 were free of relapses. Significant improvements were noted in neurological disability, as determined by EDSS score ($p<0·0001$), neurological rating scale score ($p=0·0001$), paced auditory serial addition test ($p=0·014$), 25-foot walk ($p<0·0001$), and quality of life, as measured with the short form-36 (SF-36) questionnaire ($p<0·0001$).**

Interpretation **Non-myeloablative autologous haemopoietic stem cell transplantation in patients with relapsing-remitting MS reverses neurological deficits, but these results need to be confirmed in a randomised trial.**

Funding **Division of Immunotherapy, Northwestern University.**

Introduction

Most patients with multiple sclerosis (MS) present with intermittent symptoms that are commonly, at least partially, reversible; this form of the disease is termed relapsing-remitting MS. Over time, most patients eventually develop secondary-progressive MS, which manifests as irreversible and gradual neurological impairments that often progress without acute relapses.[1] Autologous haemopoietic stem cell transplantation for MS was first done by Fassas and co-workers[2] and has subsequently been repeated in many countries. These studies were done mostly in patients with late secondary-progressive MS, although some have included patients with primary-progressive, relapsing-remitting, or relapsing-progressive MS.

In most patients, haemopoietic stem cell transplantation for secondary progressive MS did not improve the neurological disability;[3–14] consequently, outcome was generally reported as duration of stabilisation of neurological disability (ie, results were reported as progression-free survival, defined as the probability of no sustained increase in Kurtzke expanded disability status scale [EDSS] score by at least 1 point after transplantation compared with pretransplantation score) rather than improvement. Post-transplantation progression-free survival varies substantially between studies and has been reported as either 61% or 73% at 2 years,[3,4] between 36% and 77% at 3 years,[5–11] and between 58% and 75% at 3 to 6 years.[12–14]

However, the EDSS, which is a validated and widely used scale to measure outcomes in MS, is not a linear scale. Without a transplant, the time for a patient to progress by 1 point on the EDSS varies in accordance with the baseline score and the duration of MS. For example, the mean time

to progression by 1 point on the EDSS is longer for patients with scores at the lower and higher ends of the scale and shorter for patients with scores of between 3 and 5 points.[15] An analysis of the natural history of a group of patients with MS who did not have haemopoietic stem cell transplantation suggested that the risk of sustained progression (a sustained increase in EDSS score of at least 1 point) was 50% by 7 years and 70% by 15 years.[16] The likelihood of progression is also affected by baseline EDSS score: 34% of patients with an EDSS score of 3·0 to 3·5 points at entry progressed compared with 62% of patients with EDSS scores of 4·0–5·5 points.[16]

The investigators in many transplantation studies for progressive MS selected patients whose EDSS score had increased by 1 point in the year before treatment. Whether this criterion selects for patients with more rapidly progressive disability or selects for patients whose new EDSS score is less likely to change over the next few years because they have just reached a new plateau EDSS score is unclear. Therefore, without a randomised trial, we cannot ascertain whether haemopoietic stem cell transplantation alters the progression-free survival of patients with secondary-progressive MS. Furthermore, haemopoietic stem cell transplantation does not reverse neurological disability in the late progressive phase of MS.[3–14]

The rationale behind autologous haemopoietic stem cell transplantation for MS is to reset the immune system; that is, to produce new and self-tolerant lymphocytes from the haemopoietic stem cell (immune stem cell) transplant after chemotherapy-induced elimination of self-reactive or autoreactive lymphocytes.[17]

Neurological deficits during the late secondary-progressive phase of MS are mostly caused by neurodegeneration from axonal atrophy,[18–20] for which no immune-based therapy, including haemopoietic stem cell therapy, has been effective for reversing the deficits. By contrast, during the relapsing-remitting phase of MS, demyelination is mediated by immune cells.[21] First-line therapy for relapsing-remitting MS is immune modulation, which ameliorates the inflammatory processes that mediate the damage to the CNS. Current therapies to treat the relapsing-remitting or progressive phases of MS are designed to delay progression of the disease, rather than reverse neurological disability. In this study of autologous haemopoietic stem cell transplantation we treated patients with relapsing-remitting MS who had failed to respond to immune modulation but were still at an inflammatory stage, rather than wait until their MS becomes predominately neurodegenerative. This will enable us to ascertain

	Sex	Age at transplantation (years)	Duration of MS (years)*	Number of steroid-treated relapses in preceding 12 months	Baseline EDSS score (points)	Previous therapy
Patient 1	Male	45	8	3	2·0	Interferon beta 1a, steroids, intravenous immunoglobulin
Patient 2	Male	20	3	2	3·0	Interferon beta 1a, steroids
Patient 3	Female	29	5	2	3·5	Interferon beta 1a, glatiramer acetate, steroids
Patient 4	Male	34	8	3	3·5	Interferon beta 1a, steroids
Patient 5	Male	41	2	3	2·0	Interferon beta 1a, steroids
Patient 6	Male	22	3·5	2	5·5	Interferon beta 1a, steroids, plasmapheresis, cyclophosphamide
Patient 7	Male	36	4	2	2·5	Glatiramer acetate, interferon beta 1a, steroids, daclizumab
Patient 8	Female	33	6	2	4·0	Interferon beta 1a, glatiramer acetate, steroids
Patient 9	Female	40	4	2	4·0	Interferon beta 1a, steroids
Patient 10	Female	38	6	1	3·5	Interferon beta 1a, glatiramer acetate, steroids, intravenous immunoglobulin
Patient 11	Female	27	4	2	4·0	Interferon beta 1a, steroids
Patient 12	Male	38	5	2	2·5	Interferon beta 1a, steroids
Patient 13	Male	25	4	2	3·0	Interferon beta 1a, azathioprine, steroids
Patient 14	Male	29	1·5	2	3·5	Interferon beta 1a, steroids
Patient 15	Female	25	6	2	2·0	Interferon beta 1a, steroids
Patient 16	Female	37	10	4	3·5	Interferon beta 1a, steroids, cyclophosphamide
Patient 17	Female	53	2	2	3·5	Interferon beta 1a, steroids
Patient 18	Male	22	3	3	2·5	Interferon beta 1a, glatiramer acetate, steroids, intravenous immunoglobulin
Patient 19	Female	26	4	2	5·5	Interferon beta 1a, cladribine, steroids
Patient 20	Female	30	9	2	2·0	Interferon beta 1a, glatiramer acetate, steroids
Patient 21	Female	34	6	5	2·0	Interferon beta 1a, glatiramer acetate, steroids, cyclophosphamide

*Duration of MS from time of diagnosis. MS=multiple sclerosis.

Table 1: **Baseline characteristics**

whether haemopoietic stem cell transplantation during the relapsing-remitting phase of MS can reverse neurological disability.

Methods

Patients

Patients were eligible if they were aged between 18 and 55 years, had MS according to the revised McDonald criteria, met the Poser criteria for clinically definite MS,[22] and had failed to respond to at least 6 months' therapy with interferon beta. Failure was defined as two or more clinical relapses with documented neurological changes that were treated with intravenous corticosteroids during the year before study entry; or at least one relapse treated with methylprednisolone and, on a separate occasion within the previous 12 months, evidence of active inflammation (ie, gadolinium enhancement on MRI of the CNS). Sensory-only relapses were excluded. The EDSS was used to assess neurological function; eligible patients had EDSS scores of 2·0 to 5·5 points assessed at least 3 months after the last acute attack of MS. Patients had normal baseline renal, cardiac, pulmonary, and hepatic function, and no history of previous or active malignancy, except for localised cutaneous basal or squamous cell carcinoma or carcinoma in situ of the cervix. The protocol was approved by the Institutional Review Board of Northwestern University. All patients gave written informed consent.

***Panel:* Toxicity and events after transplantation, ranged by patient number**

1 None
2 Dermatomal zoster at 20 months; perforated peptic ulcer at 28 months
3 Grade I rash, which resolved without treatment
4 None
5 None
6 None
7 None
8 Dermatomal zoster at 22 months
9 Neutropenic fever for 2 days; grade I haemorrhagic cystitis; deep vein thrombosis at 6 months; immune thrombocytopenia at 7 months, which was treated and resolved
10 None
11 None
12 Grade II elevation of transaminase concentration, which resolved with discontinuation of fluconazole
13 None
14 Neutropenic fever for 1 day; filgrastim-related transient MS flare with left-sided paraesthesia, which resolved without treatment
15 Neutropenic fever at day 0; coagulase-negative staphylococci in blood culture, which was isolated from one specimen and was deemed a contaminant
16 None
17 Immune thrombocytopenia at 14 months, which resolved with treatment
18 Neutropenic fever at day 3 with negative culture; diarrhoea due to *Clostridium difficile* at 1 month after discharge
19 None
20 None
21 Neutropenic fever for 4 days

Procedures

All patients had a detailed history, physical examination, and baseline assessment of haematological, cardiac, renal, and pulmonary function. The neurological assessment consisted of the MS functional composite assessment,[23] which comprises the EDSS,[24] Scripps neurological rating scale (NRS),[25] paced auditory serial addition test (PASAT), nine-hole peg test, and a timed 25-foot walk. The short-form 36 (SF-36) quality of life questionnaire and MRI scans of the brain and cervical spinal cord with gadolinium enhancement were also done. Assessments were scheduled at baseline (before mobilisation of the haemopoietic stem cells), at 6 and 12 months, and annually thereafter.

Peripheral blood stem cells were mobilised with 2 g per m² intravenous cyclophosphamide followed by 10 µg per kg subcutaneous filgrastim daily from day 5. After neutrophil recovery, the mobilised cells were collected by apheresis, until a yield of at least 2×10^6 per kg $CD34^+$ cells was obtained. The recovered cells were unselected and cryopreserved. There was an interval of at least 3 weeks between the administration of cyclophosphamide to mobilise the cells and the conditioning regimen.

The conditioning regimen used was 200 mg per kg intravenous cyclophosphamide, given in four equal fractions between day –5 and day –2 with intravenous mesna, and one 20 mg dose of intravenous alemtuzumab given on day –2 with 250 mg intravenous methylprednisolone as premedication. In light of the US Food and Drug Administration report that alemtuzumab might cause immune thrombocytopenic purpura in patients with MS, this treatment was changed to rabbit antithymocyte globulin, and the last four patients received 6 mg per kg rabbit antithymocyte globulin over 5 days instead of alemtuzumab. The haemopoietic stem cells were reinfused 36 h after completion of cyclophosphamide (day 0). 5 µg per kg per day subcutaneous filgrastim was given from day 5, and was continued until neutrophil recovery.

All blood products were irradiated, filtered, and free from cytomegalovirus. Packed red blood cells were given when haemoglobin concentrations fell below 8 g per dL or when clinically indicated. Patients were transfused when their platelet count declined below 20×10^9 platelets per L or when clinically indicated.

Antibacterial prophylaxis with intravenous cefepime or piperacillin–tazobactam was given during neutro-

penia. Antifungal prophylaxis with oral fluconazole was continued for 6 months after transplantation. Antiviral prophylaxis with valaciclovir or aciclovir was continued for 12 months post-transplantation. Oral trimethoprim-sulfamethoxazole three times a week or nebulised pentamidine inhaled once a month were used for *Pneumocystis jirovecii* prophylaxis and treatment was continued for 6 months after transplantation.

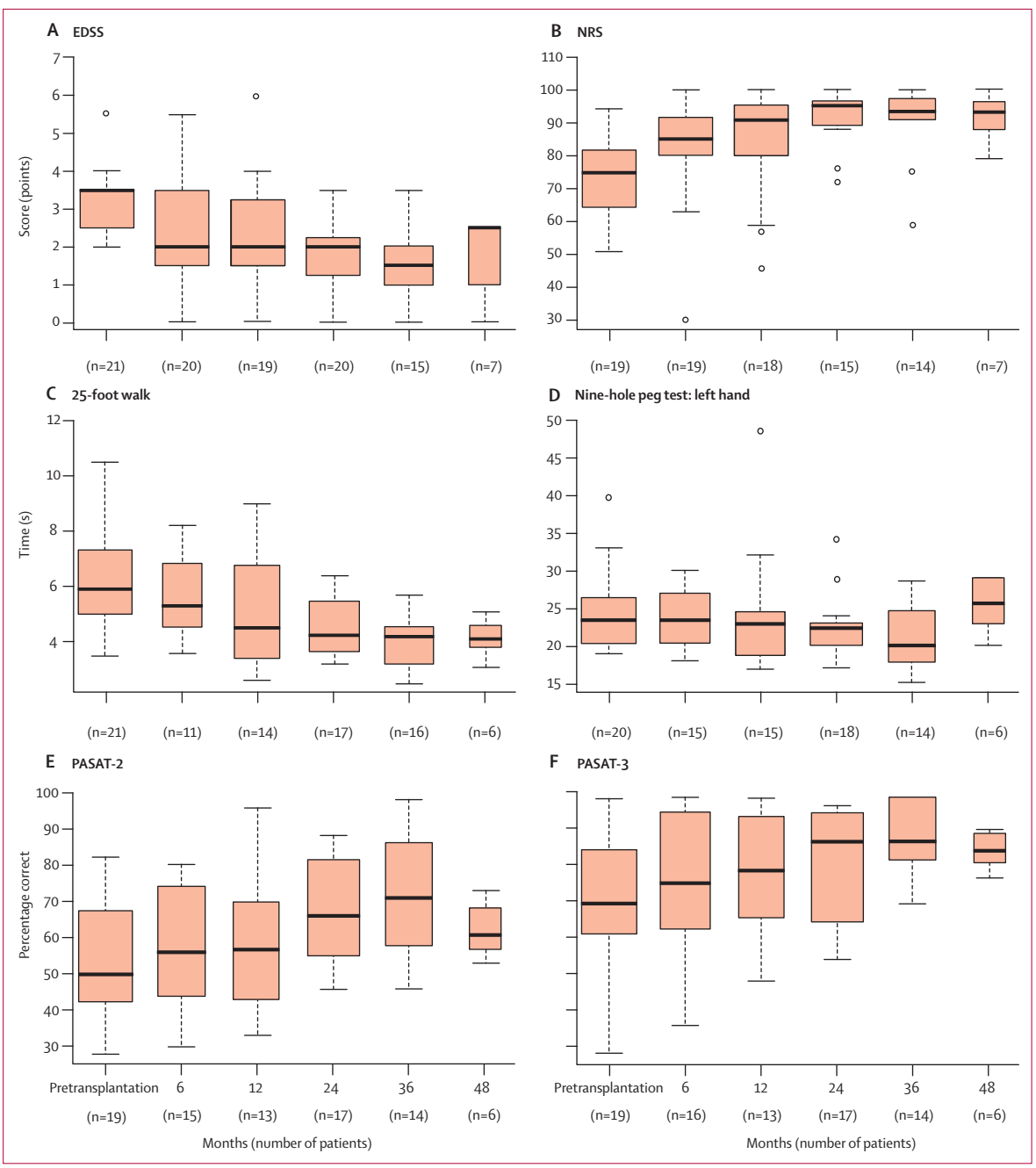

***Figure*: Neurological disability before and at 6, 12, 24, 36, and 48 months after haemopoietic stem cell transplantation**
(A) The Kurtzke extended disability status scale (EDSS) is a neurological performance scale that increases with increasing disability at intervals of 0·5 points, from 0 (healthy) to 10 (death from neurological disability). (B) The Scripps neurological rating scale (NRS) is a neurological performance scale that increases at intervals of 1 point, with decreasing disability going from 0 points (dead) to 100 points (healthy). (C) The 25-foot walk is the time (mean of three attempts) taken to walk 25 feet. (D) The nine-hole peg test is the time in seconds needed to place and remove nine pegs in a hole. Similar results were obtained for the right hand. (E, F) The 2-second (E) and 3-second (F) paced auditory serial addition test (PASAT) are cognitive tests that give the patient 2 or 3 s, respectively, to write down the sums of recorded numbers. Although all patients had EDSS score recorded before transplantation, a few missed one or more of the pretransplantation tests and a few patients declined a post-transplantation interval follow-up, but did return for the next follow-up visit. Thick lines are median; boxes are values for upper and lower quartiles. Open circles are outliers, defined as values that are more than 1·5 times the 25th or 75th percentiles. Note not all axis start at zero.

	Scale								Dimension		Total
	Physical function	Physical role limitation	Body pain	General health perception	Vitality energy fatigue	Social function	Emotional role limitation	Mental health	Physical health	Mental health	Total SF-36 score
Pretransplantation	62·0	27·5	57·0	43·1	41·5	58·8	61·6	65·8	46·2	54·1	52·2
Post-transplantation	87·2	80·0	80·1	74·3	68·3	80·6	83·3	77·8	77·9	76·8	78·9
Mean difference	27·5	52·5	25·3	29·8	27·7	23·1	21·6	12·6	32·5	22·9	26·7
SD	23·0	39·6	22·0	26·7	21·7	23·7	64·2	22·4	19·3	22·5	18·6
p value	<0·0001	<0·0001	<0·0001	0·0001	<0·0001	0·0004	0·15	0·024	<0·0001	0·0003	<0·0001

Table 2: **Mean differences between pretransplantation (baseline) and last post-transplantation score for the short-form 36 (SF-36) quality of life questionnaire**

Toxicity was evaluated in accordance with the National Cancer Institute common toxicity criteria scale, version 2. Neutrophil engraftment was defined as the first day after transplantation when the absolute neutrophil count was greater than 500 cells per μL. Platelet engraftment was defined as the first day after transplantation when the platelet count was greater than 20000 platelets per μL without platelet transfusion.

At each follow-up visit, the patients had a neurological examination done by a neurologist (BC, DS, RB, or GK).

	T2 lesions	T1 lesions	Width of third ventricle (mm)	Gd-enhancing lesions	EDSS score
Patient 1					
Pretransplantation	50	27	8·0	0	2·0
6 months	50	27	8·0	0	1·0
1 year	50	27	8·0	0	0·0
2 years	50	27	8·0	0	0·0
3 years	50	27	8·0	0	0·0
4 years	50	27	8·0	0	0·0
Patient 2					
Pretransplantation	55	28	5·0	2	3·0
6 months	55	28	5·0	0	1·5
1 year	55	28	5·0	0	1·5
2 years	55	28	5·6	0	0·5
3 years	55	28	5·6	0	..
Patient 4					
Pretransplantation (6 months)	59	55	8·0	4	3·5
Pretransplantation (1 month)	60	55	8·0	12	3·5
6 months	60	55	8·0	0	3·5
1 year	60	55	8·0	0	3·5
2 years	60	55	8·0	0	2·5
3 years	..	..	..	..	3·0
4 years	..	..	..	..	2·5
Patient 5					
Pretransplantation (10 months)	45	7	4·0	10	..
Pretransplantation (3 months)	50	10	4·0	1	2·0
1 year	50	7	4·0	0	2·0
2 years	..	..	..	..	2·0
3 years	..	..	..	..	2·0
4 years	..	..	..	..	2·0

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Worsening or improvement in neurological function was defined as a change in EDSS of 1 point or more on a minimum of two occasions that were at least 3 months apart. Although routine neurological examinations were done every 6 months, patients were advised to report new symptoms immediately. If the condition of a patient had declined on routine examination, or they reported new symptoms or worsening between scheduled visits, they were assessed immediately and again at 3 months. A relapse of MS was defined as an acute deterioration in neurological function that lasted for more than 24 h without intercurrent illness or another cause for neurological impairment and with objective changes on neurological examination.

The study was designed to assess safety and clinical outcomes. MRI analysis was a tertiary outcome and depended on insurance approval. MRI scans were done on Siemens scanners with a field strength of 1·5 Tesla (Siemens Avanto and Symphony) and 3·0 Tesla (Siemens Trio). Transverse axial T2-weighted turbo spin echo, transverse axial and sagittal fluid-attenuated inversion recovery (FLAIR), and pregadolinium-enhanced and post-gadolinium-enhanced T1 spin echo pulse sequences were obtained for the brain and cervical spine at baseline and for the brain at follow-up visits. Pregadolinium and post-gadolinium transverse axial fat-suppressed T1-weighted turbo spin-echo and 3D magnetisation-prepared rapid gradient echo (MPRAGE) images were also acquired. Post-contrast imaging was done with triple dose (0·3 mmol per kg) gadolinium diethylene triamine penta-acetic acid (DTPA).

All scans were reviewed by the same neurologist (JS), and comparisons were made between baseline and subsequent scans in an unblinded manner. At each examination, supratentorial hyperintense lesions were evaluated on the basis of a review of all FLAIR and T2-weighted scans. Owing to the limitations of FLAIR imaging in the posterior fossa, infratentorial region, and brainstem, lesions were better detected and characterised in some cases with T2-weighted turbo spin-echo images. The number of T1 hypointense lesions was evaluated on the precontrast 3D-MPRAGE sequence. Enhancing lesions were evaluated on contrast-enhanced T1-weighted and 3D-MPRAGE images. The diameter of the third ventricle was

measured on the axial 3D-MPRAGE sequence, with electronic calipers on the workstation that was used for image review and analysis (GE Centricity PACS), to obtain a linear measurement of the diameter of the largest visualised transverse axial plane.

Statistical analysis

To provide appropriate longitudinal analysis, we applied Proc Mixed in SAS (Version 9.2), with random intercepts and time in months as fixed effects predictors. The test for the slope parameter (with two-sided alternative hypotheses) is the test of (upward or downward) linear trends. Overall significance is shown with p values.

Role of the funding source

No company or outside agency was involved in sponsoring the study, had a role in the design, data collection, data analysis, data interpretation, or writing of the report. The statistician (BJ) and corresponding author (RKB) had access to all the data. The corresponding author is responsible for the decision to submit the manuscript for publication.

Results

Between January, 2003, and February, 2005, 21 patients (11 women and 10 men) with MS had autologous haemopoietic stem cell transplantation. The median age at the time of transplantation was 33 years (range 20 to 53 years) and the median duration of disease before transplantation was 5 years (range 1·5 to 10·0 years). The median baseline EDSS score was 3·1 points (range 2·0 to 5·5 points). Table 1 summarises the pretransplantation characteristics of the patients.

The median infused dose of peripheral blood stem cells was $11{\cdot}40\times10^6$ $CD34^+$ cells per kg (range $2{\cdot}22\times10^6$ to $25{\cdot}91\times10^6$), and the median time to neutrophil engraftment was 9 days (range 8 to 11 days). The platelet counts for three patients did not decline below 20 000 cells per µL; for the other patients, the median time to platelet engraftment was 9 days (range 7 to 11 days). Eight patients did not require a transfusion of packed red blood cells, whereas 13 patients each received between one and four units. Six patients did not receive any platelet transfusions, whereas 15 patients received an average of two units of single-donor apheresed platelets (range 1 to 3 units). The mean discharge day was 11 days after infusion of haemopoietic stem cells (range 8 to 13 days).

A summary of the adverse events during and after transplantation are shown in the panel. The mobilisation and transplantation procedures were well tolerated. There were no non-haematological toxicities of grade III severity or greater during transplantation; however, five patients had neutropenic fever without identification of a pathogen or other clinical signs of infection. One patient had transient neurological symptoms that manifested as left-sided hypoaesthesia, which was attributed to a filgrastim-related flare; the neurological symptoms resolved when the drug was discontinued. All blood cultures and surveillance cultures were negative for bacterial growth, with the exception of one patient, who had a blood culture with coagulase-negative staphylococcus; this was isolated in one specimen of four sent concurrently, which suggests a probable contaminant.

	T2 lesions	T1 lesions	Width of third ventricle (mm)	Gd-enhancing lesions	EDSS score
(Continued from previous page)					
Patient 8					
Pretransplantation (4 months)	125	110	5·0	30	4·0
Pretransplantation (1 month)	130	110	5·0	7	4·0
6 months	120	110	5·0	0	4·0
1 year	120	110	5·0	0	4·0
2 years	120	110	5·0	0	3·5
3 years	120	110	5·0	0	3·5
4 years	..	..	..	..	2·5
Patient 9					
Pretransplantation (6 months)	51	18	8·0	0	..
Pretransplantation (1 month)	51	18	8·0	0	4·0
6 months	51	18	8·0	0	..
1 year	..	..	..	..	..
2 years	51	18	8·0	0	1·5
3 years	..	..	..	..	1·0
Patient 10					
Pretransplantation (6 months)	75	55	4·0	4	..
Pretransplantation (1 month)	75	55	4·0	1	3·5
6 months	75	55	4·0	0	2·0
1 years	75	55	4·0	0	2·0
2 years	75	55	4·0	0	2·0
3 years	75	55	4·0	0	1·0
Patient 11					
Pretransplantation (6 months)	35	28	4·0	0	..
Pretransplantation (1 month)	36	28	4·0	1	4·0
6 months	36	28	4·0	0	4·0
1 year	44	28	4·0	0	3·0
2 years	44	28	4·0	0	3·0
3 years	44	28	4·0	0	2·5
4 years	..	..	..	..	2·5
Patient 12					
Pretransplantation	30	11	2·5	0	2·5
6 months	30	11	2·5	0	2·5
1 year	30	11	2·2	0	1·5
2 years	30	11	2·2	0	1·5
3 years	..	..	..	..	2·0
Patient 14					
Pretransplantation (3 months)	30	12	11·0	4	3·5
Pretransplantation (1 month)	35	12	11·0	9	3·5
6 month	35	10	12·0	0	2·0
1 year	35	10	12·0	0	2·0
2 years	35	19	12·0	0	3·0
3 years	..	..	..	..	1·5

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	T2 lesions	T1 lesions	Width of third ventricle (mm)	Gd-enhancing lesions	EDSS score
(Continued from previous page)					
Patient 15					
Pretransplantation (6 months)	27	18	2·0	0	2·0
Pretransplantation (1 month)	31	20	2·0	3	2·0
6 month	31	20	3·0	0	1·0
1 year	31	20	3·0	0	1·0
2 years	31	20	3·0	0	1·5
3 years	..	..	..	..	1·0
Patient 16					
Pretransplantation (3 months)	55	42	4·0	11	3·5
6 months	55	40	4·0	0	1·5
1 year	55	40	4·0	0	1·5
2 years	55	40	4·0	0	1·5
3 years	55	40	4·0	0	1·5
Patient 17					
Pretransplantation (8 months)	40	18	5·0	2	..
Pretransplantation (7 months)	45	20	5·0	1	..
Pretransplantation (2 months)	50	23	5·0	2	3·5
6 months	50	23	5·0	0	2·0
1 year	50	23	5·0	0	..
2 years	53	23	5·0	0	0·0
3 years	..	..	..	..	0·0
Patient 18					
Pretransplantation (6 months)	60	45	4·0	6	..
Pretransplantation (1 month)	60	45	4·0	8	2·5
6 months	55	45	4·0	0	2·5
1 year	55	45	4·0	0	2·0
2 years	55	45	4·0	0	2·0
Patient 20					
Pretransplantation	45	5	4·0	2	2·0
6 months	45	5	4·0	0	0·0
1 year	45	5	4·0	0	0·0
2 years	..	..	..	..	0·0
Patient 21					
Pretransplantation	70	20	5·0	6	2·0
6 months	70	20	5·0	0	2·0
1 year	70	20	5·0	0	1·5
2 years	..	..	..	..	2·0

Table 3: **Changes in pretransplantation and post-transplantation MRI and EDSS in patients without clinical relapse**

Two patients developed dermatomal zoster at 20 and 22 months post-transplantation, respectively, which resolved with oral antiviral therapy. One patient developed diarrhoea due to *Clostridium difficile* 1 month after hospital discharge that resolved with oral metronidazole. No other early or late opportunistic infections were documented. Two of the patients who were treated with alemtuzumab developed grade IV thrombocytopenia at 7 and 14 months, respectively, after transplantation; the thrombocytopenia presented as easy bruising and petechiae, although haemoglobin concentrations and white blood cell counts were normal, and the clinical impression was of immune thrombocytopenic purpura. These patients were treated with intravenous immunoglobulin, prednisone, and rituximab; one patient also required pulsed cyclophosphamide. Both patients had remission from immune thrombocytopenia and are no longer on immunosuppression.

The patients were followed up for a mean of 37 months (range 24–48 months). At the most recent assessment, 17 patients (81%) had an improvement in EDSS score of 1 point or more compared with baseline (seven patients had improvements of 1·0–1·5 points, six patients had improvements of 2·0–2·5 points, and four patients had improvements of 3 points or more), two patients had an improvement of 0·5 points, and two patients had no change in EDSS score. None of the patients had a final EDSS score that was lower than the score at baseline. EDSS scores improved in all patients after haemopoietic stem cell transplantation compared with baseline scores ($p<0·0001$). Progression-free survival, defined as no increase in EDSS score of at least 1 point between pretransplantation and last post-transplantation assessment, was 100% at a mean of 3 years follow-up.

Mean EDSS scores improved by 0·8 points at 6 months, 0·9 points at 1 year, 1·5 points at 2 years, 1·6 points at 3 years, and 1·7 points at 4 years. Mean EDSS scores in the four patients who were treated with rabbit antithymocyte globulin and cyclophosphamide instead of alemtuzumab and cyclophosphamide improved by 1 point at 6 months, 1·3 points at 1 year, and 1·5 points at 2 years.

The NRS score at baseline was not included for one patient and not evaluated in one patient; for the other 19 patients, the score improved by 10 or more points in 14 patients (10–20 points in seven patients, 20–30 points in four patients, and by more than 30 points in three patients) and by fewer than 10 points in five patients, at the last follow-up compared with baseline. None of the patients had a final NRS score that was worse than their pretransplantation score. The figure shows the change in NRS scores between baseline and final follow-up ($p=0·0001$).

The timed 25-foot walk was assessed in all patients except for one, and was improved after transplantation ($p<0·0001$). Scores on the 2-second and 3-second PASAT improved after transplantation ($p=0·009$ and $p=0·014$, respectively). Scores on the right-hand nine-hole peg test and the left-hand nine-hole peg test improved but did not change significantly ($p=0·10$ and $p=0·12$, respectively).

Patients perceived their general health status to be improved during the study period, and the differences between pretransplantation and post-transplantation scores were statistically significant within all domains except emotional role (table 2). A comparison of mean pretransplantation scores with mean post-transplantation scores taken at the last post-transplantation assessment showed an increase of 26·7 points (52·2 *vs* 78·9; $p<0·0001$) in the overall general health status score (table 2).

Comparison between the number of pretransplantation and post-transplantation T1, T2, and gadolinium-enhancing lesions was done unblinded by the same neuroradiologist (JS). 16 patients did not have a clinical relapse, and no gadolinium-enhancing lesions were found on any of the post-transplantation scans for these patients (table 3). Of the patients who did not relapse, the number of T2 lesions was unchanged in 12 patients, increased in two patients, and decreased in two patients, whereas the number of T1 lesions was unchanged in 13 patients, higher in one patient, and lower in two patients. The diameter of the third ventricle was unchanged in 13 patients and higher in three patients (table 3). Five patients had a clinical relapse. Three patients with clinical relapses had new gadolinium-enhancing lesions. All relapses occurred 6–16 months post-transplantation. Of the five patients who had relapses, new T2 lesions were seen in three patients and new T1 lesions in one patient (table 4). Because three patients who did not have a clinical relapse or documented enhancing lesions had an increase in non-enhancing T1 or T2 lesions between the pretransplantation and most recent follow-up (table 3), at 3-year mean follow-up the disease-activity-free survival defined as no disease progression by EDSS, no clinical relapses, and no MRI evidence of new lesions was 62% (13 patients).

Five patients (23%) relapsed at an average of 11 months after transplantation after an initial improvement in neurological function. Patients who relapsed were retreated with immunosuppressants: one was treated with daclizumab, one with mycophenolate mofetil, two with intravenous cyclophosphamide for 6 months, and one with intravenous cyclophosphamide for 6 months followed by maintenance interferon beta. The patients have all achieved further remissions with no further relapses and continued improvement in EDSS score (table 4).

	T2 lesions	T1 lesions	Width of third ventricle (mm)	Gd-enhancing lesions	EDSS
Patient 3*					
Pretransplantation	135	130	5·0	0	3·5
6 months	135	130	6·0	0	2
1 year	145	130	6·0	29	3·5
1·5 years	145	130	7·6	0	..
2 years	147	130	8·0	0	..
3 years	147	130	8·0	0	2·5
Patient 6†					
Pretransplantation	100	90	9·0	16	5·5
6 months	100	90	9·0	0	5·5
1 year	100	90	9·0	0	6·0
2 years	100	90	9·0	0	2·0
Patient 7‡					
Pretransplantation	30	15	5·0	0	2·5
6 months	30	15	5·0	0	1·5
1 year	30	15	5·0	0	1·5
2 years	30	15	5·0	0	1·0
2·5 years	30	14	5·0	0	1·5§
Patient 13¶					
Pretransplantation (10 months)	58	21	7·0	3	..
Pretransplantation (1 month)	58	21	7·5	2	3·0
6 months	58	21	7·5	8	3·5
1 year	63	28	8·0	3	3·0
2 years	63	28	8·0	1	3·0
3 years	63	28	8·0	0	2·0
Patient 19*					
Pretransplantation (1 month)	30	20	7·0	20	5·5
6 months	30	20	7·0	0	3·5
1 year	40	20	7·0	10	3·5
2 years	..	..	..	..	2·0

The pretransplantation conditioning regimen for patients 3, 6, 7, and 13 was cyclophosphamide and alemtuzumab, whereas patient 19 received cyclophosphamide and antithymocyte globulin. EDSS=Kurtzke expanded disability status scale. ..=data not available. *Relapse at 12 months, treated with six doses of cyclophosphamide. †Relapse at 12 months, treated with methylprednisone and mycophenolate. ‡Relapse at 16 months, treated with daclizumab. §At 3 years. ¶Relapse at 6 months, treated with six doses of cyclophosphamide and patient maintained on interferon.

Table 4: **Changes between pretransplantation and post-transplantation MRI and EDSS in patients with clinical relapse**

Discussion

We report the results of autologous haemopoietic stem cell transplantation for relapsing-remitting MS. After a mean follow-up of 3 years, progression-free survival was 100%. By contrast with haemopoietic stem cell transplantation in patients with progressive MS, 81% of our patients had reversal of neurological disability and a sustained improvement in EDSS score of 1 point or more. However, the recruitment of patients with recent relapsing disease might not enable a true baseline EDSS score to be assessed because their neurological function might improve after an acute exacerbation. Because disability during an acute attack is not fixed, we waited until 3 months after an acute clinical attack, to enable the EDSS score to improve and reach a true baseline before enrolment.

Continued improvement in neurological function was seen for up to 2 years after transplantation and was sustained for up to 4 years in the longest follow-up (mean 37 months). The results of tests of neurological disability improved on serial assessments after transplantation; however, some improvements occurred or continued after a delay of 1 year or more, suggesting that the total treatment-related benefit might be apparent only with prolonged observation. Improvements in neurological disability after haemopoietic transplantation correlated with improvements in quality of life, as seen in the SF-36 scores.

The initial clinical data from transplantation studies in patients with progressive MS prompted us to move away from intense myeloablative regimens (ie, the regimens that contain total body irradiation or full-dose busulfan) because of the high mortality reported for intense myeloablative regimens for autoimmune diseases in general,[17] and MS[4,5,26] in particular. Short-term disease-related mortality from MS, equivalent to an EDSS score of

10 points, is relatively uncommon. However, patients with MS are three times more likely to die than the general population,[27] and long-term disease-related mortality increases with increasing baseline EDSS scores.[28] In one study with 20 years of follow-up, MS-related mortality was 14% for patients with an EDSS score of 0 to 3 points at enrolment; 19% for patients with an EDSS score of 3·5 to 5·5 at enrolment; 36% for patients with an enrolment EDSS of 6·0 to 7·5 points; and 62% for patients with an enrolment EDSS of 8·0 to 9·5 points.[28] Therefore, any treatment that is designed to affect the natural history of MS, particularly when given during the early inflammatory stages of the disease, must not cause excessive acute mortality.

Non-myeloablative regimens, such as those used in this study, followed by autologous haemopoietic stem cell transplantation have been done with less than 1% mortality for several autoimmune diseases, including some with multiorgan dysfunction and a history of long-term, high-dose, pretransplantation immune suppression.[17] For a disease such as relapsing-remitting MS, in which patients have normal organ function outside the CNS and are not exposed to chronic high-dose immune suppression, transplantation-related mortality with a non-myeloablative regimen should be well below 1%. In this study, there were no transplantation-related deaths, few episodes of neutropenic fever, and no cases of neutropenic-related opportunistic infections or cytomegalovirus reactivation.

We initially used a lymphocyte-depleting but non-myeloablative approach, with alemtuzumab and cyclophosphamide; however, two patients had immune thrombocytopenia. We had previously reported that secondary autoimmune cytopenias might arise in many autoimmune diseases after the use of alemtuzumab but not when antithymocyte globulin is used in the haemopoietic stem cell transplantation conditioning regimens.[29] For this reason, we stopped including alemtuzumab in the conditioning regimen, and the last four patients to be treated received antithymocyte globulin, which was a similar regimen to that used in our previously reported studies of haemopoietic stem cell transplantation in patients with systemic lupus erythematosus[30] or type I diabetes.[31]

Despite immune analysis that showed immune reset after myeloablative transplantation regimens during the progressive phase of MS,[32] EDSS scores did not improve. By comparison, EDSS scores in the current study improved for more than 2 years post-transplantation. Because immune reconstitution is still being evaluated in these patients, improvements might have been related to either immune reset or transient immune suppression without regeneration of an immunologically distinct immune system. Although further studies are needed, immune reset seems probable, owing to the duration of ongoing improvement after transplantation. The intense myeloablative regimens used in patients with progressive MS also cause near-total suppression of MRI enhancement.[33] During the progressive phase of MS, MRI enhancement normally decreases; therefore, comparison of the suppression of MRI activity due to the different regimens used in the different phase of MS is difficult.

Whether the data from this trial are superior to those achieved with continuation of standard therapies for relapsing-remitting MS can be assessed only in a randomised trial. A recent randomised trial of standard immune-modulating versus immune-suppressing therapy for patients with relapsing-remitting MS who had EDSS scores of less than 3 points showed that the mean EDSS score worsened by 0·38 points in patients who were maintained on beta interferon, and improved by 0·39 points in patients who were treated annually with alemtuzumab.[34]

Autologous non-myeloablative haemopoietic stem cell transplantation for patients with relapsing-remitting MS with active inflammatory disease and frequent exacerbations is a feasible procedure that not only seems to prevent neurological progression, but also appears to reverse neurological disability. The current multiple sclerosis international stem cell transplant (MIST) trial[35] is investigating patients with relapsing-remitting MS who have not responded to interferon therapy and are randomly assigned to autologous non-myeloablative haemopoietic stem cell transplantation or continued standard therapy.

Contributors

RKB, WHB, BAC, YO, and JV contributed to the design of the study. RB, DS, RKB, AT, YL, YO, GK, and JMB participated in care of patients. RB, RKB, JWR, DS, ER, PM, and JS participated in collection and assessment of data, and BJ and FM did the statistical analysis. AT contributed to the figure. RB, DS, PM, RKB, BAC, JWR, JS, YL, and ER participated in the writing and editing of the manuscript.

Conflicts of interest

We have no conflicts of interest.

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