CD34+ selected autologous peripheral blood stem cell transplantation for multiple sclerosis: report of toxicity and treatment results at one year of follow-up in 15 patients

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sBackground and Objectives. Autologous stem cell transplantation (ASCT) is currently being evaluated as a therapy for patients with multiple sclerosis (MS). We report the results of a phase II trial to evaluate feasibility and toxicity of CD34+ selected ASCT (CD34+/ASCT) and treatment results at one year of follow-up.

Design and Methods. Patients with advanced secondary progressive (SP) or relapsing-remitting (RR) MS and confirmed worsening of the extended disability status scale (EDSS) in the previous year despite interferon or other immunotherapies were included. Peripheral blood stem cells were obtained by leukaphereses after mobilization with cyclophosphamide (Cy) and granulocyte colony-stimulating factor (G-CSF). CD34+ selection was performed by means of an Isolex 300 or CliniMACS device. BCNU, Cy and antithymocyte globulin (ATG) were administered as conditioning regimen.

Results. Fifteen patients (9 SPMS and 6 RRMS) with a median EDSS of 6.0 (4.5-6.5) and a median of 3 (1-7) relapses in the previous year were included. Mobilization was unsuccessful in one patient. During mobilization, one patient had a transient neurologic deterioration. The main complications during ASCT were engraftment syndrome, which developed in three patients, CMV reactivation in one, and neurologic deterioration in two patients coinciding with high-fever related to ATG. Hematologic recovery was fast and complete in all cases. At 12 months, the EDSS had improved in three patients, worsened in two and remained stable in nine. Despite withdrawal of all immunosuppressive therapy only two patients had relapses. Magnetic resonance imaging showed disappearance of enhanced T1 lesions but oligoclonal bands persisted in the cerebrospinal fluid of all evaluated cases.

Interpretation and Conclusions. CD34+/ASCT using BCNU, Cy and ATG as conditioning regimen has an acceptable toxicity and clearly reduces the progression of MS. Further follow-up is necessary to establish the real impact of this procedure on the long-term evolution of the disease.

Key words: multiple sclerosis, autologous stem cell transplantation, CD34+ positive selection, autoimmune diseases.

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Multiple sclerosis (MS) is a common disease of the central nervous system affecting approximately 2.5 million adults, mostly young women, worldwide.[1] The exact pathogenesis of MS has not yet been fully defined. Genetic susceptibility, environmental triggers, and abnormal immunological reactions seem to lead to T-cell mediated focal myelin destruction and secondary damage of the axons. Several additional factors, including autoantibodies or cytokines, may also be necessary for myelin destruction to occur.[1,2] MS treatment involves immunosuppressive and immunomodulating agents. β interferons and glatiramer acetate are effective in reducing the number of relapses, but if these therapies are not successful or the disease evolves into its progressive phase no treatment has proven effective for substantially altering the clinical course.[2,3] Recently, mitoxantrone has been shown to be more effective than placebo in modifying the clinical course of secondary progressive MS.[4] However, some patients will fail to benefit from these therapeutic approaches, this justifying the evaluation of novel therapies such as stem cell transplantation (SCT).

The rationale for using SCT is based on the principle of complete ablation of an aberrant immune system followed by reconstruction of a new immune system derived from allogeneic or T-cell depleted (TCD) autologous hematopoietic progenitors.[5,6] This hypothesis is based on the theory that environmental factors play a major role in MS pathogenesis and that autologous SCT (ASCT) could be used to *reset* the autoimmune cell line, thus restoring self-tolerance.[7] However, if genetic predisposition plays a major role in MS development and autoimmunity is a consequence of a hematopoietic stem cell disorder, ASCT would have only a temporary anti-inflammatory effect.[8]

In April 1998 we initiated a phase II clinical trial to evaluate the feasibility and toxicity of CD34+ selected autologous peripheral blood stem cell transplantation (CD34+/ASCT) for MS with unfavorable prognosis. In this paper we report the results of this trial and the effect on the course of MS at one year after CD34+/ASCT.

Design and Methods

Patient eligibility

Patients with MS were considered candidates to be included in the trial if they fulfilled all of the following criteria: 1) aged between 18 and 60 years; 2) clinically definite secondary progressive MS (SPMS) or relapsing

remitting MS (RRMS) with a Kurtzke's Expanded Disability Status Scale (EDSS) of 4.0 to 6.5; 3) an increase in the EDSS by 1.0 point with an EDSS of 5.5 or less, or 0.5 with an EDSS >5.5 during the previous year in spite of treatment with interferon or other immunotherapy. This treatment was stopped at least one month before CD34+/ASCT. The trial was approved by the institutional Ethical and Research Committee and the Spanish Ministry of Health.

Stem cell mobilization and collection

Cyclophosphamide (Cy) at a dose of 3 g/m^2 followed by daily G-CSF (Filgrastim; Amgen, Thousand Oaks, CA) 5 μg/kg subcutaneously until the end of leukaphereses were used for hematopoietic cell mobilization. Leukaphereses were started when leukocyte and CD34+ cells counts were in excess of 1×10^9/L and 0.01×10^9/L, respectively. It was planned that patients underwent two leukaphereses by means of a Fenwall CS3000-Plus (Baxter, Fenwall Division, Deerfield, IL, USA) or a GAMBRO Spectra (Gambro-BCT, Denver, CO, USA) cell separator. In each procedure a volume of 10-12 L was processed for a target collection of, at least, 6 and 2.5×10^6 CD34+ cells/kg, respectively. The first procedure was T-cell depleted (positive selection) and the second one was cryopreserved unmanipulated as a back-up.

T-cell-depletion

In the first two patients, CD34+ positive selection was carried out using an Baxter Isolex 300 system. The remaining cases were depleted using a CliniMACS (Miltény, Biotec Bbergisch Gladbach, Germany) method. The minimum number of CD34+ cells after positive selection required to perform the transplant was 2.5×10^6/kg.

Conditioning regimen

As soon as possible after hematopoietic cell harvest the following conditioning regimen was administered: BCNU 300 mg/m^2 on day –6, Cy 50 mg/kg on days –5 to –3 and ATG (Lymphoglobuline, Merieux) 15 mg/kg plus methylprednisolone (2 mg/kg in the first 8 patients; 500 mg in the remaining 6) on days –5 to –2. Mesna® and alkaline hyperhydratation were administered to avoid Cy bladder toxicity.

Supportive care

Low microbial diets and oral ciprofloxacin, fluconazole and acyclovir (if positive herpes simplex virus serology) were administered as infection prophylaxis during the neutropenic period. During this phase patients were admitted in rooms equipped with HEPA filters and laminar airflow. Inhaled pentamidine (300 mg) was administered on days – 6 and +21 and oral trimethoprim and sulfamethoxazole (two days per week) was given from this date until counts exceeded 0.2×10^9/L CD4+ cells. Immunoglobulins (Flebogamma, Grifols, Spain) were administered intravenously at a dose of 100 mg/kg per week until day +90. G-CSF (Filgrastim; Amgen), was administered at 5 μg/kg per day on day +1 and continued until the absolute neutrophil count was greater than 1×10^9/L for 3 consecutive days. No additional immunosuppressive drugs were administered after CD34+/ASCT unless required.

Clinical evaluation

The hematologic and neurologic (neurologic examination, EDSS, Ambulatory Index Score) conditions of the patients were evaluated at baseline, at the end of hematopoietic cell mobilization, and at 1, 3, 6, 9, and 12 months post-SCT. The two neurologists involved in the study evaluated each case independently. The immunization schedule after treatment was given according to previously reported guidelines.[9] Systemic adverse effects were scored using Bearman's scale.[10]

Magnetic resonance imaging evaluation

Magnetic resonance imaging (MRI) brain scans were obtained at baseline and at 1, 3, 6 and 12 months post-SCT with a 1.5 T unit Siemens Magnetom SP (Erlangen, Germany) with a quadrature head coil. The methodology used to identify and count the number of hypointense and enhanced lesions in pre- and post-contrast T1-weighted images and hyperintense lesions in T2-weighted images has been previously reported.[11]

CSF analysis

Paired serum/CSF samples were obtained at baseline, and 3 and 12 months after CD34+/ASCT and stored a –80°C. The method used to identify the IgG-specific oligoclonal bands in CSF and serum has been previously reported.[11]

Immune reconstitution

Peripheral blood was examined at baseline and 3, 6 and 12 months post-SCT. T-cells (CD3+), helper/inducer T-cells (CD3+CD4+), naive (CD4+ CD45RA+) and memory (CD4+CD45RO+) helper/inducer T-cells, cytotoxic T-cells (CD3+CD8+), B cells (CD19+), and natural killer cells (CD3–CD56+) were analyzed using a FAC-Scan [Becton Dickinson Immunocytometry Systems (BDSI), San José, CA, USA], as reported elsewhere.[12]

Results

Patients

Between April 1998 and April 2001, 15 patients were included in the trial. The patients' main characteristics are shown in Table 1. Their median age was 30 years (range 22-45). Nine patients had

Table 1. Patients' characteristics.

Inclusion period:	April 1998 - April 2001
Patients included:	15
Age,* years	30 (22-45)
Male/female	2/13
MS type - secondary progressive	9
- relapsing – remitting	6
Interval diagnosis-SCT*	8 (1-19) years
EDSS score before CD34+/ASCT*	6.0 (4.5-6.5)
EDSS increase in previous year*	1.0 (0.5-4,5)
Relapses in the previous year*	3 (1-7)

**Median (range).*

SPMS and six, RRMS. During the 12 months prior to CD34+/ASCT, the median number of MS relapses was 3 (range 1-7) (6 for patients with RRMS), and the median increase in EDSS 1.0 (range 0.5-4.5). The median EDSS at CD34+/ASCT was 6.0 (range 4.5-6.5) (Table 1).

Stem cell mobilization and harvest

In one patient the minimum required leukocyte and CD34+ cells counts were not reached after mobilization and leukaphereses were not performed. An unsuccessful second attempt was performed several weeks later using the same mobilization scheme. In the remaining cases the target yield of CD34+ cells was reached. In one patient it was necessary to perform three leukaphereses. Conversely, in three cases enough cells for CD34+ selection and back-up were collected from a single procedure. After positive selection the graft contained a median of 5.1 (range 2.5-12.9) $\times 10^6$/kg CD34+ cells. The number of CD3+ cells after selection with CliniMACS (12 cases) resulted in a median of 0.29 (range, 0.06-4.3) $\times 10^4$/kg; being 30 and 7×10^4/kg in the two Isolex procedures.

Toxicities observed during mobilization were a transient leg paresis (patient #5) on day +2 of G-CSF, a mild hemorrhagic cystitis (related to an inadequate administration of Mesna®), and a febrile episode during neutropenia, which resolved with empirical antibiotics.

Stem cell transplantation

CD34+/ASCT was performed in the 14 patients from whom sufficient CD34+ cells had been collected. In 13 cases ASCT was performed at a median of 13 days (range, 10-30) after the first leukapheresis; in one, it was delayed 73 days due to non-medical reasons.

Conditioning toxicity. One patient (#5) developed grade II liver toxicity (transaminase level $\times$ 20) after BCNU administration. For this reason the administration of the remaining drugs of the conditioning regimen was delayed 6 days and restarted when transaminase levels had returned to normal values. Six of the first eight patients developed high-fever with ATG administration. In addition to fever three patients developed a skin rash and one a vasculitis that required prolonged treatment with steroids. For this reason, the last six patients received 500 mg of methylprednisolone before each ATG dose: none of them presented these complications. Six patients developed grade I and two grade II mucositis.

Neurologic complications. Patient #2 developed severe persistent paraparesis that increased her EDSS from 6.5 to 8.0 and patient #7 a transient deterioration of her hemiparesis. These episodes coincided with the high-fever related to ATG administration.

Infections. During neutropenia 12 patients developed fever. In seven of these patients all cultures were negative; in the remaining five *E. coli* (one case), MARSA (one case) and coagulase-negative *Staphylococci* (3 cases) were isolated in blood and *E. coli* (one case) in urine. One patient developed two consecutive episodes of CMV positive antigenemia on days +20 and +38, both successfully treated with gancyclovir. Two patients (#9 and 12) developed herpes zoster 7 and 10 months after CD34+/ASCT, respectively.

Other complications. Three patients (#3, 7 and 8) developed fever resistant to empirical antibiotics, mild skin rash and weight gain coinciding with neutrophil recovery. They were classified as having engraftment syndrome and treated with steroids until complete resolution. One patient (#12) had an episode of uveitis that resolved with steroid treatment 2.5 months after CD34+/ASCT.

Hematopoiesis recovery. All patients reached and maintained values of $>0.5 \times 10^9$/L neutrophils and $>20 \times 10^9$/L platelets after a median of 10 (range, 9-18) and 11.5 (range, 7-15) days, respectively.

Immune reconstitution. Figure 1 shows median values of CD3+CD4+, CD3+CD8+, CD4+CD45RA+, CD4+CD45RO+, CD19+ and CD3-CD56+ at baseline and 3, 6 and 12 months after CD34+/ASCT. The number of CD4+ T-lymphocytes decreased from a baseline median (range) of 1.01 (0.41-2.84) cells $\times 10^9$/L to a median of 0.10 (0.08-0.71) cells $\times 10^9$/L by the third month after transplantation. As shown in Figure 1a, CD4+ cell counts increased at 6 months, achieving a median (range) of 0.18 (0.08-0.34) cells $\times 10^9$/L. Although normal pretreatment values were not achieved throughout the period of the study, all patients but one had CD4+ cell counts over 0.20 cells $\times 10^9$/L at 12 months after transplant (median 0.35 cells $\times 10^9$/L, range 0.11-1.06) (Figure 1a). The reconstitution of the CD4+CD45RA+ subtype, defined as the immature, naive CD4+ cell population, regenerating through a thymus-dependent pathway, remained low during the period

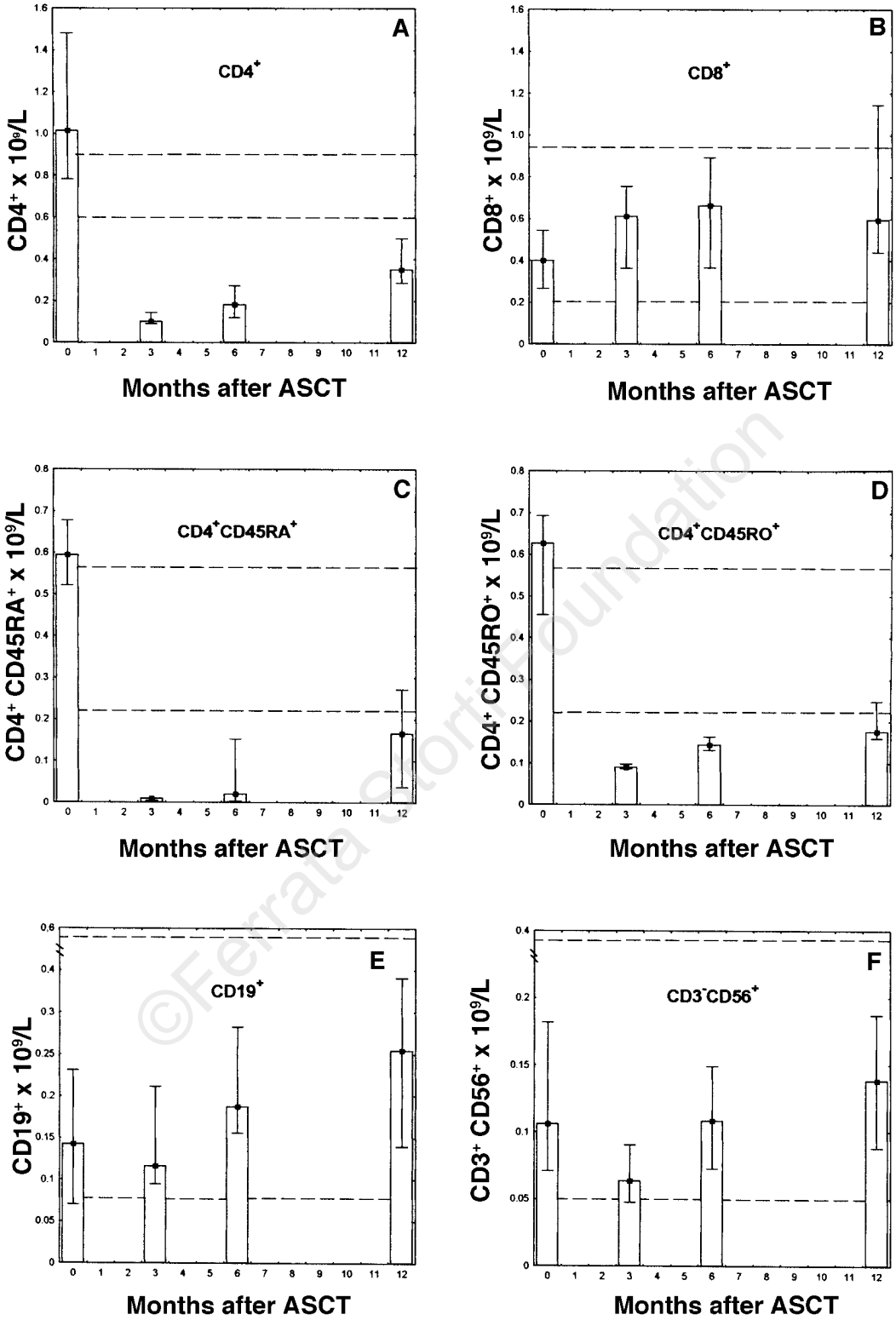

Figure 1. Median values of $CD3^+CD4^+$ (a), $CD3^+CD8^+$ (b), $CD4^+CD45RA^+$ (c), $CD4^+CD45RO^+$ (d), $CD19^+$ (e) and $CD3^-CD56^+$ (f) at baseline and 3, 6 and 12 months after $CD34^+$/ASCT. Dashed lines in each figure indicate the normal range of each subtype of lymphocytes. Error bars represent 25th and 75th percentiles.

Table 2. Main data of MS evolution in patients undergoing CD34⁺/ASCT.

	Patient		Disease		MS relapses			EDSS			MRI	
#	Sex	Age	MS type	Dx-Tx (years)	previous year	at 12 m	↑prev. year	pre-SCT	after ASCT	at 12 m	Gd⁺ pre	Gd⁺ 12 m
1	F	30	SP	8	3	0	0.5	6.0	6.0	6.0 (=)	Yes	No
2	F	43	SP	7	2	0	0.5	6.5	8.0	7.5 (↑)	No	No
3	F	24	RR	9	6	2@	1.0	5.0	5.0	5.0 (=)	Yes	No
4	M	44	SP	14	2	1@	1.5	6.5	6.5	5.0 (↓)	No	No
5	F	27	RR	8	6	0	2.0	6.5	6.5	5.5 (↓)	Yes	No
6	F	45	SP	19	3	0	0.5	6.5	6.5	7.5 (↑)	No	No
7	F	23	RR	6	6	0	1.0	6.5	6.5	6.5 (=)	No	No
8	F	37	SP	3	1	0	1.0	5.5	5.5	5.5 (=)	Yes	No
9	F	31	SP	10	1	0	1.0	6.0	6.0	6.0 (=)	No	No
10	F	28	RR	1	6	1	4.5	5.5	5.5	5.0 (↓)	No	No
11	F	28	SP	9	1	0	0.5	6.5	6.5	6.5 (=)	No	No
12	M	33	SP	8	3	2	1.0	6.5	6.5	6.5 (=)	No	No
13	F	37	SP	10	1	0	1.0	5.5	5.5	5.5 (=)	No	No
14	F	22	RR	6	7	0	1.5	4.5	4.5	4.5 (=)	Yes	No

prev: previous; @: subjective sensory symptoms; Dx: diagnosis; Tx: transplantation.

studied (Figure 1c). In the early (3-6 months) period after the transplant most of the CD4⁺ cells were CD4⁺CD45RO⁺ (Figure 1d).

In contrast to CD4⁺ cells, CD8⁺ T-lymphocyte counts recovered quickly, representing the major lymphoid population during the period studied [median and range of 0.61 (0.27-0.95), 0.66 (0.26-1.66) and 0.60 (0.20-2.74) cells $\times 10^9$/L at 3, 6, and 12 months after transplant, respectively] (Figure 1b). The increase of CD8⁺ T-lymphocyte levels in conjunction with the reduced CD4⁺ levels resulted in an inversion of the usual CD4/CD8 ratio. Finally, B-cells and NK-cells, measured as CD19⁺ and CD3-CD56⁺ cells, respectively, remained at normal levels 3, 6, and 12 months after the transplant (Figures 1e and 1f, respectively). We did not observe any correlation between immune reconstitution and clinical evolution.

MS status one year after CD34⁺/ASCT

Table 2 shows MS relapses, EDSS and MRI results 12 months after CD34⁺/ASCT. The patient who did not undergo ASCT, despite the high doses of Cy received during both mobilization procedures, continued progressing and mitoxantrone was started. At 1 year her EDSS score had increased 1.0 point. Patient #2, whose condition worsened during ASCT, slowly improved to an EDSS of 7.5 over the ensuing 3 months, and then remained stable. Three patients (#4, 5 and 10) improved 1.5, 1.0 and 0,5 points, respectively, and one patient (#6) worsened 1.0 point. The remaining 9 patients remained stable throughout the follow-up.

Four patients had relapses (Table 2). Patient #3 had relapses at 5 and 10 months, and patient #4 at 4 months: these three relapses were all manifested as transient subjective sensory symptoms that lasted a few days and did not require treatment. The two other patients (#10 and #12) with relapses were treated with intravenous bolus methylprednisolone: patient #10 at 12 month, and patient #12 at 6 and 12 months. There was full recovery from all these relapses and the patients' EDSS score remained unchanged. Fatigue (although not formally measured) improved during the follow-up period in all patients. Eleven out of 14 patients had problems with urinary sphincter control before CD34⁺/ASCT. At one year this function had clearly improved in 7, was unchanged in 3 and had worsened in 1 (patient #2) coincidentally with the above mentioned complication during CD34⁺/ASCT. The follow-up MRI studies did not show enhanced T1 lesions in any of the 14 patients (four had gadolinium-enhancing lesions in the basal MR, see Table 2). In only one patient (#6), who progressed after the transplant, did the MRI at 1 year showed new hypointense T1 and hyperintense T2 lesions. In the other 13 patients no new lesions were observed on T1- or T2-weighted images in the post-transplant images. All but one patient showed oligoclonal bands at the baseline CSF examination. As previously reported in the first five patients,[11] the oligoclonal bands persisted at month 12 post-transplant in the CSF of all of the nine patients who were examined. Given the uniform results, the remaining patients in the study did not have a lumbar puncture performed to evaluate this variable.

Discussion

The present study demonstrates that ASCT with intensive T-cell depletion is feasible in MS patients and that it has an acceptable toxicity. These results contrast with a recent retrospective analysis by the EBMT/EULAR involving 85 MS patients who had undergone ASCT in 20 centers. That study reported a procedure-related mortality of 6%.[13] Most of these patients had been previously reported (Table 3).[14-19] We describe, additionally, the impact of CD34⁺/ASCT on clinical conditions, immune reconstitution, brain MRI and oligoclonal bands in CSF after one year in our group of uniformly treated patients.

When our trial was designed several aspects of the procedure were unclear. Thus, although stem cells obtained after mobilization from peripheral blood contain ten times more lymphocytes than those from bone marrow, we decided to use the former source of stem cells because of the well known advantages (easier harvest, quicker engraftment, fewer days of neutropenia, lower transplant-

Table 3. CD34⁺/ASCT toxicity in MS.

Ref.	*patients Age@ EDSS@*	*MS type*	*Mobilization*	*Positive selection Method CD3⁺ × 10⁴/kg*	*Conditioning*	*Neurological toxicity Mobilization*	*ASCT*	*Other remarkable complications[1] related with ASCT*	*Death*
Burt[15]	6 not given 7.5	PP 2 SP 4	G(10)	6/6 Cellpro 77 (23-140)@	Cy-TBI(12 Gy) + MPD 1 × 4	No	No	No	0
Fassas[14]	24 40 6.0	SP 16 PP 8	Cy(4)⁺ G(5-10)	9/24 Cellpro 7 (+/-4)#	BEAM-ATG	Seizure [1]	Transient neurologic flares [10]	VOD [1], TTP [1], CMV [1]. AID [1]	IA [1]
Kozak[17]	8 41.5 6.5	SP	Cy(4)⁺ G(10)	7/8 Cellpro [5], Clini MACS [2] 0.4 (0.3-3.5)@	BEAM (+ATG in 1)	No*	No	VZV [1] Late cellulitis [1]	0
Openshaw[18]	5 44 6.5	SP 5	G(10)°	5/5 Isolex 29 (5-120)@	Bu-Cy-ATG [4] BEAM-ATG [1]	Hemiplegia [1]°	Transient hemiparesis [1]	Influenza A pneumonia [1]	ARDS [1] Sepsis [1]
Mancardi[19]	10 35.5 6.5	SP 6 SPR 4	Cy(4)⁺ G(5)	None	BEAM-ATG	No	No⁺⁺	CMV [3] VZV [2]	0
Present series	14 30 6.0	SP 9 RR 6	Cy(3)⁺ G(5)	14/14 CliniMACS [12], Isolex, [2] 0.29 (0.06-4.3)@&	BCNU-Cy-ATG	Transient leg paresis [1]	Persistent paraparesis [1] Transient hemiparesis [1]	CMV [1], VZV [1], ES [3], Uveitis [1]	0

*1: excluding usual infectious complications occurring during the neutropenic phase; [ ]: in brackets number of patients; *in 11 mobilized patients; °one patient had a severe MS flare after G-CSF, and was finally successfully mobilized with etoposide + Cy + G-CSF; ++: transient neurologic worsening defined as "common"; SP: secondary progressive; PP: primary progressive; RR: relapse-remitting; SPR: secondary progressive with relapses; G= G-CSF; Gy: cyclophosphamide; Cy(x): in parenthesis dose of Cy in g/m²; Bu: busulfan; BEAM: BCNU + etoposide + cytarabine + melphalan; TBI: total body irradiation; VOD: veno-occlusive disease; TTP: thrombotic thrombocytopenic purpura; AID: autoimmune disease; VZV: varicella zoster virus; CMV: cytomegalovirus; IA: invasive aspergillosis; ARDS: adult respiratory distress syndrome; ES: engraftment syndrome; @median (range); #mean (±SD); &referred to CliniMACS selection.*

related mortality, effective methods to perform T-cell depletion).[20] However, our main concern was the reported episodes of clinical flares of autoimmune diseases following G-CSF administration.[21] To prevent a G-CSF-related disease flare, combined Cy and low-dose G-CSF were used.[6,22] With this approach only one transitory relapse occurred in a single patient; this patient had had multiple relapses in the months prior to the transplant and a coincident relapse not strictly related to the use of G-CSF cannot be ruled out. Similar results were observed by other groups using this mobilization scheme.[6,13,17] Mobilization failed in one patient; this observation could not be correlated with high previous interferon doses or other causes. Although described to be useful by other authors,[17] two courses of high-dose cyclophosphamide given to this patient did not produce any apparent clinical improvement.

A second aspect was the design of the conditioning regimen. As our intention was to perform CD34⁺/ASCT as soon as possible after harvest, we decided to distribute the scheduled dose of Cy between mobilization (3 g/m²) and conditioning (150 mg/kg). We excluded total body radiation (TBI) due to the neurotoxic effect described in animals,[23] the exacerbation of demyelinating polyneuropathy reported after SCT[24] and the increased production of tumor necrosis factor by astrocytes and microglial cells after radiation.[25] However, further reports from several groups have shown that TBI has no adverse effect in humans with MS.[6,15] Similarly, despite their crossing the blood-brain barrier we decided not to use busulfan, etoposide or cytosine-arabinoside, as has been recommended by other authors,[14,17,19,26,27] because of the well known neurotoxicity or potential late effects of these drugs. Finally, we decided to associate Cy, BCNU and ATG. BCNU was selected because of its capacity to cross the blood-brain barrier and its powerful immunosuppressive activity.[28] ATG was used in order to maximize *in vivo* lymphocyte depletion and methylprednisolone added as prophylaxis of allergic reactions. As expected, the toxicity of our conditioning regimen was low. Mucositis was mild and brief in all cases. Hepatic toxicity

attributable to BCNU was transitory and, fortunately, did not preclude restarting the conditioning regimen. Despite prophylactic methylprednisolone, most patients developed high-fever after ATG administration and two of them had neurological deterioration. For this reason, the last 6 patients received a higher dose of corticosteroids and no complications were observed. Febrile episodes not associated with ATG did not differ from those observed in ASCT for other indications and were controlled with empirical treatment. The only unexpected events were the three episodes of engraftment syndrome. This relatively infrequent complication, observed mainly in SCT for solid tumors, seems to be a relatively frequent complication in MS patients.[6,29]

The graft was T-cell depleted based on the understanding of the im-munopathology of MS and on previously reported data showing early relapses after unmanipulated SCT in animals and humans.[6,7] Theoretically, purging the graft of lymphocytes could prevent the infusion of potential disease-causing cells and favor regeneration of new lymphocytes from stem cells that result in immune tolerance. In our study, the reconstitution pattern of T-lymphocytes was similar to that previously reported in other ASCT settings[30,31] and in MS patients.[15,17] Thus, the numbers of $CD4^+$ cells were characteristically low during the first year post-transplant, when they began to return towards normal; the recovery was mainly due to the mature subset ($CD4^+CD45RO^+$) with a slow recovery of naive $CD4^+$ cells ($CD4^+CD45RA^+$). This is in agreement with the notion that, in adult patients with decreased thymic function, T-cell regeneration occurs primary via thymic-independent pathways.[32,33] According to this notion, it is unlikely that the reason for the clinical improvement in the MS is the *re-education* of the immune system resulting in immune tolerance to myelin components, but rather is more likely to be due to the profound immunosuppression caused by ASCT. T-cell depletion could contribute significantly to this immunosuppression since it not only seems to slow down quantitative T-cell reconstitution but it is also associated with long-lasting functional immune defects.[34,35] In this regard, recent observations showing an apparent absence of correlation between T-cell depletion and disease relapse[13] could be explained by the less intensive depletion used in these trials (Table 3). Besides this, aggressive lymphocyte depletion may increase the risk of serious post-transplant opportunistic infections.[35,36] Consequently, we adopted all well established prophylactic measures to prevent infections including periodic CMV antigenemia checks. In this series no serious infectious complications were observed and only one patient developed a CMV reactivation without CMV disease. Our data suggest that the decision of whether or not deplete the graft of T-cells should be based on efficacy against MS rather than toxicity.

Although the trial was not designed to evaluate the efficacy of the procedure, one year after the $CD34^+$/ASCT, the EDSS of twelve of our patients had not worsened. Of note, the median increase of the EDSS during the previous year in these patients had been 1.0 point (range 0.5-4.5). The EDSS improved in three patients by 1.5, 1.0, and 0.5 points (the increases in the previous year had been 1.5, 2.0 and 4.5 points, respectively) and remained stable in nine. The score worsened in two patients: the first one had a severe deterioration during $CD34^+$/ASCT, and despite improving at 12 months, did not reach the previous baseline level; the second one had a clearly increased EDSS after $CD34^+$/ASCT.

Undoubtedly, the most relevant aspect for all our patients was the evolution of MS relapses after $CD34^+$/ASCT. This aspect was not evaluated in most other studies because patients with relapsing-remitting MS were usually excluded.[6] In our series, despite withdrawal of all immunosuppressive therapy, only two patients had 3 relapses that required treatment. This is a strong reduction taking into account the fact that in the year prior to $CD34^+$/ASCT, all 14 patients had had relapses, and that the number of treated relapses was 51. Additionally, in 7 out of 11 patients with urinary sphincter disturbance this function improved after $CD34^+$/ASCT. All these facts contributed to a subjective improvement in the patients' quality of life; however no validated instruments of measurement of this improvement were used.

The disappearance of enhanced T1 lesions and the lack of new or enlarging hyperintense T2 lesions, suggest that $CD34^+$/ASCT had a positive impact on active inflammation. Unfortunately the persistence of oligoclonal bands in CSF supports the idea that the B-cells responsible for their synthesis in the CNS survived the conditioning regimen.[11] Apparently no pre-ASCT variable had an impact on clinical outcome, but the low number of clinical events after ASCT precluded any statistical analysis.

In conclusion, this trial shows that $CD34^+$/ASCT is a feasible therapeutic approach for MS patients. Our more immunosuppressive than myeloablative conditioning regimen has an acceptable toxicity, clearly reduces the advance of the disease and improve the patients' quality of life. Long-term follow-up is necessary to establish the real impact of this procedure on the evolution of the disease.

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Contributions

FG was the principal investigator and responsible, together with AS, for the clinical management of patients, acquisition of clinical data and reviewing the manuscript from a neurological point of view; EC was responsible for the hematologic aspects of the trial, SCT clinical data acquisition and wrote the paper; PM was responsible for the mobilization, $CD34^+$ selection and cryopreservation; ML was responsible for leukaphereses; CM, NV and MA were responsible for the immune reconstitution studies. CM, MR, AUI and FFA were responsible for the patients during BMT; EM promoted this trial and critically reviewed the manuscript. The authors acknowledge the BMT Unit nursing staff members for their help in the realization of this trial, and neurologists from several cities of Spain for referring us their patients.

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Disclosures

Conflict of interest: none.

Redundant publications: the neurologic aspects of the first five patients of this series were described in the paper *MRI and CSF oligoclonal bands after autologous hematopoietic stem cell transplantation in MS* published in Neurology 2001; 56:1084-9. Additionally, the first eight patients were included in a recent analysis published by A. Fassas *et al.* in the Journal of Neurology 2002; 249:1088-97; however, because of the multicenter origin of patients in that analysis, it is not possible to draw any conclusion on the clinical outcome of our subset of patients.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Athanasios Fassas, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Fassas and the Editors. Manuscript received on September 4, 2002; accepted January 23, 2003.

In the following paragraphs, Dr. Fassas summarizes the peer-review process and its outcomes.

What is already known on this topic

High-dose immunosuppression and autologous stem cell transplantation is a feasible therapy for severe forms of multiple sclerosis. It has a prominent anti-inflammatory effect in the CNS, which is evident on MRI and is superior to the effect of any other immunosuppressive or immunomodulating therapy. Disease forms with inflammatory rather than neurodegenerative lesions are likely to benefit. The clinical superiority of transplantation remains to be demonstrated in comparative trials, in view, also, of a small therapy-associated risk of death.

What this study adds

Transplantation for multiple sclerosis is an investigational therapy and a controversial issue. Centers usually treat few numbers of patients in a phase-I trial context. The series of 15 patients presented in this work can be regarded as one of the large existing series of patients and shows that, if properly selected, patients can be treated with an intensive regimen without mortality. Patients with the relapsing-remitting form of the disease are, too, included in this study and appear to experience fewer relapses after transplantation.

Caveats

It is notoriously difficult to show that a therapy is clinically efficacious in multiple sclerosis, i.e. it can prevent or halt progression of disability, and this phase I-II trial is no exception. In a previous publication, the same group of investigators reported on the MRI results using objective criteria. However, the clinical benefit of transplantation can only be validated in a prospective randomized trial against a standard therapy.