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A Feasibility Study of the Full Outpatient Conduction of Hematopoietic Transplants in Persons with Multiple Sclerosis Employing Autologous Non-Cryopreserved Peripheral Blood Stem Cells

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Keywords
Autografts · Autotransplants · Multiple sclerosis

Abstract
Background: With the goal of achieving immune system reset, autologous hematopoietic stem cell transplantations have been performed in patients with multiple sclerosis (MS). ***Material and Methods:*** Two hundred and eighty-six consecutive patients with MS were autografted in a single center using non-frozen peripheral blood stem cells (PBSCs), on an outpatient basis and conditioning with cyclophosphamide and rituximab. The protocol was registered in ClinicalTrials.gov identifier NCT02674217. ***Results:*** One hundred and ninety-four females and 92 males were included; the median age was 47. All procedures were started on an outpatient basis and only 8 persons needed to be admitted to the hospital during the procedure. In order to obtain at least 1×10^6/kg viable CD34 cells, 1–4 aphereses were performed (median 1). The total number of viable CD34+ cells infused ranged between 1 and 19.2×10^6/kg (median 4.6). Patients recovered above 0.5×10^9/L absolute granulocytes on median day 8 (range 0–12). Two individuals needed red blood cells but none needed platelet transfusions. There were no transplant-related deaths and the 128-month overall survival of the patients is 100%. In 82 persons followed up for 3 or more months, the Expanded Disability Status Scale diminished from a mean of 5.2–4.9, the best results being obtained in relapsing-remitting and primary progressive MS. ***Conclusions:*** It is possible to conduct autotransplants for patients with MS employing non-frozen PBSCs and outpatient conduction. Additional information is needed to assess the efficacy of these procedures in the treatment of patients with MS.

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Background

Multiple sclerosis (MS) is a chronic, inflammatory, debilitating disease that causes the destruction of the central nervous system myelin, with varying degrees of axonal damage. It mainly affects young adults and is twice as common in women than it is in men [1]. Studies published from the 1990s brought animal models and theoretical considerations of hematopoietic stem cell transplantation (HSCT) being useful in the prevention and treatment of autoimmune diseases, with clinical responses in some patients, suggesting that high-dose chemotherapy followed by HSCT rescue could "reset" the immunological changes through the control of autoreactive clones, followed by immunological tolerance after immune reconstitution [2]; this led to the conclusion that HSCT may be a viable therapeutic option for MS [1–6]. Autologous HSCT have been given to patients with MS since 1996 and more than 700 HSCTs have been performed around the world [1–6]. Most patients have been treated in small trials or in multicenter studies. In retrospective analysis, a progression-free survival of more than 5 years after transplant has been observed, the neurological outcomes being considerably more favorable in patients with the relapsing-remitting type and/or those who showed an inflammatory pattern in magnetic resonance imaging during the pre-transplant screening. Reports of good results, particularly in the aggressive forms of MS, reinforce the effectiveness HSCT in MS patients with prominent inflammatory activity. The risk of transplant-related mortality in HSCT for MS was conventionally considered very high but has declined since 2001 to less than 1.3% [2–6]; this may be due to the result of the changes in the conditioning regimens, thus reducing toxicity. Recent data, with more than 700 autologous transplants for MS in Europe, showed an overall survival (OS) of 92% in 5 years and a progression-free survival of 46%, the main cause of mortality and morbidity being the recurrence of the autoimmune disease [2–6]. The consensus provides an indication of HSCT in patients with progressive MS unresponsive to conventional therapy and Expanded Disability Status Scale (EDSS) [1] between 3 and 6. The forms of the disease that might benefit from transplantation are relapsing-remitting, primary or secondary progressive, and the "malignant" form, provided there is evidence of inflammatory activity at the time of transplant indication.

Since 1993, we have engaged in practicing HSCT using novel methods to both decrease the toxicity of the procedures and diminish costs [7–14]; we have done over 800 HSCT for different diseases such as acute leukemia, chronic leukemia, aplastic anemia, myeloma, lymphoma, myelodysplasia, and autoimmune diseases, including MS. Within the subset of autologous HSCT, the salient features of our method is that we conduct them on an outpatient basis [8, 9, 15, 16], we avoid freezing and thawing the hematopoietic cells in order to both increase viability of hematopoietic cells in the graft as well as to reduce costs [8, 9, 15, 16], and we always employ peripheral blood stem cells (PBSCs). All these changes have turned the practice of autografting in our hands in an affordable procedure, which can be offered to individuals living in underprivileged circumstances such as those prevailing in developing countries [17]. Having gained experience autografting hematological malignancies [8, 9, 15], we have enrolled in a program of grafting non-cryopreserved autologous hematopoietic stem cells in patients with MS, employing a modification of the autografting conditioning regimen used in malignant diseases. In this study, we report the results of a feasibility study on how to conduct, on an outpatient basis and using non-frozen PBSCs, autologous hematopoietic stem cell transplants in individuals with MS.

Material and Methods

Patients

All consecutive patients with MS referred to our center for an HSCT between November 2006 and February 2017 were prospectively entered in the study. Individuals with a relapsing-remitting MS (RRMS) course, secondary progressive MS (SPMS), primary progressive MS (PPMS), or progressive relapsing MS were included. Patients should have a Karnofsky performance status [18] above 70% and an EDSS score [1] of 7 or below in the 2 weeks prior to transplantation. None of the patients had received marrow-damaging agents before being included in the study and all had a normal complete blood cell count when the mobilization was started. All patients had a wash-out period of at least 3 months of other immunosuppressive agents. The study was approved by the Ethics Committee of the Clinica RUIZ (*Conbioetica* 21CEI00120130605, Registry No. 13 CEI 21 114 126). All patients signed a consent form after being fully informed about the procedure and possible complications. All patients were included in the analysis. The primary endpoints of the study were OS and hematopoietic recovery. The protocol is registered in ClinicalTrials.gov identifier NCT02674217.

PBSC Mobilization and Apheresis

The PBSC mobilization schedule was done with cyclophosphamide (Cy) and filgrastim (granulocyte colony stimulating factor, G-CSF). Intravenous Cy (50 mg/kg) was delivered in a 120-min period on days –11 and –10. Subcutaneous G-CSF (10 μg/kg/b.i.d.) was delivered on days –9 to –1. Using either a peripheral vein or a Majurkar-type subclavian catheter, the apheresis procedure was

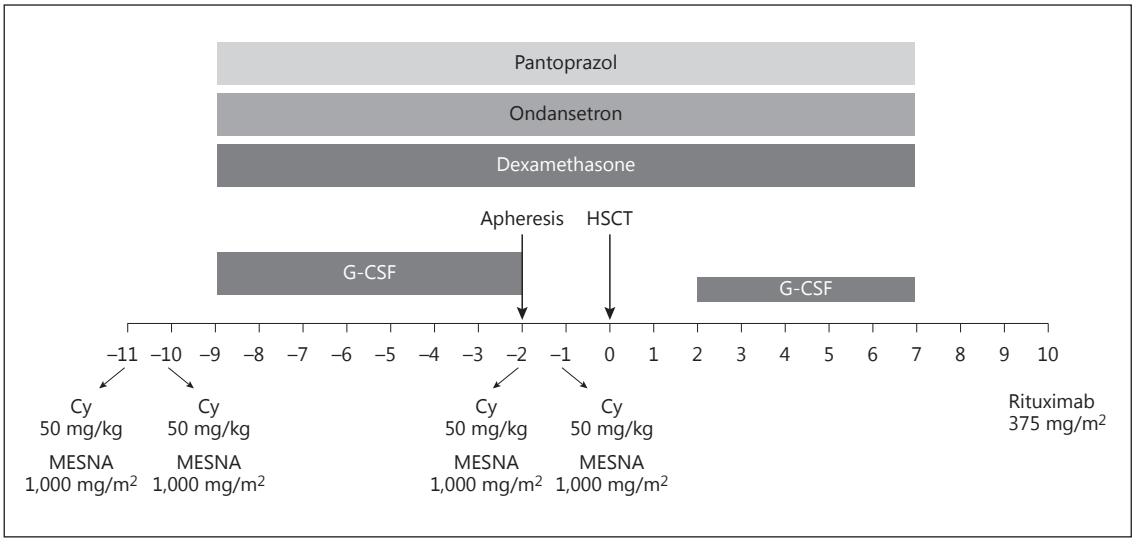


Fig. 1. Details of the "Mexican method" to conduct hematopoietic stem cell transplantation (HSCT) in persons with multiple sclerosis, Cy, cyclophosphamide; G-CSF, granulocyte colony stimulating factor.

performed on day –2, using an Amicus machine (Fresenius Kabi, Deerfield, IL, USA) or a Spectra Optia machine (Terumo BCT, Lakewood, CO, USA) and the Spin-Nebraska protocol [19]. The apheresis objective was to reach at least 1×10^6 viable CD34+ cells/kg. CD34+ cells in peripheral blood were not measured before the apheresis procedures.

Conditioning and Autografting

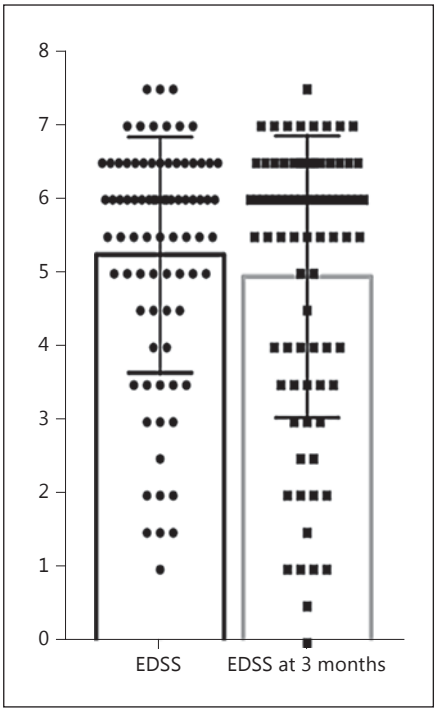
As outpatients and after collecting the targeted number of peripheral blood CD34+ cells, intravenous Cy (50 mg/kg) was delivered along a 120-min period, on days –2 and –1 followed by MESNA (1,000 mg/m^2 along a 180-min period), ondansetron 8 mg, dexamethasone 4 mg, and pantoprazole 40 mg. Figure 1 summarizes these data. After the intravenous Cy, ondansetron (4 mg every 12 h after chemotherapy), oral cotrimoxazole (800/160 mg every 24 h), oral fluconazole (200 mg), and oral acyclovir (400 mg every 12 h) were used in all patients until granulocytes were greater than 0.5×10^9/L; in this period, all patients had laboratory workup and clinical studies every 48 h. After the recovery of the granulocytes, patients were given rituximab (375 mg/m^2 along a 3-h period). As prophylaxis of both infections and MS relapses, in the following 6 months, cotrimoxazole 800/160 mg b.i.d. 3 times a week, acyclovir 800 mg daily and rituximab (100 mg) every 2 months along a 12-month period were recommended. The cumulative dose of Cy is 200 mg/kg. The informed consent decision to be given rituximab despite known John Cunningham virus antigenemia was taken by each individual patient.

Apheresis Product Preservation, Studies, and Infusion

The products of the apheresis and 1 mL aliquots were kept in ACD-A (Baxter Healthcare, Deerfield, IL, USA) at 4°C in 1,000 mL transfer packs (Baxter Healthcare, Deerfield, IL, USA) composed of gas impermeable, polyvinyl chloride plastic film for up to 96 h. Enumeration of the total white mononuclear cells (MNCs) and CD34-positive cells was done by flow-cytometry [20] in an EPICS Gallios apparatus (Coulter Electronics, Hialeah, FL, USA) using phycoerythrin labelled anti-CD34 HPCA-2 monoclonal antibody (Becton Dickinson, San José, CA, USA) and a fluorescence isothiocyanate tagged anti CD45 monoclonal antibody (Beckman Coulter, Hialeah, FL, USA), gating in 7'amino-actinomycin-D-excluding cells. Viability studies of the stored MNC used propidium iodide exclusion and anti-cell antibodies on the flow cytometer. The apheresis products obtained on days –2 and –1 were reinfused to the patients on days 0 and +1, respectively, after keeping them in a conventional blood bank refrigerator (Thermoforma, Marietta, OH, USA).

Results

Between 2006 and 2016, 286 patients with MS were prospectively accrued in the study. There were 194 females and 92 males. The median age was 47 with a range 24–60. The EDSS score of these patients had a median of 5 points (range 1–7). There were 110 RRMS, 62 PPMS, and 114 SPMS. All the autografts were started on an outpatient basis and only 8 persons needed to be admitted to the hospital during the procedure, 3 as a result of persistent nausea and/or vomiting, 3 more to have a chest tube placed to solve a pneumothorax, one after experiencing a minor stroke and one because of neutropenic fever; these patients stayed in the hospital for a maximum of 48 h. In order to obtain a minimum of 1×10^6 viable CD34+ cells/kg 1–4 aphereses were needed (median 1). The total num-



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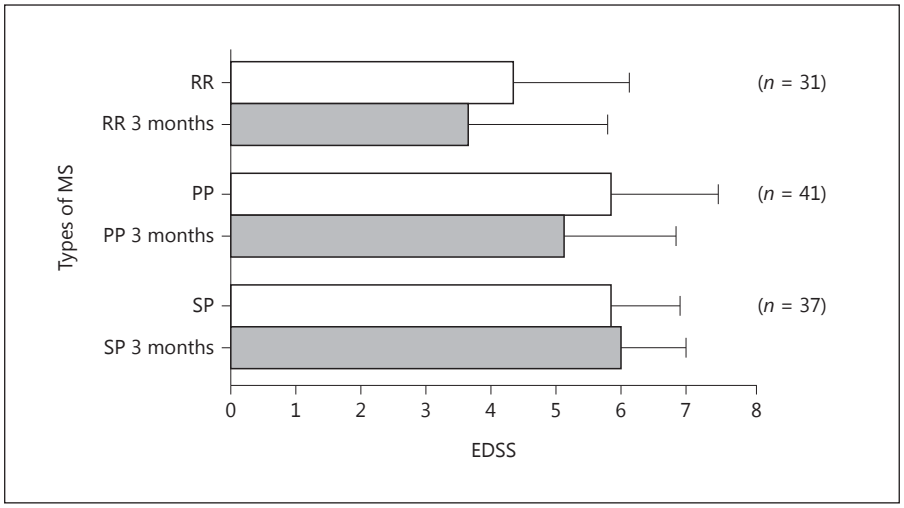

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Fig. 2. Changes in the Expanded Disability Status Scale (EDSS) score 3 months after the autograft in 109 persons followed up for more than 3 months and providing the information.

Fig. 3. Changes in the Expanded Disability Status Scale (EDSS) score 3 months after the autograft in 109 persons, according to the type of multiple sclerosis (MS). RR, relapsing-remitting; PP, primary progressive; SP, secondary progressive.

ber of viable CD34+ cells infused to the patients ranged between 1 and 31.7 × 10^6/kg (median 4.6 × 10^6/kg). A single apheresis procedure was enough to collect at least 1 × 10^6/kg CD34+ cells in 81% of individuals. Patients recovered above 0.5 × 10^9/L absolute granulocytes on median day 8 (range 0–12). The lowest platelet count in the patients ranged between 14 and 282 × 10^9/L (median 102). Two individuals needed transfusions of red blood cells but none required platelets. The 128-month OS of the autografted patients was 100%. No opportunistic infections have been recorded. Of the 286 patients, 155 (54%) could be given the subsequent, low-doses of prophylactic rituximab.

Despite the fact that this is a feasibility and not an efficacy study, in 109 persons, the EDSS was assessed 3 months after the graft; it dropped from a mean of 5.2 to a mean of 4.9. The EDSS score improved in 45 patients (41%), remained stable in 44 (40%), and worsened in 20 (19%); it improved or stabilized in 92% of PPMS, 83% of RRMS, and 70% of SPMS. Figure 2 depicts the changes in EDSS score after the autograft and Figure 3 refers to the changes in the EDSS score according to the variant of MS. Since many patients come from overseas and then do not return for follow-up, the delivery of the subsequent, prophylactic low-doses of rituximab have not been delivered in all individuals (only 54%) and the assessment of the EDSS score has been done by either neurologist of the patients themselves and this is considered a drawback of the study.

Discussion

Autologous HSCT has been employed in the treatment of some forms of MS, even though it is not considered to be the standard treatment of the disease [1–3]. There are still controversies about its indication, which range between total skepticism and opinions about the unethical behavior of neurologists not offering it to patients with MS [4]. The mortality rate of autologous HSCT had been used as an argument against the procedure, but it has decreased substantially as the result of several changes done to the autografting procedures. We and others have shown in hematological malignancies that the mortality rate of the autotransplants can be substantially lowered to figures below 5% [9, 14–17] if the transplant procedures are conducted with PBSC on an outpatient basis and using non-frozen, non-thawed stem cells [7–9]. After gaining expertise since 1993 autografting patients with hematological malignancies, mainly multiple myeloma [15] and lymphomas [21], we embarked on a pilot study to assess the feasibility of conducting auto-

grafts in patients with MS, considering that these individuals had never been exposed to chemotherapy and that in turn, would both mobilize stem cells and recover hematopoiesis more promptly than individuals previously exposed to chemotherapeutic agents. It is important to emphasize that we have used a novel low-intensity conditioning regimen quite different from those recommended in other parts of the world.

Considering that this is a feasibility study, we have not made a detailed assessment of the clinical response in the patients; however, in 68 cases who have been followed up for 5 or more months, a positive response has been observed.

Our results indicate that it is possible to conduct HSCT autografts in patients with MS employing this method, which is endowed with the following salient features: (a) employs PBSC, (b) uses non-frozen/thawed stem cells, and (c) is conducted on an outpatient basis, thus diminishing costs and nosocomial infections and in turn, morbidity and mortality. In the series of 286 patients that we are presenting here, there were no transplant-related deaths, no serious complications, and only 8 instances (2.8%) in which patients were admitted to the hospital for 48 h or less. Additional studies are needed both to confirm the feasibility of the method to autograft individuals with MS and to assess the efficacy of the procedure in the treatment of patients with MS and to compare the results of auto-HSCT with those of other forms of immunosuppression [22]. The objective of publishing this feasibility study is to create awareness about our method and prove that it can be done. Subsequent studies will deal with long-term results and more detailed MS subsets studies.

Note Added in Proof

By May 8, 2017 the number of patients which have been autografted is 354, the results being similar.

Disclosure Statement

The authors declare no conflicts of interest.

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