Research Article

Non-myeloablative Autologous Haematopoietic Stem Cell Transplantation with Consolidation Therapy using Mitoxantrone as a Treatment Option in Multiple Sclerosis Patients

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Abstract

High-dose immunosuppressive therapy with autologous haematopoietic stem cell transplantation (AHSCT) is a new and promising approach to multiple sclerosis (MS) treatment. Recently, the rationale of evolution from myeloablative to non-myeloablative (NM) transplant regimens has been discussed. We aimed to study clinical outcomes in MS patients after NM -AHSCT with consolidation therapy using Mitoxantrone.

55 MS patients were included in this study (mean age - 29.1; male/female - 23/32). Median EDSS at base-line - 4.0 (1.5-8.0), the mean follow-up duration - 26 months (range 9.0 - 50). No transplant related deaths were reported. There were no deaths in the study throughout the follow-up period. The mobilization and transplantation procedures were well tolerated. All the patients responded to the treatment. At long-term follow-up in the group with relapsing-remitting MS improvement was demonstrated in 15 patients (58%) and stabilization in 11 (42%). No relapses throughout the whole follow-up period were found. In the group with progressive MS improvement was achieved in 15 patients (82%) and stabilization in 3 (18%). No active new or enlarging lesions were found according to MRI data.

Thus, NM-AHSCT with consolidation therapy by Mitoxantrone appears to be a safe and effective treatment for MS. The results of our study support the feasibility of NM-AHSCT with consolidation therapy in this patient population.

Introduction

MS is a chronic inflammatory disorder of the CNS caused by autoimmune reactivity of T cells towards CNS myelin components. MS progression inevitably leads to the loss of motor function, sensitive disturbances and cognitive impairment because of the immune-mediated demyelination and axon degeneration [1]. MS is one of the most common neurological disorders, which mainly affects young adults, and causes gradual decrease of their quality of life. Ten years after onset about 50% of patients have a chronic progressive course [2, 3] with this proportion increasing to 70 % after 15 years from disease onset and to 85 % after 25 years [4].

Conventional disease-modifying treatments do not provide satisfactory control of MS due to their inability to eradicate self-specific T cell clones. Immunosuppressive treatments, which are frequently used as second-line therapy, have also only partial beneficial effects [4,5]. Recently, high-dose immunosuppressive therapy (HDIT) with AHSCT was proposed as a new and promising therapy for MS patients [6-8]. The rationale for this method is that ablation of aberrant immune system followed by reconstitution of the new immune system from haematopoietic stem cells may alter the characteristics of the T-cell responses and other immunological properties which may improve the clinical course of MS. By now centers in Europe, North and South America, Russia, China, Israel and Australia have the experience of use of HDIT+AHSCT for MS treatment.

Since 1995, several clinical studies have addressed the issue of feasibility and efficacy of HDIT+AHSCT in MS and a certain clinical benefit has been shown [9-14]. Fifteen years later, despite the promising clinical results, there is still a number of questions to be clarified before recommending HDIT+AHSCT as a treatment choice for MS patients. First, the selection of conditioning regimen is of much importance as there are concerns that HDIT+AHSCT is accompanied with the risk of mortality and adverse effects.

According to the Guidelines for Autologous Blood and Marrow Stem Cell Transplantation in Multiple Sclerosis [15], the definition of the risk/benefit ratio for such a treatment is perceived as a major issue for the neurological community worldwide [15]. Transplant-related mortality in centers with relatively "large" series was 0-4%; during the last years it did not exceed 1% [16]. The intensity of HDIT and the effectiveness of T-cell purging may have been important factors contributing to increased morbidity and mortality. The analysis of the data in the Autoimmune Disease Working Party (ADWP) registry of the European Group for Blood and Marrow Transplantation (EBMT) has shown that intensive HDIT regimens have been associated with

increased toxicity, including transplant-related mortality [17,18]. Recently, the rationale of evolution from myeloablative to non-myeloablative transplant regimens has been discussed [19]. At the same time there are considerations that the intensity of conditioning may be associated with a sustained long-term response and control of disease activity [20]. In connection with this, consolidation therapy sounds reasonable in case of non-myeloablative transplant regimen. As far as Mitoxantrone exerts cardiotoxicity and bone marrow toxicity, and is accompanied with the risk of secondary leukemia [21], the total lifetime dose chosen for our study is at least twice less than the recommended upper limit 140 mg/ m^2 used for MS treatment. Mitoxantrone in the total dose of 60-72 mg/m^2 within the first year after transplantation might be considered as a good option.

At present the most promising results of HDIT+AHSCT have been obtained in MS patients with BEAM as a conditioning regimen [7,12,13,22-25]. Thus, we aimed to study if reduced-intensity BEAM with consolidation therapy using Mitoxantrone is a safe and effective treatment approach for MS.

Another important issue to be considered is the patient selection criteria for HDIT+AHSCT. Moreover, the timing for transplantation is still unclear. In spite of some evidence that primary progressive (PP) MS patients are less responsive to HDIT+AHSCT as compared to both secondary progressive (SP) and relapsing-remitting (RR) MS patients information about the outcomes of transplantation in patients with various types of MS is limited. In addition, the majority of patients included in the above-mentioned studies had SP MS, and were severely disabled with an average EDSS score of 6.5. In our study we included patients with different types and stages of MS. We also aimed to test the hypothesis that HDIT+AHSCT is more effective in younger patients at early stages of MS.

To date, we report the results of a prospective open-label single center study with the analysis of the safety and efficacy of NM-AHSCT with reduced-intensity conditioning regimen followed by consolidation therapy in 55 patients with different types and stages of MS.

Patients and Methods

Fifty five patients were treated with NM-AHSCT in the Transplantation Unit, Department of Hematology and Cellular Therapy, National Medical Surgical Center in Moscow from September 2006 to March 2010. The study was conducted according to the principles of the Helsinki Declaration, and was approved by the Institute Research Board and local Ethics Committee before initiation. All patients gave written informed consent. Patients were eligible if they were aged between 18 and 55 years and met the Poser criteria for clinically definite MS [26]. Other criteria for patient selection were: EDSS score 1.5-8.0; normal mental status; absence of severe concomitant diseases, and no treatment with with Interferons or immunosuppressive agents within 3 months before enrollment. Patient characteristics are shown in Table 1. There were 32 RR MS, 13 SP MS, 9 PP MS and 1 progressive relapsing (PR MS) patients. Female/male ratio was 23/32. Age at the time of AHSCT was 17-49 (mean 29.1). The vast majority of patients (84%) were under 35 years old. Median Expanded Disability Status Scale (EDSS) at base-line was 4.0 (range 1.5 – 8.0). Half of the patients (n=27) had EDSS ≤3.5; 36% of patients (n=20) - EDSS from 4.0 to 5.5; 14% of patients (n=8) – EDSS >5.5. MS duration was from 0.5 to 19 years (mean 5.2). The vast majority of patients (78%) with low EDSS scores from 1.5-2.5 (n=23) received standard therapy before NM-AHSCT which was ineffective. The mean follow-up was 26 months (range, 9-50 months).

Neurological assessment using EDSS was performed at baseline,

Characteristics	Value
Number of patients	55
Gender (F/M)	23/32
Age (years), mean (range)	29.1 (17-49)
age under 35 years, number of patients (%)	46 (84%)
Type of MS, number of patients (%):	
SP MS	13 (24%)
PR MS	1 (2%)
PP MS	9 (16%)
RR MS	32 (58%)
MS duration (years), mean (range)	5.2 (0.5-19)
Duration of follow-up (months), mean (range)	26 (9-50)
EDSS before AHSCT, median (range)	4.0 (1.5-8.0)
EDSS ≤3.5, number of patients (%)	27 (50%)
EDSS from 4.0 to 5.5, number of patients (%)	20 (36%)
EDSS >5.5, number of patients (%)	8 (14%)

Table 1: Base-line characteristics of the patient population.

at discharge, at 3, 6, 9, and 12 months after transplantation, every 6 months thereafter up to 48 months, and then at yearly intervals. MRI scans of the brain and spinal cord with gadolinium enhancement were performed at baseline, at 3, 6, 9, and 12 months after transplantation, every 6 months thereafter up to 48 months, and then at yearly intervals.

Hematopoietic stem cells were mobilized with G-CSF at 10 g/kg +/- cyclophosphamide at 4 g/m^2 according to EBMT/EULAR guidelines [27]. The mobilized cells were collected by apherisis, until a yield of at least 2x10^6 per kg CD34+ cells was obtained. The grafts were not manipulated. Reduced-intensity conditioning regimen based on BEAM was used. The conditioning regimen, called BM, included BCNU (300 mg/m^2) or CCNU (200 mg/m^2) and melphalan (50 mg/m^2) on day -1. It was followed by autologous hematopoietic stem cell transplantation (day 0) +/- horse anti-thymocyte globulin (ATG) in dose 30 mg/kg on days 1 and 2 for in vivo T cell-depletion. Five g/kg s.c. of G-CSF were administered from day 5 post-infusion until granulocyte recovery. For infection prophylaxis oral ciprofloxacin and fluconazole were used.

After NM-AHSCT all the patients were administered consolidation therapy using Mitoxantrone. The total dose of Mitoxantrone was 60-72 mg/m^2 within the first year after transplantation. Injections with Mitoxantrone were conducted every 3rd , 4th , 5th month after transplantation, and then three times every 3 months.

Toxicity was evaluated in accordance with the National Cancer Institute common toxicity criteria, version 2. Neutrophil engraftment was defined as the first day after transplantation when the absolute neutrophil count was greater than 500 cells per ml. Platelet engraftment was defined as the first day after transplantation when the platelet count was greater than 20, 000 platelets per ml. without platelet transfusion.

According to the EBMT criteria of response, patients with either steady EDSS scores representing a halt of disease progression or with improved EDSS scores representing subsidence of inflammation in the CNS were regarded as responding to treatment [28]. Improvement in neurological function was defined as a decrease in the EDSS score of at least 0.5 points on two consecutive visits 3 months apart as compared with baseline. Disease stabilization was defined as no changes in EDSS score during follow-up. Disease progression was defined an increase in the EDSS score of 0.5 points or more on a minimum of two occasions that were at least 3 months apart. For RR MS, in addition, number of relapses per year were determined to evaluate treatment outcomes. A decrease in number of relapses per year was defined as clinical improvement; no changes or an increase in number of relapses per year - as worsening. A relapse of MS was defined as an acute deterioration in neurological function that lasted for more than 24 hours without

intercurrent illnesses or another causes for neurological impairment and with objective changes on neurological examination.

Transplant related mortality definition included every death occurring within 100 days from transplantation [12,13].

Outcomes are reported as of October 2010, based on the last follow-up of each patient. Progression-free survival (PFS) was calculated using the Kaplan-Meier method.

Results

Safety

No transplant related deaths were reported among the 55 MS patients, irrespective of their clinical condition at the time of transplant. In addition, there were no deaths in the study within all follow-up. The mobilization and transplantation procedures were well tolerated. Mobilization was successful in all cases, with a median number of 2.1 $x10^6$/kg (range 1.5–5.5 $x10^6$/kg) collected $CD34^+$ cells, and no major clinical adverse events were observed during this phase. Unmanipulated grafts were infused without complications. Engraftment was uneventful, and no signs of an engraftment syndrome were reported. Median of neutropenia with polymorphonuclear leukocytes (PMN) < $0.5x10^9$ was 7 days (range from 4 to 10) and thrombocytopenia with Plt < $50x10^9$ - 7 days (range from 0 to 11).

There were no non-haematological toxicities of grade III severity or greater during transplantation. Early adverse effects following the NM-HDIT+AHSCT were: alopecia in 44 patients (80%), febrile neutropenia without clinical signs of infection in 12 patients (21.8%), hepatic toxicity grade I and II in 18 patients (32.7%), transient neurological dysfunction in 5 patients (9.0%), enteropathy in 5 patients (9.0%), skin allergy in 5 patients (9.0%), stomatitis in 3 patients (5.5%), pneumonia in 1 patient (1,8%).

Late adverse events during the first year after transplantation were as follows: frequent viral respiratory infections in 9 patients (16.4%), herpes simplex in 1 patient (1.8%), dysmenorrhea in 9 (16.4%) patients.

Efficacy

All fifty five patients were included in the clinical outcome analysis. The follow-up period varied from 9 to 50 months (mean follow-up duration - 26 months). All patients responded to the treatment. At 6 months post transplant 30 patients (55%) achieved an objective improvement of neurological symptoms; 25 patients (45%) had disease stabilization (Table 2). In those who improved there were 23 patients with base-line EDSS below 5.5. Among them 13 patients had decrease of EDSS by 0.5 points, and 10 patients – by ≥ 1.0.

The following distribution of patients with relapsing remitting MS according to their clinical response was observed: 16 patients (50%) improved, and other 16 patients (50%) were stable. In the group with progressive MS 14 patients (61%) achieved an objective improvement of neurological symptoms, and 9 patients (39%) had disease stabilization.

Characteristics of outcomes	Number of patients/ Total patients number (%)		
	6 months after transplantation	12 months after transplantation	Follow-up > 12 months after transplantation (mean 26 months)
Improvement	30/55 (55%)	30/44 (68%)	30/44 (68%)
Stabilization	25/55 (45%)	14/44 (32%)	14/44 (32%)
Progression	0/55 (0%)	0/44 (0%)	0/44 (0%)

Table 2: Efficacy of NM-AHSCT with Mitoxantrone consolidation therapy in MS patients.
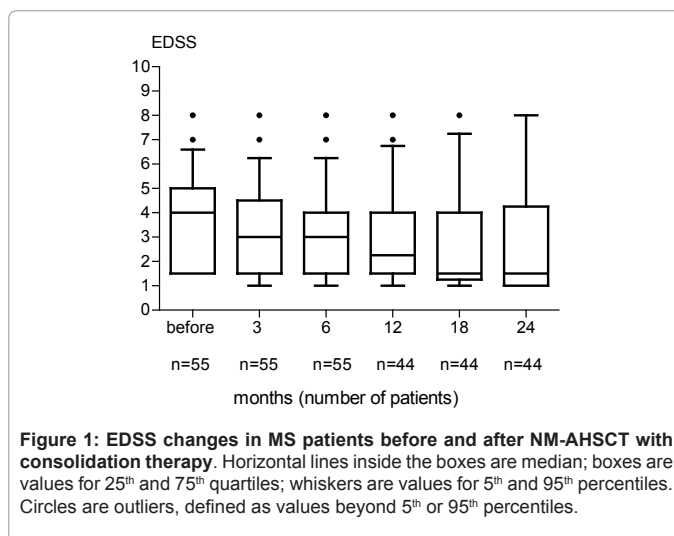

Figure 1: EDSS changes in MS patients before and after NM-AHSCT with consolidation therapy. Horizontal lines inside the boxes are median; boxes are values for 25th and 75th quartiles; whiskers are values for 5th and 95th percentiles. Circles are outliers, defined as values beyond 5th or 95th percentiles.

Forty four patients had follow-up for more than 12 months. Among them 30 patients (68 %) experienced improvement as compared with base-line, and others (32%) had disease stabilization. In the group with improvement there were 21 patients with base-line EDSS below 5.5: 12 patients had decrease of EDSS by 0.5 points, and 9 patients – by ≥ 1.0.

EDSS score changes after NM-AHSCT are reported in Figure 1. As it is seen from the figure, significant decrease of EDSS after NM-AHSCT took place.

In the group with relapsing-remitting MS improvement was demonstrated in 15 patients (58%) and stabilization in 11 (42%). No relapses throughout the whole follow-up period were found. In the group with progressive MS improvement was achieved in 15 (82%) patients, and stabilization - in 3 patients (18%). In addition, patients with PP MS (n=9) either improved or were stable: 8 patients had improvement of neurological symptoms, and one patient had disease stabilization.

Results of MRI scans were available in 54 patients. Thirty nine patients (72%) had active lesions at baseline and all turned to inactive status. No gadolinium-enhancing lesions were found on any of the post-transplantation scans for these patients. In the group of patients without active lesions at base-line no active lesions were observed throughout the whole follow-up.

Thus, overall clinical response at long-term follow-up was observed in all the patients.

Progression-free survival at 4 years after NM-AHSCT was 100%.

Discussion

High-dose immunosuppressive therapy with autologous hematopoietic stem cell transplantation shows promising results in the treatment of severe autoimmune diseases, in particular, multiple sclerosis. Since 1995, more than 700 transplantations in MS patients have been performed worldwide. The results of single-center studies [7,9,13,29,30] and multi-center cooperative studies [14,22,28,31] demonstrated the benefits of HDIT+AHSCT in MS. However, the treatment is associated with a number of side effects and, of major concern is the transplant-related mortality. In respect with this, the choice of conditioning regimen is the crucial issue of HDIT+AHSCT. At present the most promising results of HDIT+AHSCT have been obtained in MS patients with BEAM as a conditioning regimen

[19,32]. BEAM is an intermediate-intensity conditioning regimen, pioneered by Fassas et al. [33]. According to EBMT data from years 2001-2006 mortality in MS patients treated with intermediate-intensity conditioning regimens is 0.9% [16]. Importantly, 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. Therefore, serious concerns of neurological community worldwide that HDIT+AHSCT is accompanied with the risk of mortality and adverse effects, as well as published EBMT data about the cases of mortality in MS patients treated with BEAM conditioning regimens, prompted us to move away from conventional BEAM regimen and implement the reduced-intensity conditioning regimen, based on BEAM, the so called mini-transplantation. As far as there are considerations that the intensity of conditioning may be associated with a sustained long-term response and control of disease activity, consolidation therapy might be reasonable in case of non-myeloablative transplant regimen. In our study we used Mitoxantrone within the first year after transplantation as a consolidation therapy. To note, the total dose of Mitoxantrone was 60-72 mg/m^2 which is at least twice less than the recommended upper limit 140 mg/m^2 [34]. We report the results of NM-AHSCT with consolidation using Mitoxantrone for 55 patients with different types and stages of MS.

The results of safety of NM-AHSCT obtained in our study are encouraging. Among our 55 patients there were no transplant-related deaths. In addition, there were no deaths in our study within overall follow-up. As for the adverse effects, the majorities of them were limited to the post-transplant period and were short-lived. Early adverse events following NM-AHSCT were less pronounced than following intermediate-intensity BEAM regimen [35]. It is also important to note, that there were no severe neurological complications related to NM-AHSCT. Analysis of late adverse effects did not reveal serious cardiotoxicity and bone marrow toxicity that could be expected as a result of Mitoxantrone treatment. In our opinion, no serious adverse effects were observed because the cumulative dose of Mitoxantrone was twice less than the recommended upper limit. These data are consistent with the results of the study by Stuve et al. [36].

The results of our study have also demonstrated the efficacy of NM-AHSCT for MS patients.

All the patients responded to the treatment. The majority of patients achieved clinical improvement, others had disease stabilization. Notably, there were no relapses in the patients with RR MS. At 4 years follow-up, progression-free survival was 100% which exceeds the results of studies with intermediate and high-intensity conditioning regimens [6,10,28].

MRI lesions are a major marker of inflammatory activity in the brain tissue. Seventy two percent of patients included in the study had active lesions at baseline and all turned to inactive status. No active new or enlarging lesions were found throughout the whole follow-up.

Thus, these data support the hypothesis of feasibility of consolidation therapy with Mitoxantrone within the first year after NM-AHSCT.

It is worth mentioning that the issues surrounding the patient selection criteria for HDIT+AHSCT are still unclear. The advantage of our study is that we included patients with different types of MS. In spite of some evidence that PP MS patients are less responsive to HDIT+AHSCT as compared to SP MS and RR MS [12], the information about the outcomes of HDIT+AHSCT in patients with various types of MS is limited. According to our results all 9 patients with PPMS included in the study either improved or were stable at long-term post transplantation period. These results might be explained exactly by combination of NM-AHSCT with consolidation therapy with Mitoxantrone. Thus, in our experience, patients with different types of MS might benefit from this treatment.

Another advantage of our study is the performance of transplantation in patients with different stages of MS, including early stages, while most patients in the previous studies had late stages of MS. Moreover, the encouraging results obtained may be partly explained by the fact that the vast majority of the patients in our study were under 35 years old and at early stages of MS.

Thus, the risk/benefit ratio of NM-AHSCT in our population of MS patients is very favorable. The consistency of our clinical and MRI results, together with the persistence of improvement is in favor of the efficacy of NM-AHSCT with consolidation therapy using Mitoxantrone in MS patients. At the same time, long-term follow-up is worthwhile to search for carcinogenic potential Mitoxantrone exposure and evaluate long-term treatment outcomes. Thus, relevance of consolidation therapy with Mitoxantrone is to be validated in further studies and confirmed by means of a randomized controlled trial.

Overall, the results of our study support the feasibility of NM-AHSCT with consolidation therapy using Mitoxantrone in MS patients. Multicentre cooperative studies should be done for optimization treatment protocol of NM-AHSCT in MS patients.

Acknowledgements

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