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Research Article

Treatment Outcomes and Factors Associated with the Success of Autologous Hematopoietic Stem Cell Transplantation (AHSCT) in Multiple Sclerosis

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Abstract

AHSCT is highly effective and promising treatment option for MS. We evaluated clinical outcomes and associated factors in patients with MS undergoing non-myeloablative AHSCT. Patients with various types of MS who underwent AHSCT from 2006 to 2023 were enrolled in a single-center study. Three non-myeloablative conditioning regimens were used - BEAM-based with ATG or Rituximab (25%), Cyclophosphamide with Rituximab (62%), and Fludarabine + Cyclophosphamide with Rituximab (13%). For assessment of clinical outcomes EDSS evaluation and MRI were performed, for QoL assessment SF-36 was used at baseline and at different time points after AHSCT. Totally, 898 patients with relapsing-remitting (RRMS, n = 477, 53%), primary progressive (PPMS, n = 141, 16%), and secondary progressive MS (SPMS, n = 280, 31%) were enrolled. Median age - 40 yrs, 40% were males. Mean (SD) disease duration - 6.8 (5.9) yrs. Median baseline EDSS - 4 (range 1.5-8.5). There were 6 cases of TRM: RRMS - 3, SPMS - 2, PPMS - 1; Cyclophosphamide was used in all cases. Toxicity profile was the best for Fludarabine-based conditioning. Median follow-up after AHSCT was 30 months (Q1; Q3 - 12; 54). EDSS improvement for the entire group at all-time intervals after transplantation as compared with baseline was observed ($p < 0.001$). At 12 months after AHSCT the decrease of EDSS in RRMS was from median 3.0 to 1.5, in SPMS - from 6.0 to 5.0, and in PPMS - from 5.5 to 4.5. The estimated 5-years EFS probability for the entire group was 83.6%, EFS for RRMS - 87.3%, EFS for SPMS - 80.4%, and for PPMS - 77.6%. No differences in EFS depending on conditioning regimen were found. Factors associated with AHSCT failure were - age ≥ 40, progressive MS, baseline EDSS ≥ 4, previous standard treatment, baseline physical health component score < 40 by SF-36. Three risk groups were identified: in high risk group EFS was worse than in low and in intermediate risk groups (low risk group - 89.7%, intermediate risk group - 79.3%, and high-risk group - 58.2%). AHSCT was accompanied by significant improvement in patient's QoL. QoL dramatically improved by all SF-36 scales at 24 months after AHSCT as compared to baseline and at long term follow-up. The results demonstrate that non-myeloablative AHSCT is effective in patients with different types of MS in terms of clinical response and QoL improvement. The safest non-myeloablative conditioning is Fludarabine-based. The best candidates for AHSCT are patients aged < 40 years, with relapsing MS, treatment-naive, with baseline EDSS < 4, and with baseline physical health summary score ≥40. EFS is better in MS patients of low and intermediate risk than in patients of high risk.

Keywords: Autologous Hematopoietic Stem Cell Transplantation; Low-Intensity Conditioning Regimens; Relapsing-Remitting Multiple Sclerosis; Progressive Multiple Sclerosis; Quality of Life; Prognostic Value

Abbreviations

MS: Multiple Sclerosis; CNS: Central Nervous System; RRMS: Relapsing-Remitting Multiple Sclerosis; SPMS: Secondary Progressive Multiple Sclerosis; PPMS: Primary Progressive Multiple Sclerosis; HDIT: High-Dose Immunosuppressive Therapy; AHSCT: Autologous Hematopoietic Stem Cell Transplantation; DMT: Disease-Modified Treatment; G-CSF: Granulocyte Colony-Stimulating Factor; QoL: Quality of Life; NEDA: No Evidence of Disease Activity; EDSS: Expanded Disability Status Scale; MRI: Magnetic Resonance Imaging; TRM: Transplantation-Related Mortality; RFS: Relapse-Free Survival; PFS: Progression-Free Survival; EFS: Event-Free Survival; ANOVA: Analysis of Variances; PF: Physical Functioning; RPF: Role Physical Functioning; BP: Bodily Pain; GH: General Health; V: Vitality; SF: Social Functioning; REF: Role Emotional Functioning; MH: Mental Health; ATG: Antithymocyte Globulin, BEAM-based: Conditioning Regimens Based on BEAM Included Mini-BEAM and BM; R-Cph: Conditioning Regimens Based on Cyclophosphamide; R-Flu/Cph: Conditioning Regimens Based Fludarabine, Cyclophosphamide and Rituximab; PCS: SF-36 Physical Component Summary Score; MCS: SF-36 Mental Component Summary Score

Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease based on a complex of autoimmune inflammatory and neurodegenerative processes leading to multiple focal and diffuse lesions of the central nervous system, resulting in disability of patients and a significant decrease in their quality of life. There is an increase in the number of MS cases worldwide, which is associated with both improved diagnosis and increased opportunities for pathogenetic and symptomatic therapy, as well as a true increase in the incidence for reasons that are still unclear. According to the publication "Atlas of MS of the International Federation of Patients with Multiple Sclerosis" (MSIF), from 2008 to 2013, the prevalence of MS increased by 10% in 5 years from 30 to 33 cases per 100,000 population. In the absence of adequate modern treatment, after an average of 10 years, up to 50% of patients have difficulties in performing professional duties, after 15 years, more than 50% have difficulties in independent movement, and with a duration of MS of more than 20 years, they have problems in self-care [12,16,20]. MS is the most common immune-mediated neurological disease. There is a polygenic hereditary predisposition to MS. Currently, more than 200 genetic factors have been identified that form this predisposition. The predisposition is realized with the participation of external factors, among which viral infections (especially retroviruses and Epstein-Barr virus), vitamin D deficiency, early onset of smoking, changes in the intestinal microbiome, and other factors are considered in the first place [16,20]. Proinflammatory cytokines produced by T and B cells systemically and locally in the tissue cause activation of autoreactive T lymphocytes lead to autoimmune inflammatory damage to CNS tissue. Neurodegenerative changes are already noted in the early stages of the disease. Activation of clones of sensitized cells, along with a lack of anti-inflammatory and regulatory systems, contributes to the chronization of the process. Secondary activated macrophages and microglia also secrete pro-inflammatory cytokines. Modern disease-modified drugs (DMD) are limited to halt MS due to inability eliminate deeply pathological clones of T and B-cells [13,15]. Term "DMD" means that the disease will be changed, modified, but not cured. Unfortunately, modern medications pursue surrogate goals such as reducing the frequency of exacerbations, decreasing the number of active lesions and reducing the time to disability, but they do not solve the global problem of stopping the disease and preventing irreversible critical disability. Autologous hematopoietic stem cell transplantation (AHSCT) is very promising approach which can stop MS in high percent of patients and prevent further disability. Also, it's very important that after AHSCT, patients stop using any specific immunomodulating and immunosuppressive treatment. Treatment effectiveness in AHSCT is a result of deep immunosuppression or immunoablation due to high-dose chemotherapy as a conditioning regimen. Hematopoietic stem cells are only decrease the length of neutropenia, so AHSCT should be considered as immune-based treatment [1,7,10,30]. Patients' selection for AHSCT is another core issue [23,27]. The careful selection of patients for AHSCT is extremely important, so analyzing the factors that affect the success of a transplant is extremely important**.** The study with the focus on optimization of transplantation procedure for MS and increase its safety and effectiveness is worthwhile. Also, the identification of factors influencing the success of treatment could make it

possible to unify the criteria for selecting patients in daily clinical practice and facilitate decision-making by physicians for prescribing this treatment. In the future, wider implementation of AHSCT as an effective treatment for MS will help thousands of patients, especially if standard therapy is ineffective or the disease is aggressive. Preventing disability of people of working age is of great social and economic importance.

In this paper we aimed to study the long-term outcomes of AHSCT with reduced intensity regimens in patients with various types of MS and to identify factors associated with success of transplantation. In this study we report both clinical and patient-reported outcomes at long-term follow-up after AHSCT.

Materials and Methods

All the patients underwent AHSCT in the Transplantation Unit, Department of Hematology and Cellular Therapy, Pirogov's National Medical and Surgical Center in Moscow from October 2006 to December 2023. The follow-up period lasted till October 2024. The study was conducted according to the principles of the Helsinki Declaration, and was approved by the Institute Research Board and local Ethics Committee of Pirogov's National Medical and Surgical Center before initiation. All patients gave written informed consent. Patients were eligible if they were aged > 18 years old and met the McDonald criteria for clinically definite MS [16,28]. Other criteria for patient selection were: normal mental status, absence of severe concomitant diseases and signed informed consent to participate in the study. Acute infection, psychiatric disorders, severe concomitant pathology, and refusal to participate in the study were exclusion criteria. The vast majority of patients were refractory to 2-4 different variants of conventional treatment including interferons, copaxone, mitoxantrone, cladribine, monoclonal antibodies therapy, azathioprine, intravenous immunoglobulin, steroids and other. The study was approved by the Scientific Council of the Pirogov Center and the Local Ethics Committee (Protocol №15 from September 21, 2005).

Hematopoietic stem cells were mobilized with granulocyte colony-stimulating factor (G-CSF) 10 mg/kg with steroids (Methylprednisolone 500 mg) during 4-5 days. The mobilized cells were collected by apheresis after 4 days of stimulation until a yield of at least 2.0 x 10^6/kg CD34+ cells.

Three low-intensity regimens were applied. The first one was based on BEAM (BEAM-based, 2005 - 2009 - 25% of patients): BCNU 300 mg/m^2 D-6, Etoposide 100 mg/m^2 D-5 - D-2, Ara-C 200 mg/m^2 D-5 - D-2, Melphalan 100 mg/m^2 D-1 +/- horse ATG (ATGAM) 30 mg/kg on D+1 - D+2 or Rituximab 500 mg/m^2 - D+8.

The second regimen was based on Cyclophosphamide (R-Cph, 2009 - till now, 62% of patients): Cyclophosphamide 200 mg/kg (50 mg/kg, D-5-D-2) and Rituximab 500 mg/m^2 - D+8.

The last regimen was our new program based on Fludarabine (R-Flu/Cph, 2020 - till now, 13% of patients): Fludarabine 150 mg/m^2 (25 mg/m^2, D-6-D-1), Cyclophosphamide 100 mg/kg (50 mg/kg, D-6, D-5) and Rituximab 1000 mg on D+8. This program was developed to optimize AHSCT safety and effectiveness, to decrease length of neutropenia and risk of infections and cardiotoxicity [3,19].

Five micrograms per kilogram G-CSF were administered from D+1-D+2 till granulocyte recovery. For infection prophylaxis oral levofloxacin, fluconazole, co-trimoxazole and acyclovir were used.

Toxicity was evaluated in accordance with National Cancer Institute Common Toxicity Criteria (version 2). Neutrophil engraftment was defined as the first day after transplantation when absolute neutrophil count was > 500 cells/mL. Platelet engraftment was defined as the first day after transplantation when the platelet count was > 20 000 platelets/mL without platelet transfusion. Transplantation-related mortality (TRM) definition included every death occurring within 100 days of transplantation [10].

The primary end-point was disability defined by the EDSS score [20]. Other study end-points included event-free survival (EFS), proportion of patients who achieved NEDA-3 (NEDA - no evidence of disease activity), safety, and quality of life (QoL) changes. EFS was considered as relapse-free survival (no acute relapses) for RRMS (RFS), and as progression-free survival for SPMS and PPMS (PFS). NEDA was referred broadly to stabilization of disease as evidenced by lack of clinical relapses, lack of disease progression measured by expanded disability status scale (EDSS) and absence of new disease activity (new T2 lesions/enhancing lesion) on magnetic resonance imaging (MRI) over a period of observation

[12,20]. For clinical outcomes neurological assessment and MRI scans were performed. Neurological assessment using EDSS was performed at baseline, at discharge, at 3, 6, and 12 months after transplantation, every 6 months thereafter up to 48 months, and then at yearly intervals. A decrease of 1.0 or greater was considered significant improvement and an increase of 1.0 or greater is considered significant worsening. MRI scans of the brain and cervical spinal cord with gadolinium enhancement were performed at baseline, at 3, 6, and 12 months after transplantation, every 6 months thereafter up to 48 months, and then at yearly intervals. QoL was assessed using RAND SF-36. SF-36 is generic tool for QoL assessment widely used in patients with chronic diseases, including MS [9,24]. Measurements were conducted before AHSCT, at 6 and 12 months after AHSCT, then every 6 months during 2 years after AHSCT and every 12 months after 2 years during 5 years after AHSCT. QoL was analyzed by eight scales as well as by two summary components of health - physical component summary score (PCS) and mental component summary score (MCS).

For the initial study no sample size was calculated as it aimed to monitor outcomes of AHSCT at early and long-term follow-up in a real-world setting. The data collected within this study from 2006 till now allow us to conduct different types of analysis and highlight important research and practical issues for AHSCT in MS.

Descriptive statistics were presented as numbers and percentages (n, %) for categorical variables or as means (SD), medians (Q1; Q3), ranges (min-max) for continuous variables. For comparisons paired t-test, Wilcoxon test and ANOVA were used. Univariate regression analysis was performed to identify the risk factors for poor efficacy of AHSCT. The univariate logistic regression analysis was performed according to basic demographic characteristics, clinical and patient-reported characteristics (independent variables), with summary components of health (PCS and MCS) as dependent variables. The odds ratio (OR) with a 95% confidence interval (CI) were used to quantify the strengths of associations between variables. EFS in terms of RFS and PFS after AHSCT were evaluated using Kaplan-Meyer method and presented as percentages (%) and 95% CI. For comparison of survival log-rank criterion and Tarone-Ware criterion were applied. P values of less than 0.05 were used as a cut-off point for statistical significance and all statistical tests were two-sided. Medcalc v.19.0.5 and IBM SPSS 23.0 software's were applied.

Results

In total, 898 patients with MS were enrolled in to the study; 477 RRMS (53%), 280 SPMS (31%), and 141 PPMS (16%). Mean age - 40.0 years old; male/female - 359/539 (40%/60%). Median EDSS before transplantation was 4.0 (range 1.5-8.5); mean duration of the disease was 6.8 (5.9) years. In the group with RRMS median EDSS before transplantation was 3.0 (range, 1.5-4.5), in SPMS - 6.0 (range, 4.5-6.5), in PPMS - 5.5 (4.0-6.0). In the total sample 63.3% had active lesions at baseline.

Safety

Transplantation procedure was well tolerated in vast majority of patients.

Mobilization was successful in all cases with median number of 2.1 x 10^6/kg (range 2-10.9 x 10^6/kg) collected CD34+cells; no major clinical adverse events were observed during this phase.

Median duration of neutropenia was 10 days in R-Cph group and 8 days for BEAM-based group. Duration of neutropenia was much less in R-Flu/Cph group (from 3 to 10 days, median - 6 days). The median duration of thrombocytopenia was 7 and 8 days in R-Cph and BEAM-based groups and 4 days in R-Flu/Cph group. Platelet transfusion received 63% of patients in R-Cph group, 23.3% patients in BEAM-based group and only 8.2% of patients in R-Flu/Cph group. Anemia grade I-II was observed in 82.0% in BEAM/BEAM-based group and 90% of patients in R-Cph and R-Flu/Cph groups.

Oral mucositis was observed in 26% patients in R-Cph+R group (Grade II - 38.5 %), in 4.3% in BEAM-based group (grade 2 - 38.5%) and in 4% of patients in R-Flu/Cph group (only grade I - 100 %). Grade 2 enteropathy was observed in 19% of patients in BEAM-based group and in 16% in R-Cph group to compare with 11% in R-Flu/Cph group (mainly grade I).

The incidence of infectious complications was higher in BEAM-based and R-Cph groups - 27% and 26% of patients to compare with R-Flu/Cph group - 11.4% of patients. Bacterial infection rate was much less in R-Flu/Cph group (10.0%) to compare with BEAM-based and R-Cph groups (15.0 and 26.0% correspondingly). Hepatic toxicity in R-Flu/Cph group had 10% of patients (grade I and II). In R-Cph and BEAM-based group hepatic toxicity was observed in 18% and 35.9% of patients correspondingly (grade II).

TRM in whole group was 0.67%. Tragically 6 patients died, the main reason was sepsis (sepsis - 4, cardiotoxicity - 1, related to corticosteroid induced psychosis and self-harm - 1). Median EDSS in these patients was 4.5; MS variants - RRMS-3, SPMS-2, PPMS-1. Cyclophosphamide was used in all cases (TRM - 1.07% in this group and 0% in BEAM-based and R-Flu/Cph groups).

Toxicity of AHSCT in MS patients (BEAM-based vs R-Cph vs R-Flu/Cph) is shown in table 1.

	BEAM-based (Conditioning regimens based on BEAM included mini-BEAM and BM)	R-Cph (Conditioning regimens based on Cyclophosphamide)	R-Flu/Cph (Conditioning regimens based Fludarabine, Cyclophosphamide and Rituximab)
Febrile neutropenia (%)	27.0	26.0	11.4
Bacterial infection (%)	15.0	26.0	10.0
Hepatic toxicity (%)	35.9	18.0	10.0
	(grade 1-2)	(grade 1-2)	(grade 1)
Enteropathy (%)	19	16	11
	(grade 2)	(grade 2)	(grade 1)
Oral mucositis (%)	4.3	26.0	4.0
	(grade 2 - 38.5%)	(grade 2 - 38.5%)	(grade 1)
Duration of neutropenia (median, days, PMN < 0.5 x 10*9/L)	8.0	10.0	6.0
Duration of thrombocytopenia (median, days, Plt < 50 x 10*9/L)	8.0	7.0	4.0
Platelets transfusion (%)	23.3	63.0	8.2
Anemia (grade 1-2, %)	82.0	90.0	90.0
TRM (%)	0	1.1	0

Table 1: Toxicity profile for different conditioning regimens.

PMN: Polymorphonuclear Leukocytes; Plt: Platelets

Finally, R-Flu/Cph conditioning program was much less toxic as compared to BEAM-based and R-Cph conditioning and was accompanied with less rate of neutropenia, less rate of thrombocytopenia and less need of transfusions. Also, infection complications and organ toxicity were less in R-Flu/Cph group.

Efficacy

Median follow-up after AHSCT was 30.0 months (Q1; Q3 - 12; 54). Significant improvement in EDSS for RRMS, SPMS and PPMS at all-time intervals after transplantation as compared with baseline was observed (Figure 1 a-c). The decrease of EDSS in RRMS took place from median 3.0 to 1.5 at 12 months and at long term follow-up after AHSCT. In SPMS EDSS decreased from median 6.0 to 5.0 at 12 months after AHSCT and exhibited further improvement to 4.0 at long term follow-up. In PPMS the decrease of EDSS took place from median 5.5 to 4.5 at 12 months after AHSCT and exhibited further improvement to 4.0 at long term follow-up.

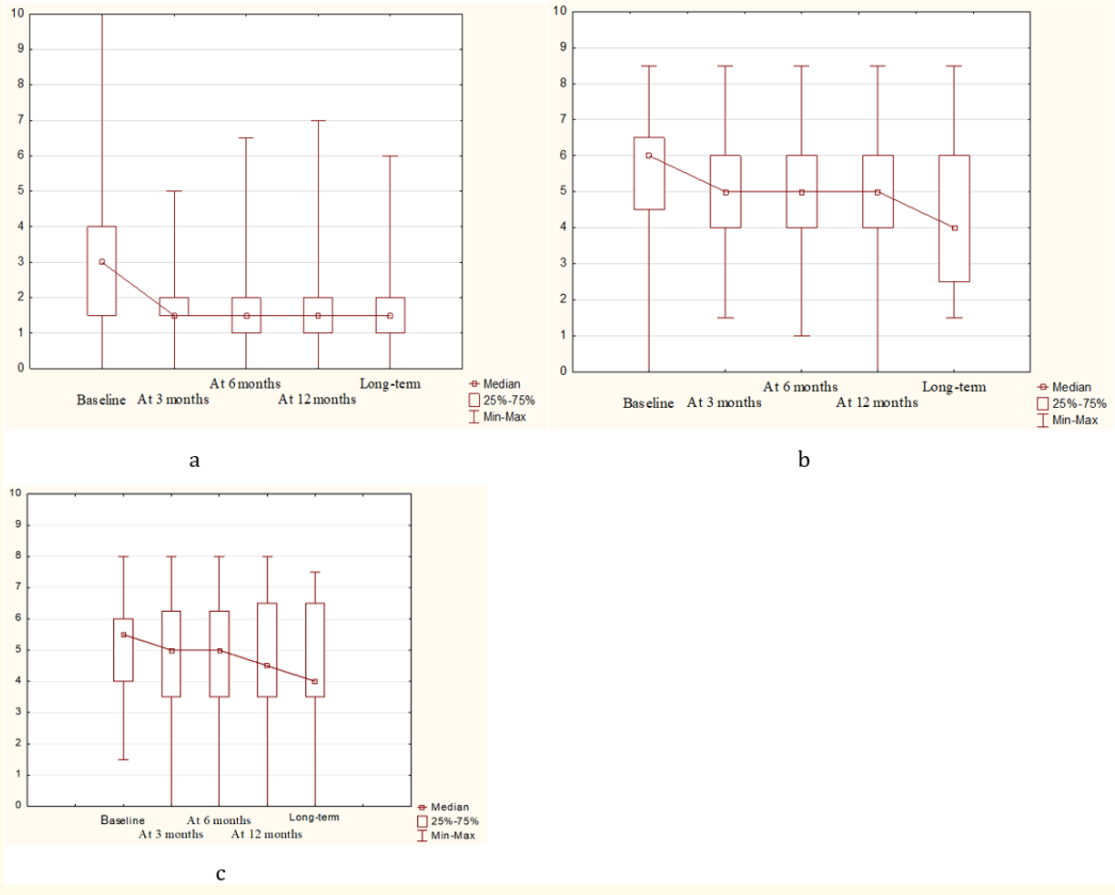

Figure 1: EDSS medians in patients with RRMS (a), SPMS (b) and PPMS (c) before and at different time-points after AHSCT. RRMS: Relapsing-Remitting Multiple Sclerosis; SPMS: Secondary Progressive Multiple Sclerosis; PPMS: Primary Progressive Multiple Sclerosis

The estimated 5-years EFS for the entire group was 83.6% (95% CI 79.8-87.4). Differences in EFS were found depending on MS type: RRMS 87.3% [95%CI 82.3-92.4] vs SPMS 80.4% [95%CI 73.3-87.4], log-rank, $p < 0.001$; RRMS 87.3% [95%CI 82.3-92.4] vs PPMS 77.6% [95%CI 67.7-87.6], log-rank, $p = 0.002$ (Figure 2).

Noteworthy, no active, new or enlarging lesions were registered in RRMS patients who were relapse free and in SPMS and PPMS without disease progression. At follow-up of 12 months after transplantation, 244 (94.6%) RRMS patients and 137 (85.6%) SPMS patients were without relapse or progression free. Many of them had MRI improvement and stabilization. All patients with clinical response to AHSCT at 12 months didn't have new and active lesions on MRI which corresponds to achievement of NEDA-3.

Separate analysis for EFS in the groups of patients with different conditioning regimens was performed. Comparison was made between the conditioning regimens based on BEAM,

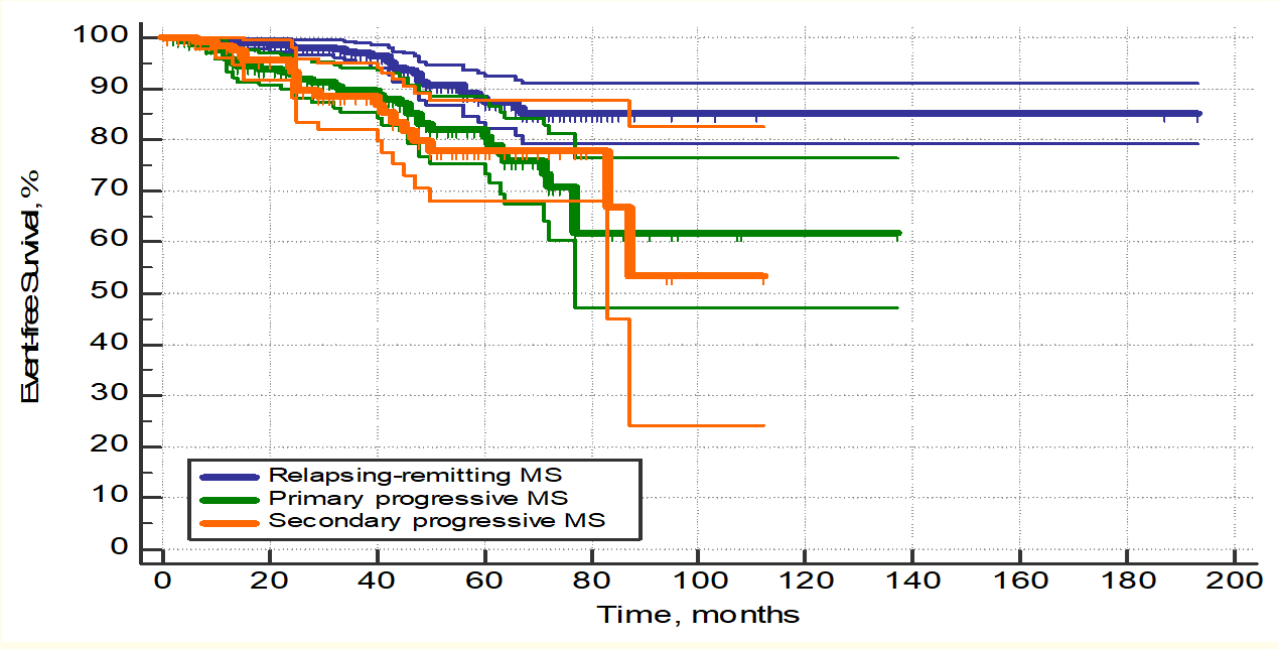

Figure 2: Event-free survival Kaplan-Meyer curves in MS patients depending on MS type.

Cyclophosphamide+Rituximab and R-Flu/Cph. Previously it was shown that the outcomes for mini-BEAM and BM were similar [17], thus the group BEAM-based included mini-BEAM and BM conditioning regimens. No differences in EFS for RRMS, SPMS, PPMS were found between patients who received BEAM-based and those who received Cyclophosphamide+Rituximab and R-Flu/Cph (log-rank, p = 0.515), Figure 3.

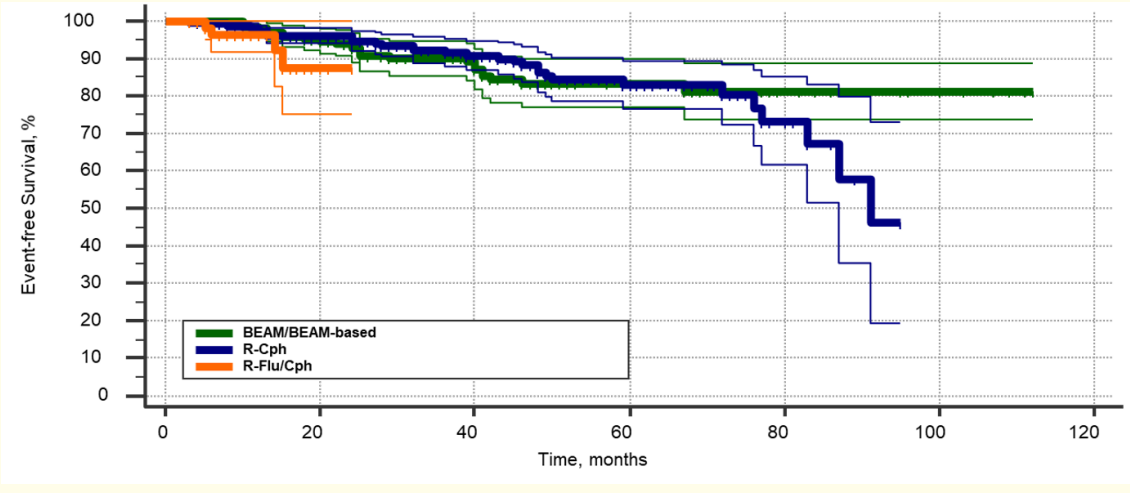

Figure 3: Event-free survival Kaplan-Meyer curves in MS patients who received BEAM-based, Cyclophosphamide+Rituximab and R-Flu/Cph.

BEAM-based: conditioning regimens based on BEAM included mini-BEAM and BM; R-Cph: conditioning regimens based on Cyclophosphamide; R-Flu/Cph: conditioning regimens based Fludarabine, Cyclophosphamide and Rituximab.

Further analysis of EFS was performed for the subgroups of patients with different age, EDSS and disease duration. EFS Kaplan-Meyer curves for patients younger 40 years old and aged ≥40 years old are presented in Figure 4 a. The estimated 5-years EFS was 85.1% for patients younger 40 years vs 81.3% aged ≥40 years old (log-rank, p = 0.06, Breslow, p = 0.043, Tarone-Ware, p = 0.054). The differences in EFS were also statistically significant between patients with disease duration less 10 years (89.5%) and disease duration ≥ 10 years (78.5%), log-rank, p = 0.028 (Figure 4 b). The estimated 5-years EFS probability depending on previous treatment by disease modifying therapies (DMT): 89.2% in treatment naïve patient's vs 78.6% in patients who received DMT (log-rank, p = 0.085), Figure 4 c. The estimated 5-years EFS probability depending on the time of transplantation (early, conventional or salvage AHSCT): 92.6% patients after early AHSCT vs 77.7% after conventional AHSCT vs 53.3% after salvage AHSCT (log-rank, p < 0.001), Figure 4, d.

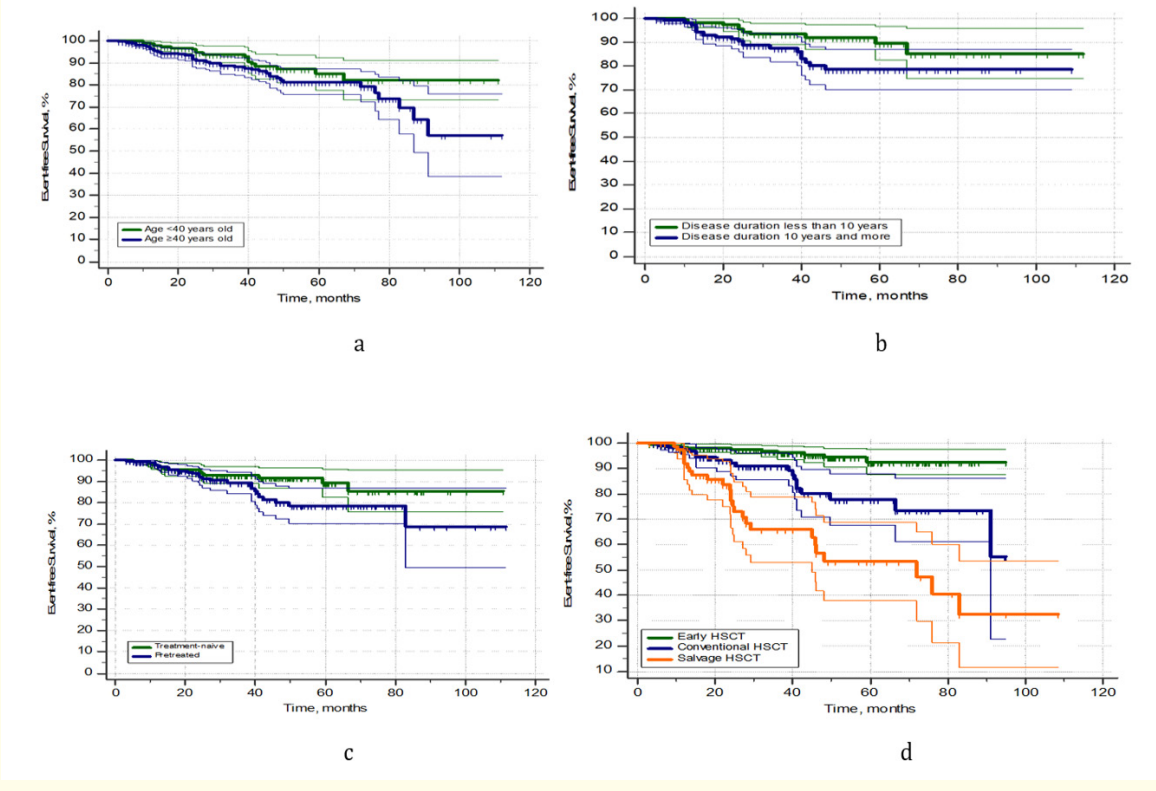

Figure 4: EFS Kaplan-Meyer curves for different groups of patients: patients aged < 40 years and patients aged ≥ 40 years (a); patients with disease duration < 10 years and disease duration ≥ 10 years (b); treatment naïve patients vs patients who received DMT before AHSCT (c); patients who received early, conventional or salvage AHSCT (d).

Early AHSCT: EDSS 1.0-3.5 + unfavorable factors; conventional AHSCT: EDSS 4.0-6.5 +/- noneffective DMT +/- unfavorable factors; salvage AHSCT: EDSS > 6.5 + high disease activity [33,35].

Early AHSCT: EDSS 1.0-3.5 + unfavorable factors; conventional AHSCT: EDSS 4.0-6.5 +/- noneffective DMT +/- unfavorable factors; salvage AHSCT: EDSS > 6.5 + high disease activity.

Quality of life

AHSCT was accompanied by significant improvement in patients QoL. QoL dramatically improved by all SF-36 scales at 24 months after AHSCT as compared to baseline and at long term follow-up: PF - 40.15 vs 64.17 ($p < 0.001$), RPF - 35.17 vs 62.04 ($p < 0.001$), BP - 71.49 vs 81.28 ($p = 0.001$), GH - 54.83 vs 64.42 ($p < 0.001$), V - 45.88 vs 58.69 ($p < 0.001$), SF - 61.71 vs 80.28 ($p < 0.001$), REF - 64.65 vs 86.26 ($p = 0.001$), MH - 67.64 vs 74.84 ($p < 0.001$). Physical health by PCS (37.50 vs 42.60; $p < 0.001$) as well as mental health by MCS (46.67 vs 51.27; $p < 0.001$) improved significantly post-transplant. Improved QoL was preserved during the entire period of follow-up ($p \leq 0.001$).

QoL profiles in MS patients in entire group at baseline and at different time periods after AHSCT are presented in Figure 5, respectively.

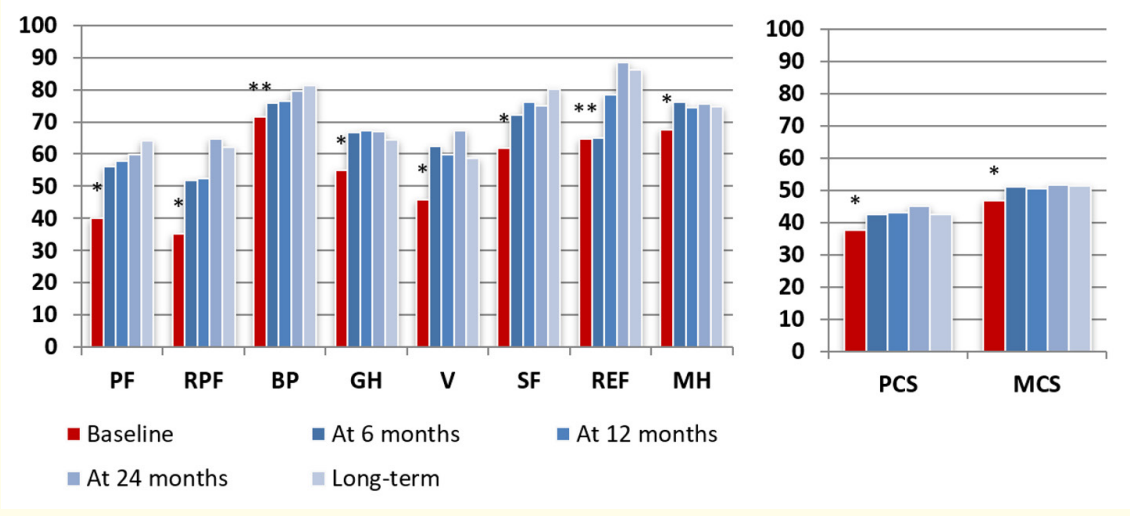

Figure 5: QoL profiles in MS patients at baseline and at 24 months and at long term follow-up after AHSCT.
SF-36 scales: physical functioning (PF), role physical functioning (RPF), bodily pain (BP), general health (GH), vitality (V), social functioning (SF), role emotional functioning (REF), mental health (MH); physical component summary score (PCS), mental component summary score (MCS); *: Statistically significant at $p < 0.001$; **: Statistically significant at $p = 0.001$.

Factors associated with the success of AHSCT

A univariable logistic regression analysis was performed to identify factors associated with poor efficacy of AHSCT. The following factors were analyzed: age, gender, strategy of AHSCT (early, conventional, salvage) based on EDSS, disease course, disease duration before AHSCT, Gd+/Gd- before AHSCT, conditioning regimens, previous DMT treatment and QoL before AHSCT in terms of PCS and MCS.

Age ≥ 40 years (OR 2.34, $p = 0.002$), progressive MS (OR 2.51, $p < 0.001$), pre-treated patients (OR 2.31, $p = 0.031$), conventional/salvage AHSCT (OR 4.73, $p < 0.001$), baseline physical health by PCS < 40 at base-line (OR 1.04, $p = 0.004$) were significantly associated with poor efficacy of AHSCT, while gender, disease activity before AHSCT, disease duration before AHSCT, conditioning regimen, and mental health by MCS < 40 at base-line and were not (Table 2).

Variables	B	Standard error	Wald	*p*	Odds ratio	95% confidence interval
Age ≥40 years	0.85	0.27	9.85	0.002	2.34	1.38-3.99
Female gender	0.29	0.28	1.03	0.310	1.33	0.77-2.32
Progressive MS	0.92	0.25	14.01	< 0.001	2.51	1.55-4.05
Pre-treated patients	0.84	0.39	4.64	0.031	2.31	1.08-4.95
Disease duration before AHSCT < ≥10 years	0.68	0.40	2.93	0.087	1.97	0.91-4.27
Baseline physical health by PCS < 40	1.14	0.44	6.92	0.009	3.14	1.34-7.36
Baseline mental health by MCS < 40	0.36	0.50	0.52	0.470	1.44	0.54-3.84
Conventional/salvage AHSCT (EDSS ≥4.0)	1.56	0.28	3.64	< 0.001	4.73	2.73-8.21
Gd- before AHSCT	0.48	0.25	3.79	0.051	1.61	1.00-2.6
Conditioning regimen*						
R-Cph	0.03	0.29	0.01	0.925	1.03	0.58-1.82
R-Flu/Cph	0.50	0.77	0.42	0.518	1.64	0.37-7.38

Table 2: Results of univariate logistic regression analysis of risk factors of EDSS worsening

Gd: Gadolinium; p: p value for Odd`s ratio. *: Reference category: BEAM-based; Statistically significant at p < 0.05 are highlighted by bold print

Based on these risk factors, patients were divided into three risk groups (Figure 6).

EFS depending on the risk group is presented in Figure 7.

The following differences in EFS were found: high risk group - 58.2% [95%CI 34.9-81.5] vs low risk group - 89.7% [95%CI 79.7-99.7], log-rank, p = 0.002; high risk group - 58.2% [95%CI 34.9-81.5] vs intermediate risk group - 79.3% [95%CI 64.4-94.2], log-rank, p = 0.019; low risk group - 89.7% [95%CI 79.7-99.7] vs intermediate risk group - 79.3% [95%CI 64.4-94.2], log-rank, p = 0.304. In high risk group EFS was worse than in low and in intermediate risk groups 58.2% vs 89.7% and 79.3%.

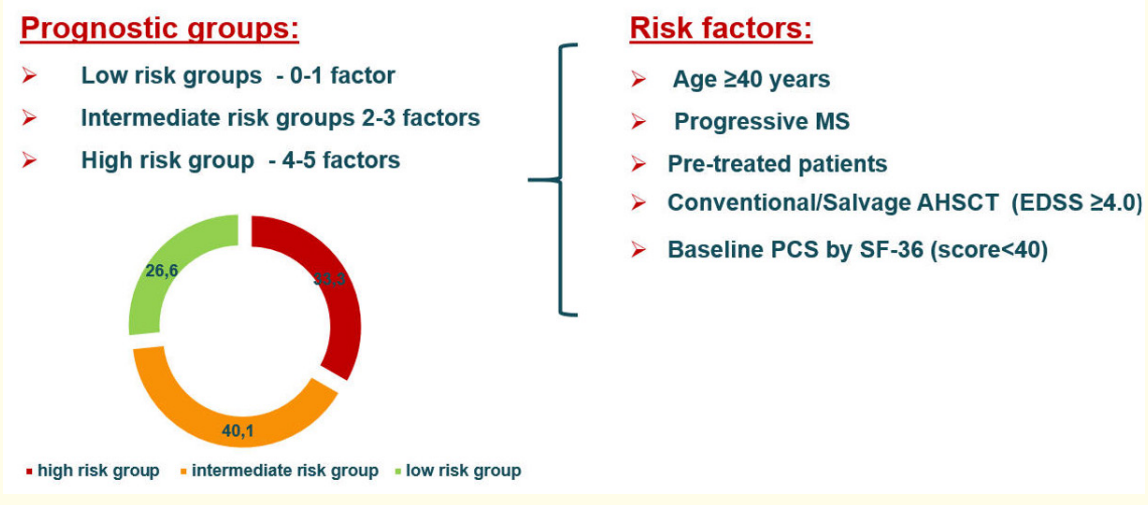

Figure 6: Risk group stratification of MS patients undergoing AHSCT.

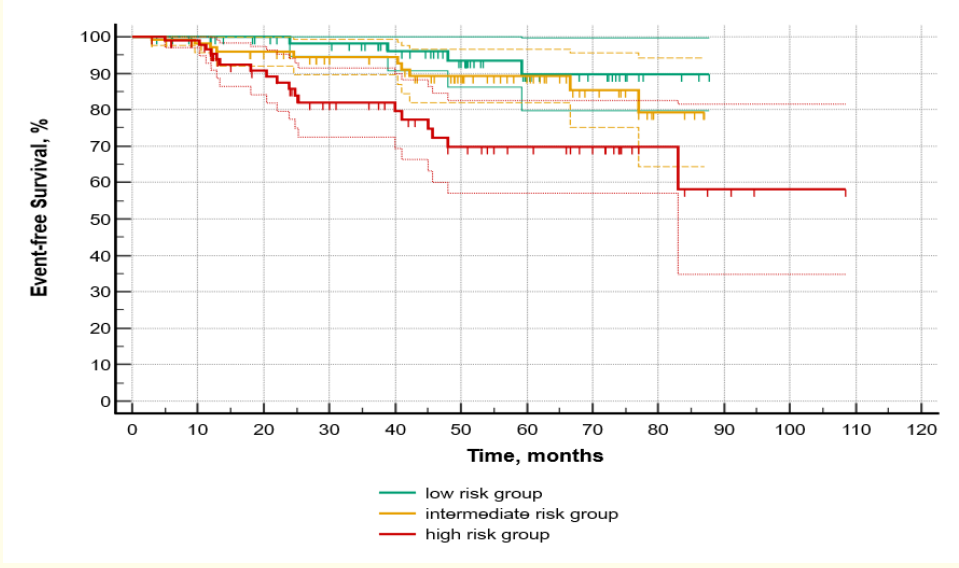

Figure 7: Event-free survival in MS patients depending on the risk group.

Thus, factors associated with success of transplantation are as follows: early AHSCT (EDSS < 4), RRMS, treatment-naive, age < 40 years, good physical health (PH by PCS ≥ 40) before AHSCT.

Discussion

MS is a huge socially significant problem, especially in the young population [25]. Modern conventional treatment has limited effectiveness due to the impossibility of complete elimination of pathological T and B lymphocyte clones. To date, a number of large clinical studies have shown the advantage of AHSCT over the most effective DMD in remitting MS [4,8,22]. Also, AHSCT remains the only method to prevent the transition to a secondary progressive form of MS [6,10,29]. It is important that after transplantation, the patient should not receive specific immunomodulatory/immunosuppressive treatment. Recent studies have also confirmed the pharmacoeconomic benefits of AHSCT in MS [5]. Of course, AHSCT does not negate modern standard treatment, which has its place and indications in MS, and in some cases is more appropriate than transplantation. Therefore, careful selection of patients, determination of indications and contraindications is a very important question, the answer to which determines the place of transplantation in the treatment of MS. A number of studies have also noted the controversial effectiveness of transplantation in progressive forms of MS [2,18,22]. For the first time, we presented unique long-term monitoring data for 898 patients with various MS types, conducted a comparative characteristic of the conditioning regimens, as well developed and tested a prognostic stratification of patients. The determination of factors influencing the outcome of transplantation and the stratification of patients by prognostic groups were carried out in this group of patients for the first time. We have shown that transplantation can be effectively used not only for the remitting course of MS, but also for primary and secondary progressive disease varices. Totally 898 patients with RRMS, PPMS, and SPMS received non-myeloablative AHSCT, using three non-myeloablative conditioning regimens - BEAM-based with ATG or Rituximab (25%), Cyclophosphamide with Rituximab (62%), and Fludarabine + Cyclophosphamide with Rituximab (13%). Median age was 40 yrs, and mean disease duration - 6.8 yrs. We have shown high efficiency of transplantation in both remitting MS and primary and secondary progressive variants. EDSS improvement for the entire group at all-time intervals after transplantation as compared with baseline was observed. At 12 months after AHSCT the decrease of EDSS in RRMS was from median 3.0 to 1.5, in SPMS - from 6.0 to 5.0, and in PPMS - from 6.0 to 4.0. The estimated 5-years EFS probability for the entire group was 83.6%, RFS for RRMS - 87.3%, PFS for

SPMS - 81.9%, and for PPMS - 78.7%. No differences in EFS depending on conditioning regimen were found. Our results obtained are comparable to clinical studies conducted earlier [6,11,14,21,25]. It should be noted that the results of earlier use of myeloablative conditioning programs based on BEAM showed similar results, but were accompanied by greater toxicity and mortality (1,6-5%) with similar efficacy (long-term RFS for RRMS - 71-85%) [10,26]. Nowadays, myeloablative programs aren't commonly used for AHSCT in MS. Lymphoablative programs are much less toxic, on the other hand they are the same effective as myeloablative. In the study of R. Burt et al. 118 patients with remitting MS were transplanted. Cyclophosphamide in a total dose of 250 mg/kg and rabbit ATG was used as a conditioning regimen. The authors did not record any serious complications or deaths. The 2-year disease-free survival rate was 89% and the 4-year - 80%. The median EDSS index dropped from 4.0 to 3.0 during the first year. Subsequently, randomized clinical trials (MIST and others) were conducted to evaluate the effectiveness of a cyclophosphamide-based conditioning regimen at a dose of 200 mg/kg and ATG, as well as a comparison with standard immunosuppressive therapy [5,8,10,22]. There were striking differences in favor of transplantation to compare with the most effective standard treatment within 2 years according to the NEDA criterion (95% vs 20-45%, respectively) [4,5]. However, due to the modern development of the pharmacological industry and the use of modern immunosuppressive drugs, low-dose chemotherapy, and monoclonal antibodies, a thorough analysis of the benefit-risk ratio is required when selecting patients for AHSCT due to a possible increase in the risk of severe complications and mortality. So, as optimization of modern lympoablative protocols, as a more careful patients selection are extremely important [3,22]. In our study, new Fludarabine + Cyclophosphamide with Rituximab (TRM - 0%) compared to the Cyclophosphamide-based program (TRM - 1.1%) and BEAM/BEAM-based (TRM - 0%) was much safer in terms of hematological and organs toxicity. R-Flu/Cph conditioning program was accompanied with less rate of neutropenia, less rate of thrombocytopenia and less need of transfusions. In general, mortality in the general group (0.7%) does not differ from that in large international studies (0 - 1.5%), but the toxicity profile of the Fludarabine-based program was significantly lower to compare with all other conditioning regimens. So, we consider that previously Fludarabine-based conditioning is the most suitable for MS patients. Actually, further studies are needed for more careful assessment new R-Flu/Cph program during longer follow-up and larger sample size.

To optimize patents selection, we are the first who has developed patient's prognostic stratification analyzing factors, associated with effectiveness of AHSCT, including QoL. Among different factors, only age ≥ 40, progressive MS, baseline EDSS ≥ 4, previous standard treatment, baseline physical health < 40 scores by PCS were associated with poorer results of AHSCT. Hereinaftere three risk groups were identified: in high risk group (4-5 factors) EFS was worse than in low (0-1 factor) and in intermediate risk groups (2-3 factors). In the low risk group EFS was 89.7%, at the same time as - intermediate risk group - 79.3% and high risk group - only 58.2% of patients. This stratification can be very important and useful tool to optimal decision making before AHSCT, significantly improving future effectiveness and safety.

Also, AHSCT was accompanied by significant improvement in patient's QoL. QoL significantly improved by all SF-36 scales at 24 months after AHSCT as compared to baseline and at long term follow-up. PCS (37.50 vs 42.60) as well as MCS (46.67 vs 51.27) improved significantly post-transplant. Improved QoL was preserved during the entire period of follow-up. The data obtained are in line with the results of clinical studies which demonstrated QoL improvement after transplantation in patients with remitting MS [6, 24, 28]. We have previously also shown a positive effect of transplantation on QoL of patients with both remitting MS and primary and secondary progressive forms. Improvement of QoL in terms of SF-36 scales was observed at 3-6 months after transplantation and was maintained for 12 months or more. In this study, similar results were obtained on a larger group of patients and over a longer follow-up period [17]. However, this is the first study to provide data on the prognostic value of QoL, in particular physical health, before treatment in terms of 5-year EFS.

The limitations of the study are the lack of randomization between different conditioning regimens and the smaller sample size of patients who received fludarabine-based conditioning. Further research is needed to more accurately determine the prognostic

factors and benefits of separate non-myeloablative conditioning programs.

The results on toxicity and effectiveness of various conditioning programs for AHSCT in patients with MS are practically important, which makes it possible to recommend the introduction of fludarabine-based programs into clinical practice in the near future. The prognostic stratification of patients including both clinical and patients-reported outcomes is of great clinical importance, making it possible to clearly identify indications and contraindications for transplantation in daily clinical practice, and to assess possible risks and benefits of the procedure. The long-term and sustained improvement in QoL demonstrated in the study is of great medical and social importance for MS patients. The use of QoL measures must necessarily be included as an important diagnostic component both before the start of therapy and in the early and late stages after transplantation as well as during rehabilitation.

Conclusion

AHSCT with low-intensity conditioning regimens based on BEAM, Cyclophosphamide and Fludarabine is a safe and effective treatment for patients with both relapsing-remitting and progressive MS. The safest non-myeloablative conditioning is R-Flu/Cph. Event-free survival was better in RRMS (87.3%) than in SPMS (80.4%) and PPMS (77.6%). AHSCT results in long-term improvement of QoL. Factors associated with success of AHSCT are early transplantation (EDSS < 4), RRMS, treatment-naive, age < 40 years, and good physical health before transplantation

Conflict of Interest

Authors declare no conflict of interest.

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