Mobilization Strategies for Autologous Collection and Cryopreservation of Peripheral Blood Stem Cells in Patients with Lymphoproliferative Diseases

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Abstract

Background. Peripheral blood stem cells (PBSC) have largely replaced conventional, unprimed bone marrow (BM) as source for autologous transplantation.

Aim. To present the mobilizing strategies in patients with lymphoproliferative diseases taking in consideration demographic and treatment related variables that have influence as prognostic factors.

Material and Methods. The study was accomplished with patients treated with autologous transplantation at University Hematology Hospital, Skopje, and Republic of Macedonia during 5 year period. A total of 70 patients with lymphoproliferative diseases were included in this study.

Results. Patients transplanted with PBSC, compared with patients transplanted with BM showed p<0.0001 for febrile days, number of microbiological solates, days of hospital stay, engraftment. Analysis of the parameters concerning the mobilization of PBSC in the analyzed group of patients revealed p<0.001 for WBC count before aphaeresis, number of procedures, day of aphaeresis in the G-CSF mobilized group and higher lymphononocyte count in the group mobilized with CT plus G-CSF.

Conclusion. There are a number of factors that have been associated with the likelihood of successful stem cell mobilization. Such prognostic factors can assist clinicians in designing treatment regimens leading up to the transplant procedure and also identifying patients at high risk for failing the mobilization regimen.

Introduction

Mobilized peripheral blood stem cells (PBSC) have largely replaced conventional, unprimed bone marrow (BM) as source during the autologous transplant setting, because of a faster hematopoietic reconstitution (engraftment), less transfusion requirements, less infective complications and earlier hospital discharge (1). Mobilized PBSC have become the main source for autologous stem cell transplantation (ASCT) following myeloablative therapy in patients with lymphoproliferative diseases (2, 3).

Classical strategies for PBSC mobilization include administration of growth factors (GF), mainly granulocyte colony stimulating factor (G-CSF) alone or in combination with other cytokines or marrow suppressive chemotherapy (CT) (7). The combination of CT and GF mediated expansion has been shown to be synergistic. But, still it is uncertain which regimen is optimal regarding the maximum yield of progenitor cells and tumour cell contamination. Patients that fail to mobilize PBSC with GF alone might have benefit from CT plus GF. These strategies have
been optimized and described in numerous clinical trials, but still a significant proportion of transplant recipients mobilize an insufficient number of PBSC, resulting in multiple aphaeresis procedures and inadequate graft (6).

The priming strategy should be considered as two step process with obtaining optimal disease control before GF (or chemotherapy based) priming and focusing on stem cell (SC) harvest and graft quality. This tumour specific therapeutic approach was evaluated also during the creation of European Bone Marrow Transplant (EBMT) “the gold standard” for priming and harvest strategies (8, 9).

The risk factors that have influence on the autologous harvest features and engraftment of PBSC (three lineage recovery) were divided into demographic, disease and therapy related variables such as: patient age and sex, disease type at diagnosis, disease stage, bone marrow cellularity, type of prior chemotherapy, priming regimen, mononuclear cell count (MNC) in the harvest, CD34+ cell blood level, CD34+ harvest cell count and aphaeresis strategy. Such prognostic models will ultimately allow us to calculate an individual patient-specific index of major clinical impact (10).

Cyclophosphamide (CTX) 3 to 5 gr/m² plus G-CSF 10 mg/kg is commonly used regimen for mobilizing PBSC to support high dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) in patients with lymphoproliferative diseases. This schedule is associated with high rate of organ toxicities, such as haemorrhagic cystitis, prolonged neutropenia and infective complications (11).

Etoposide (VP-16) 2000 mg/m² plus G-CSF 10 mg/kg is one of the mobilizing schedules used in patients with Hodgkin’s disease (HD) and also non-Hodgkin’s lymphoma (NHL). It’s effectiveness in different dose schedules should be more evaluated, especially concerning the late effects of secondary malignancies or myelodisplasia (12).

GF alone or in combination with other cytokines showed to be effective mobilizers of PBSC in hematological malignancies, considering the possible side effects especially when administered in healthy donors during allogeneic transplant setting (13, 14). Statistical data revealed that 5% from potential transplant donors and 30% from patients are in the group of “poor mobilizers”, in which the sufficient graft with optimal number of MNC can not be obtained. Remobilization strategies consist of doubling the dose of GF, combination of two cytokines sequential or during the same setting or using BM as MNC source. Recent data showed the effectiveness of the newly discovered mobilizing agent AMD-3100 in reducing the number of aphaeresis procedures, minimal toxicities and receiving MNC with optimal engraftment potential (15, 16).

The aim of this study is to present the mobilizing strategies in patients with lymphoproliferative diseases taking in consideration demographic and treatment related variables that have influence as prognostic factors in the number of MNC in the autologous graft, as well as in the duration of engraftment period during the autologous transplant setting. Second aim of the study was to compare BM and PBSC as source during autologous transplant procedure.

Material and Methods

The study was accomplished with patients treated with autologous transplantation at University Hematology Hospital, Skopje, Republic of Macedonia during 5 year period (from 2002 to 2007). A total of 70 patients with lymphoproliferative diseases were included in this study.
The analyzed group of 46 patients was divided into two subgroups:
- patients with MM transplanted with cryopreserved autologous PBSC;
- patients with malignant lymphoma (NHL and HD) transplanted with cryopreserved autologous PBSC.

Controlled group consisted of 24 patients with hematological malignancies that were treated with HDT and autologous transplantation with fresh BM as stem cell source.

All patients signed informed consent before aphaeresis procedures, cryopreservation of autologous grafts and autologous transplantation with previously administered HDT (according to the EU regulatives for biomedical research) (9).

The collection and aphaeresis of PBSC were performed at Transfusiology Department, with large volume aphaeresis on cell separator Cobe Spectra.

BM was collected with multiple aspirations from iliac crest 10-15 mL/kg in the operation theatre under general anesthesia. Unmanipulated fresh BM was kept at +4 °C for 48-72 hours in Earle's medium and then infused on day 0 during autologous transplantation (17).

PBSC were cryopreserved in 5% cryoprotective solutions with dimethyl sulfoxide (DMSO). Cryopreservation of PBSC was performed with computer controlled rate freezing until -80°C with Nicool plus PC, Liquid (Criogenie) freezing operating system. The cells were stored in liquid nitrogen on -190°C (18).

Optimal accepted amount of MNC for successful harvest aphaeresis procedure was ≥ 2.0 x10⁸/kg MNC. Engraftment was defined by enlargement of Ne > 0.5x10⁹/L and Tr >20x10⁹/L.

Conditioning before ASCT in PBSC group consisted of myeloablative therapy Melphalan 200 mg/m² for MM patients and BEAM regimen (Carmustine 300 mg/m², Etoposide 300 mg/m² days 3-7, ARA-C 2 x 100 mg mg/m² days 3-7, Melphalan 140 mg/m²) for patients with NHL and HD.

Conditioning before ASCT in the controlled group with BM consisted of modified BEAM regimen administered during 48h schedule consisted of Carmustine 300 mg/m², Etoposide 2x300 mg mg/m², ARA-C 2x3000 mg/m² Melphalan 140 mg/m².

Mobilization strategies for PBSC for myeloma patients were performed with Cyclophosphamide 3-5 gr/m² and G-CSF 10 mg/kg starting from day +1 after completing the CTX dose, until nadir of WBC>5,0x10⁹/L on day +12, +13 or +14 of aphaeresis. In patients with complete remission (CR) after first line CT (VAD or C-ThalDex) a single agent G-CSF 10 mg/kg in a 5 days duration was administered. Aphaeresis was performed on day +5 and +6.

In patients with NHL and HD aphaeresis was performed mainly with single agent G-CSF or after HDT with DHAP (for HD patients) or DexaBEAM for NHL patients. High dose VP-16 2000 mg/m² plus G-CSF 10 mg/kg was also used as mobilizing regimen.

Statistical analysis was performed using software Statistics 7 for OS Windows. The influence of several independent variables (body weight before aphaeresis, WBC count on day of aphaeresis, lymphomonocyte percent (LyMo%) on day of aphaeresis, Plt number on day of aphaeresis, number of procedures, number of prior chemotherapy cycles, bone marrow cellularity) over one dependent variable (MNC count) was analysed in this study. Statistical comparison between control group and analyzed group in the study (BM versus PBSC) was performed by analyzing several dependent variables: sex, age and months from diagnosis until transplantation, number of febrile days, and number of microbiological isolates, number of erythrocyte (Er) and platelet (Plt) transfusions, days for hematopoietic engraftment. Transplant outcome was analysed by Kaplan Mayer survival curves.

Results

The group of patients included in the control group showed sex distribution males 13 (54%) and females 11 (64%) in 1:1 ratio and median age of 39.5 years (range 16 to 63 years). In the analyzed group with lymphoproliferative diseases sex distribution revealed presence of males 60% versus 40% females with median age 41 years (range 17 to 65 years). The period from diagnosis until transplant in the control BM group was median 7.16 months (3 to 35 months), for MM patients with PBSC 11 months (4 to 45 months) and for lymphoma patients (NHL and HD) it was 37.35 months (7 to 120 months). According to the BM cellularity in 30% (25) patients hypopcellular BM was registered before mobilization of PBSC.

The characteristics of autologous grafts from
BM and cryopreserved autologous PBSC are presented on Table 1.

### Table 1: Characteristics of autologous grafts from BM and cryopreserved PBSC.

<table>
<thead>
<tr>
<th>Bone marrow (BM)</th>
<th>Median value</th>
<th>interval</th>
<th>S.M</th>
<th>S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>947.9</td>
<td>350-1250</td>
<td>39.41</td>
<td>193.07</td>
</tr>
<tr>
<td>MNC x10^9/kg</td>
<td>3.59</td>
<td>1.63-8.0</td>
<td>0.29</td>
<td>1.45</td>
</tr>
</tbody>
</table>

- No side DMSO related toxicities were registered, except mild reactions classified as stomach cramps in 3 patients with HD, transitory cystitis in 2 patients with MM, tachycardia in 1 patient with HD and vomiting and nausea in 3 NHL patients.

### Table 2: Engraftment and transfusion requirements.

<table>
<thead>
<tr>
<th>Engraftment (Days)</th>
<th>Median value</th>
<th>interval</th>
<th>S.M</th>
<th>S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM</td>
<td>+15 day</td>
<td>10-23</td>
<td>0.83</td>
<td>4.06</td>
</tr>
<tr>
<td></td>
<td>+17 day</td>
<td>11-22</td>
<td>0.98</td>
<td>4.63</td>
</tr>
<tr>
<td>PBSC (HD-NHL)</td>
<td>+10 day</td>
<td>9-15</td>
<td>0.67</td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td>+12 day</td>
<td>9-20</td>
<td>1.00</td>
<td>4.60</td>
</tr>
<tr>
<td>PBSC (MM)</td>
<td>+10 day</td>
<td>8-13</td>
<td>0.47</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>+11 day</td>
<td>8-14</td>
<td>0.51</td>
<td>2.12</td>
</tr>
</tbody>
</table>

Median day for aphaeresis in the group of patients mobilized with CTX and G-CSF was 10.33 (range 8-12) and median day for aphaeresis in the group that received VP-16 plus G-CSF was 12.5 (range 9-17)

During the application of cryopreserved PBSC

The engraftment period in days and transfusion requirements for both groups of patients during the autologous transplantation is presented on Table 2.

### Table 2: Engraftment and transfusion requirements.

<table>
<thead>
<tr>
<th>Transfusions</th>
<th>Median value</th>
<th>interval</th>
<th>S.M</th>
<th>S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM</td>
<td>3.1</td>
<td>0.10</td>
<td>0.45</td>
<td>2.21</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.15-79</td>
<td>5.97</td>
<td>29.25</td>
</tr>
<tr>
<td>PBSC (HD-NHL)</td>
<td>3.5</td>
<td>0.6-6</td>
<td>0.55</td>
<td>2.52</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>0.15-56</td>
<td>3.63</td>
<td>16.63</td>
</tr>
<tr>
<td>PBSC (MM)</td>
<td>1.7</td>
<td>0.7</td>
<td>0.56</td>
<td>2.98</td>
</tr>
<tr>
<td></td>
<td>7.9</td>
<td>0.21-1</td>
<td>1.77</td>
<td>7.3</td>
</tr>
</tbody>
</table>

The statistical analysis preformed for analysed patients transplanted with cryopreserved PBSC, compared with patients transplanted with autologous BM showed p<0.0001 for febrile days, microbiological positive isolates, days of hospital stay, engraftment (elevation of Ne>0.5x10^9/L and Plt >20x10^9/L) statistically in favour for the group transplanted with PBSC.

The NHL group the comparison with the controlled
group for the transfusion requirements didn’t have statistical significance between the groups (Table 4).

Analysis of the difference of the parameters concerning the mobilization of PBSC and the day of aphaeresis revealed in the analyzed group of patients revealed p<0.001 and p<0.05 for WBC count before aphaeresis, number of procedures, day of aphaeresis in the G-CSF mobilized group and statistical significance in higher LyMo% in the group mobilized with CT plus G-CSF. The number of prior chemotherapy cycles, patient body weight did not have statistical impact on mobilization and aphaeresis in the patients included in this study. The multiple regression analysis showed strong correlation between the number of MNC and the number of prior chemotherapy cycles, body weight, LyMo%, and WBC before aphaeresis (Table 5).

Table 5: Statistical analysis on the prognostic factors that influence mobilization of PBSC.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G-CSF</th>
<th>CT + G-CSF</th>
<th>t-test</th>
<th>df</th>
<th>p &lt; 0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of prior CT cycles</td>
<td>25 00000</td>
<td>206 00000</td>
<td>-1.70168</td>
<td>0.088818</td>
<td></td>
</tr>
<tr>
<td>WBC x 10^9/L before aphaeresis</td>
<td>77 00000</td>
<td>154 00000</td>
<td>2.95555</td>
<td>0.003121</td>
<td></td>
</tr>
<tr>
<td>Day of aphaeresis</td>
<td>10 00000</td>
<td>221 00000</td>
<td>-3.04512</td>
<td>0.002326</td>
<td></td>
</tr>
<tr>
<td>Number of procedures</td>
<td>40 50000</td>
<td>190 50000</td>
<td>-0.31347</td>
<td>0.753826</td>
<td></td>
</tr>
</tbody>
</table>

The survival analysis of the group of patients showed better survival in PBSC group. Median period of follow up of the patient was 43 months (range 3 to 84 months). Analysis of the parameter OS (overall survival) showed in BM group survival of 33% in 43.5 months and 52% on 24 months, for MM patients 41% and 70% respectively, 50% and 68% in lymphoma group. Disease free survival (DFS) in MM group at 43.5 months was 26% and at 24 months 46%. For lymphoma patients DFS was 38%. Transplant related mortality during autologous transplantation was 5% (Fig. 2).

Discussion

Lymphoproliferative diseases are still the most common indication for treatment with ASCT. PBSC as source of stem cells versus BM was shown to be more effective from different aspects (hematological and economic as well). Since the first report of 457 patients in 1997 from the EBMT group for the benefit of autologous transplant with PBSC over BM, HDT become essential for relapsed and/or refractory aggressive NHL and relapsed or primary refractory Hodgkin’s disease (19). The PARMA study presented 109 patients that revealed chemosenitivity to HDT with DHAP regimen followed by autologous transplantation and 5 year survival 53% versus 32% in patient treated with conventional chemotherapy. Many other randomized studies confirmed the advantages of HDT and autologous HSCT over standard chemotherapy. The quality of the therapeutic response is in correlation with the incidence of achieved complete remission (CR) before autologous transplantation. Choosing the optimal regimen for HDT and mobilization of PBSC was the subject for investigation in HOVON randomized trial that confirmed the advantage of addition of CD20 antibody in combination with HDT (DHAP and R-DHAP) (20). This concept of immunotherapy and HDT and possibility for posttransplant maintenance therapy have became...
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one of the dilemmas of recently opened Coral randomized trial of the Lymphoma Working Party (LWP) of EBMT group. Younger patients with favorable IPI (international prognostic index) score can also benefit from HDT and autologous transplantation as first line therapy. For patients with primary refractory HD, HDT and ASCT is therapeutic option that has 5 year survival of 32%. For chemosensitive relapsed HD this approach is becoming a treatment standard which was described in several finished randomized trials.

Myeloma patients have better treatment response, when treated with autologous transplantation (21). Three randomized trials showed no statistical significance in the timing of the autologous transplant in correlation with survival. IFM-94 study presented the first results of double autologous transplants in MM, with better event free survival (EFS) and OS in double graft recipients (22). But the same year three other trials showed that there is no difference for the survival with or without double transplant. Nowadays double transplants in MM are routine procedure for the patients that didn’t achieved CR with the first transplant or VGPR (very good partial response). The patient that accomplished CR or VGPR after the first transplant are monitored or administered one of the regimens for aftertransplant myeloma maintenance, and the second graft can be applied during disease relapse (23, 24).

Mobilization (priming) of PBSC is an important contributing method for gaining the optimal number of MNC for autologous transplantation. Mobilized PBSC promote faster and sustained engraftment during transplant procedure. Serious mobilization problems occur with the group of “poor mobilizers” patients that can not achieve the optimal MNC number with more than 2 harvest procedures on day +14 from the mobilizing regimen (26). EBMT group started the prospective study for this group of patient with the main purpose to define mobilization strategies for poor mobilizers. In our study out of 70 included patients, in 46 patients mobilization of PBSC was performed with different regimens, with total of 140 aphaeresis procedures realized. Three patients with HD and 2 with MM gained the optimal MNC number with >2 aphaeresis procedures and more than two mobilization regimen. After failing mobilization with the combination of CT plus G-CSF, further attempts were made only with G-CSF, but in two HD patient stem cell source was changed with BM. In both group of patients unmanipulated grafts were used and in all of them an optimal number of MNC in the grafts was obtained. Successful engraftment was registered in all patients that underwent autologous transplantation. CTX plus G-CSF and VP-16 plus G-CSF, as well as the other HDT regimens that were used as mobilizing, showed to be effective in more than 80% of analyzed patients. The group mobilized only with GF, showed more safe profile in less aphaeresis procedure, less hospital stay, but lower number of lymphomonocytes in the peripheral blood on day of aphaeresis, compared with the group primed with chemotherapy.

Cryopreservation of PBSC as one of the steps during the autologous transplant setting as method for longterm storage and preservation of stem cells, in this study showed to be dependable from the difference in the concentration of DMSO and daily dose of administered amount of DMSO. In the cryopreservation regimen used in this study, cryobiological solutions were prepared depending on the cell concentration in the harvested autologous grafts with median 3 collection procedures. A 5% DMSO was the final cryoprotectant concentration. Median amount of the cell product was 224 ml, it was diluted with autologous plasma median until 480,66ml with cell concentration <100x10^6/ml. Enlargement of the amount of the grafts suggested the need for addition of higher amount of DMSO. EBMT analysis in 444 transplant centers on the influence of DMSO on the occurrence of posttransplant toxicities revealed 1 per 70 cases with DMSO related toxicity (28, 29). During 2007 EBMT promoted prospective study for infusion related cryoprotective toxicities that will define the future standards and strategies for its application. In this study PBSC were cryopreserved with DMSO solutions containing 5% and 10% concentrations of the cryoprotector. The toxicities that were registered in the analyzed group of patients were casified as mild reactions.

MNC were taken as a SC indicator in this study. All patients achieved engraftment successfully and all patients have mobilized sufficient number of stem cells. Although CD34+ count has become the gold standard for SC enumeration, due to lack of the possibility for immunocytometric count of these cells in our institution, MNC showed to be safe and effective indicator for the mobilization strategies and engraftment period in the analyzed group of patients.

With this study we can conclude that autologous PBSC are still the main source for autologous HSC in patients with lymphoproliferative diseases, with safe profile and fast engraftment. There are a number of factors that have been associated with the likelihood of successful stem cell mobilization. Such
prognostic factors can assist clinicians in designing treatment regimens leading up to the transplant procedure and also identifying patients at high risk for failing the mobilization regimen. The kinetics of the actual mobilizing agents in the terms of dose schedule, concerning the possible late effects of posttransplant secondary malignancies has to be still evaluated in a multicenter randomized studies.

References


EU 2003.


