AUTOLOGOUS STEM CELL TRANSPLANTATION FOR THE TREATMENT OF HEMATOLOGICAL MALIGNANCIES IN THE CLINICAL CENTER OF VOJVODINA

Ivana UROŠEVIĆ1,2, Ivanka SAVIĆ1, Amir EL FARRA1,2, Borivoj SEKULIĆ1,2, Aleksandar SAVIĆ1,2 and Bela BALINT3,4,5

Summary

Introduction. Autologous stem cell transplantation combined with high dose chemotherapy is an effective and safe approach in the treatment of different hematological malignancies. Nowadays, autologous stem cell transplantation represents a standard therapeutic option in the treatment of multiple myeloma, lymphomas and other hematological malignancies. Aim is to analyze the available medical data of patients with multiple myeloma, Hodgkin’s lymphoma, non-Hodgkin’s lymphoma acute myeloid and lymphoblastic leukemia, who underwent autologous stem cell transplantation, and to compare the results with published data from other similar studies. Material and Methods. A retrospective study included 90 patients with multiple myeloma, acute myeloid and lymphoblastic leukemia, non-Hodgkin’s and Hodgkin’s lymphoma who underwent autologous stem cell transplantation in the period from 2004 to August 2017. Results. In relation to the underlying disease, the distribution of the respondents was as follows: 39 patients had multiple myeloma, 25 non-Hodgkin’s lymphoma, 20 Hodgkin’s lymphoma and 6 had acute leukemia. 75 patients (89.3%) had the large volume apheresis procedure, while 9 patients (10.7%) had the conventional two-day apheresis procedure. The average number of the mononuclear cells in the apheresis product was 7.8x10⁸/kg, and the number of the CD34+ cells was about 12.11x10⁶/kg. After applying the conditioning regimen, depending on the underlying disease, neutrophils engraftment mainly occurred on the 11th while the platelet engraftment occurred on the 14th post-transplant day. Transplant-related mortality was low, and the mortality rate was 3.57%. Conclusion. Autologous stem cell transplantation is an efficient method of treatment for patients with hematological malignancies. It is associated with a low rate of complications as well as low rate of transplant-related mortality. Key words: Transplantation, Autologous; Hematopoietic Stem Cell Transplantation; Hematologic Neoplasms; Survival Rate; Graft vs Host Disease; Treatment Outcome

Sažetak

Introduction

Autologous stem cell transplantation (ASCT) is considered one of the best therapeutic methods for treating many hematological malignancies, and the number of ASCTs has increased significantly in recent decades [1, 2]. Contemporary research has made an enormous progress in understanding the hematopoietic stem cells (HSCs) biology and their great therapeutic potential, which has led to the development of new therapeutic approaches, primarily in regenerative medicine and cellular therapy. HSCs are defined as the cell population that has the ability to self-regenerate by mitosis and to differentiate into specialized blood cells [3]. From the early 1980s to the mid-1990s, the only source of HSCs was the bone marrow. Based on the results obtained by experimental research, it was confirmed that the number of HSCs is significantly higher in the stage of bone marrow recovery after applying a high-dose chemotherapy regimen, also known as the mobilization chemotherapy protocol. By introducing the hematopoietic growth factor (HGF) – usually the recombinant human growth factor of granulocytopoiesis rHGF-CSF and the granulocytic-monocyte growth factor GM-CSF – peripheral blood (PB) became the dominant source of the HSCs [4]. The ASCT process consists of: mobilization, collection, cryopreservation, and the application of a high-dose chemotherapy regimen and/or radiotherapy with the support of prior collected HDCs. The basic precondition of a successful ASCT is chemo/radiosensitivity of the underlying disease, satisfactory mobilization and apheresis of the HSCs (optimum number and quality). Chemotherapeutic mobilization protocols generally depend on the underlying disease, but mostly consist of cyclophosphamide alone or in combination with Vepeside, with the addition of HGF [4]. Apheresis of the HSCs is performed using a special apparatus, a blood cell separator. The quality of the apheresis product depends on a number of parameters: age and sex of patient, nature and stage of the underlying disease, degree of the hematopoietic tissue damage, mobilization protocol, apheresis procedure and the number of CD34+ cells before the start of apheresis [5]. The collected HSCs are re-suspended in special substances, cryoprotectants,
which preserve their vitality during the freezing process, after which they are lowered to a certain temperature (from -80 to -196°C), depending on the required length of storage. In the second phase, a high-dose chemotherapy regimen and/or radiotherapy, i.e. the conditioning regimen is applied, which is adapted to the nature of the underlying disease, followed by the reinfusion of the quickly thawed suspension of the previously frozen HSCs.

The aim of this paper was to show the results of treatment of patients with hematological malignancies (multiple myeloma, non-Hodgkin’s and Hodgkin’s lymphoma and acute leukemia) with ASCT at the Clinic for hematology, Clinical Center of Vojvodina.

**Material and Methods**

The research involved 90 patients treated with ASCT at the Clinic for Hematology, Clinical Center of Vojvodina, in the period from 2004 to August 2017.

Prior to the transplantation procedure itself, the appropriate mobilization protocol was applied, depending on the required length of storage. In the second phase, a high-dose chemotherapy regimen and/or radiotherapy, i.e. the conditioning regimen is applied, which is adapted to the nature of the underlying disease, followed by the reinfusion of the quickly thawed suspension of the previously frozen HSCs.

The aim of this paper was to show the results of treatment of patients with hematological malignancies (multiple myeloma, non-Hodgkin’s and Hodgkin’s lymphoma and acute leukemia) with ASCT at the Clinic for hematology, Clinical Center of Vojvodina.

**Results**

In the period from 2004 to August 2017, the total number of autologous stem cells transplanta-
In relation to the underlying disease, the distribution of the respondents was as follows: 39 patients had multiple myeloma (MM), 25 had non-Hodgkin's lymphoma (NHL), 20 had Hodgkin's lymphoma (HL) and 6 patients had acute leukemia (AL) (Graph 2).

The characteristics of the patients in relation to the underlying disease are shown in Tables 1 and 2.

The most commonly used mobilization protocol in patients with MM was cyclophosphamide (Cy) (22.73%) and Cy in combination with vepeside (9.1%), in NHL and HL patients DHAP protocol (21.21%), ICE and dexa-BEAM (6.2%) were used, while the most commonly used mobilization protocol in patients with AL was a high dose of cytosine arabinoside (12.12%).

After the mobilization protocols were applied, HGF was administered and after an adequate increase in leukocyte count was reached, a large-volume apheresis procedure was performed in most cases, while in others a conventional two-day apheresis procedure was performed.

In 75 patients (89.3%) a large-volume apheresis procedure was performed, while in 9 patients (10.7%) a conventional two-day apheresis procedure was performed. The mean volume of processed blood was 14922.96 ml (range 10500-18600 ml), while in the two-day conventional apheresis, an average of 25425 ml (range 19800-35840ml) was processed. The time from the apheresis procedure to ASCT ranged from 15 to 282 days (in average 68 days). The detected number of mononuclear cells (MNC) in the apheresis product ranged from 3x10^8 to 14x10^8/kg BW (in average 7.8x10^8/kg BW). The average number of CD34 + cells was 12,11x10^6/kg BW. The engraftment of neutrophils was detected in average on the 11th, and platelets engraftment on the 14th post-transplant day.

Stem cells viability was tested by the standard trypan blue method and flow cytometry with the addition of 7-Aminoactinomycin D (7AAD) in 66 patients. A statistically significant difference between the two methods was found when comparing the number of viable cells in the apheresis product pre and post cryoconservation. The standard trypan blue method detected 95.78% viable cells in the apheresis product prior to cryoconservation, and 82.58% after thawing. The flow cytometry with the addition of 7AAD prior to cryoconservation detected a 97.1% viable cells prior to cryopreservation, and 95.42% after thawing.

The type of the conditioning regimen used was based on recommendations in relation to the underlying disease. In patients with MM, melphalan was administered at a dosage of 200 or 140 mg/m^2. In 9 of them a tandem AH SCT was performed, which means that these patients underwent 2 ASCTs in less than 6 months. In patients with HL and NHL the BEAM protocol was administered, while in subjects with AL the Busulfan/Cyclophosphamide (Bu/Cy) regimen was applied. Transplant-related mortality rate was low, as 3 subjects (3.57%) had died in the phase of bone marrow aplasia due to infection.

After the ASCT, 23% of the patients had no complications whatsoever, 40% had a one or more febrile episodes, 17% developed oral mucositis, and 10% had gastrointestinal disorders. Complications such as the engraftment syndrome (7%) and ecthyma gangrenosum (3%) presented in a small number of patients.

**Discussion**

High-dose chemotherapy regimens with the support of ASCT is the standard of treatment for numerous hematological malignancies and solid tumors [1]. The results of multiple studies point to the high efficacy of this type of treatment in patients with aggressive lymphomas, multiple myeloma, and acute leukemia [6–8]. In most transplant centers, ASCT with SC obtained from the bone marrow was replaced by ASCT with SC obtained from peripheral blood, which requires the application of various mobilization protocols and apheresis procedures. A number of factors, such as age of the patient, previous exposure to chemotherapy and/or radiotherapy and the degree of bone marrow infiltration by the...
underlying disease, affect the adequate mobilization of the peripheral blood SCs. Independently of the mentioned factors, the application of different types of collecting procedures, the apheresis time, the type of blood cells separator used, and the volume of the processed blood are other factors that can affect the efficiency of the collection procedure [9].

The most commonly used mobilization protocol was cyclophosphamide (Cy) with the addition of granulocytes growth factor (G-CSF). The dose of cyclophosphamide applied varies in numerous reports and ranges from 1-7 g/m². The results of one study indicated that the application of a high dose Cy (7 g/m²) regimen with G-CSF results in a higher yield of CD34 + cells than the intermediate doses of Cy (3-4 g/m²) with G-CSF, while the results of other studies have shown that there is no difference between the high and intermediate doses of Cy. In addition, some transplant centers have shown similar efficacy in SC mobilization with the use of low-dose Cy (1-2 g/m²) in patients with MM. High dose Cy results in a greater incidence of adverse effects, including hemorrhagic cystitis, renal failure, febrile neutropenia and bleeding [10]. In patients with relapsed lymphomas, it was found that chemotherapy protocols with platinum derivatives provided better results and a more favorable mobilization outcome when compared to regimens involving the use of cyclophosphamide as monotherapy [11]. In our study, the majority of patients with MM received cyclophosphamide (Cy) or Cy in combination with etoposide (VP-16) as a mobilization protocol. As for the patients with HL and NHL, they mainly received DHAP + G-CSF as a mobilization protocol. However, an optimal mobilization regimen, with an acceptable toxicity and efficacy has yet to be defined.

The start of G-CSF application after the mobilization protocol had been applied, depends on the transplant center’s experience, as there are no recommendations on the most effective term for starting G-CSF administration. Lefrere and associates, on a group of 65 patients, applied G-CSF from +1 or +2 days in some of them and that there is no need for prolonged apheresis procedure indicate that the desired number of MNCs and CD34 + cells is reached in 75% of patients when comparing the number of conducted apheresis procedures, medians number of cells collected, the recovery of leukocytes and platelets, or the number of collected procedures, the apheresis time, the type of blood cells separator used, and the volume of the processed blood can be estimated by quantifying the number of MNCs and the number of CD34 + cells. For a successful and rapid recovery of hematopoiesis after ASCT, it is necessary that the MNCs number is at least 2-5x10⁶/kg BW and the number of CD34 + cells is greater than 2x10⁶/kg BW, while even better results are achieved if the CD34 + cells number is greater than 5x10⁶/kg BW. In our study, the MNCs number averaged 7.8x10⁶/kg BW and the number of CD34 + cells was 12.11x10⁶/kg BW in average.

The volume of the processed blood is affected by the level of blood flow and the patient’s ability to withstand the procedure. More and more centers apply the large volume apheresis (LVA), which processes three or more blood volumes of the organism in order to obtain the desired number of MNCs and CD34 + cells [13]. No significant decrease in the number of CD34 + cells in peripheral blood during LVA was detected, while the number of cells collected was proportional to the volume of processed blood.

In our study, 81 patients (89.3%) underwent the LVA procedure, while 9 patients (10.7%) had the conventional two-day apheresis procedure. Until 2016, the collection of HSCs from peripheral blood was carried out on the Cobe Spectra® (version 7.0). From 2016 the procedure is done on the Spectra Optia® separator. According to published data, there is no difference in the efficiency of collecting mononuclear cells between these two separators [14, 15]. The mean volume of processed blood in the LVA group was 14922.96 ml (range 10500-18600 ml), while in the two-day conventional apheresis group 25425 ml of blood was processed on average (range 19800-35840 ml). The time passed from apheresis to ASCT averaged 68 days (range 15-282 days).

The results of transplant centers with extensive experience in the implementation of the conventional apheresis procedure indicate that the desired number of CD34 + cells is reached in 75% of patients and that there is no need for prolonged apheresis longer than two days, as it was confirmed by our research as well. However, in a certain percentage of patients (10-20%), the first mobilization does not provide an optimal number of CD34 + cells, which requires another apheresis procedure. Ac-
According to studies conducted on NHL patients, it has been proven that it is more effective to do a second apheresis shortly after the first one, because prolonged waiting leads to a poor mobilization outcome. This approach to apheresis involves: a lower risk of infection, a lower risk of disease progression, and a faster bone marrow recovery after the initial mobilization attempt [16].

For the successful engraftment of neutrophils and thrombocytes, aside from the sufficient number of CD 34+ cells, enough viable precursor cells need to be transfused. It is well known that adding a cryoprotectant such as DMSO alters the biological characteristics of cells leading to their death via apoptosis.

In our study, aside from monitoring the viability of cells by the trypan blue method and flow cytometry with the addition of 7-Aminoactinomycin-D (7AAD), we also determined the number of the necrotic/dead cells. Using the flow cytometry method with the addition of 7AAD, the percentage of viable cells pre and post-cryopreservation was 97.10% and 95.42%, which was significantly higher than the percentage of viable cells detected by the trypan blue method, as it detected 82.58% viable cells post-cryopreservation [3].

The complications associated with ASCT include infections, especially in the period of bone marrow aplasia, as well as complications related to the applied conditioning regimen. Transplant-related mortality in our study was low, and was estimated at 3.75%. This low mortality rate can be explained by the adequate selection and pre-transplant treatment of patients undergoing ASCT, and the thorough monitoring of patients during the whole procedure.

In our study, the most common complications of ASCT were: fever, gastrointestinal complications and oral mucositis, which matches the results of other published studies while ecthyma gangrenosum was significantly less common. Although the engraftment syndrome is a pretty common complication in ASCT, only one patient suffered from it in our study.

Conclusion

Autologous stem cell transplantation is an efficient method of treatment for patients with hematological malignancies, with a low rate of transplant-related mortality, as well as low rate of complications related to the procedure itself. It is essential to carefully and thoroughly select the patients who could potentially benefit from autologous stem cell transplantation, bearing in mind the recommendations regarding the stage of the underlying disease.

References
