



Stem cell harvesting protocol research in autologous transplantation setting: large volume vs. conventional cytapheresis

Ispitivanje protokola prikupljanja matičnih ćelija za autolognu transplantaciju: veliko-volumenska nasuprot konvencionalnoj citaferezi

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Abstract

Background/Aim. The use of peripheral blood as a source of hematopoietic stem cells (SCs) is progressively increasing and has nearly supplanted bone marrow transplantation. Interpatient variability in the degree and kinetics of SC mobilization into peripheral blood is an expected event after conventional chemotherapy-based treatment, followed by sequential administration of recombinant granulocyte-colony-stimulating factor (rHu-CSF). In this study, specific factors associated with the application of two different SC-harvesting approaches, including the use of large volume leukapheresis (LVL) vs. repetitive conventional apheresis (RCA), were analyzed. The basic goal of the study was to evaluate the influence of apheresis protocol (collection timing, processed blood volume and cell yield) upon the clinical outcome of transplantation. **Methods.** Results obtained by LVL (76 pts) and RCA (20 pts – control group) were compared. The SC mobilizing regimen used was cyclophosphamide (4–7 g/m²) or polychemotherapy and rHuG-CSF 10–16 µg/kg of body mass (bm) per day. Cell harvesting was performed using COBE-Spectra (Caridian–BCT, USA). The volume of processed blood in LVL setting was ≥ 3.5 – fold of the patient's circulating blood quantity (ranged from 12.7 to 37.8 l). All patients tolerated well the use of intensive treatment, without any side or adverse effects. Our original controlled-rate cryo-

preservation was carried out with 10% dimethyl sulfoxide (DMSO) using Planer R203/200R or Planer 560–16 equipments (Planer Products Ltd, UK). Total nucleated cell (NC) and mononuclear cell (MNC) counts were examined by flow cytometry (Advia–2120 Bayer, Germany; Technicon H–3 System, USA). The CD34⁺ cell surface antigen was investigated by the EPICS XL–MCL device (Coulter, Germany). **Results.** Performing LVL-apheresis, high-level MNC and CD34⁺ cell yields (7.6±4.6 × 10⁸/kg bm and 11.8±6.5 × 10⁶/kg bm, respectively) were obtained. As a result, rapid hematopoietic reconstitution ("graft-healing") – on the 9.4th and 12.4th day for granulocytes and platelets, respectively was achieved. Using repetitive conventional apheresis (2–3 procedures), the total MNC count was high (8.2±7.0 × 10⁸/kg bm), but the total CD34⁺ yield was lower 10.8±9.9 due to inferior CD34⁺ vs. MNC ratio. **Conclusion.** The results obtained suggest that well-timed LVL-apheresis increased SC-yield in cell harvest, resulting in faster bone marrow repopulation and hematological reconstitution, as well as better overall clinical outcome of transplantation. These results necessitate additional examinations of CD34⁺ subsets ratio in cell harvest.

Key words: hematopoietic stem cells; bone marrow; transplantation; cryopreservation; hematologic diseases.

Apstrakt

Uvod/Cilj. Terapijska primena matičnih ćelija (MĆ) prikupljenih iz periferne krvi je u stalnom porastu i u velikoj meri zamenjuje upotrebu ćelija kostne srži. Varijacije u stepenu i kinetici mobilizacije MĆ u perifernu krv su očekivane pojave prilikom tretmana bolesnika hemioterapijom, sa sledećom primenom rekombinantnog faktora rasta granulocitopoeze (rHu-CSF). U ovoj studiji analizirani su činioci koji utiču na efikasnost afereze prilikom upotrebe dva protokola prikupljanja MĆ – izvođenje veliko-volumenske leukafereze (LVL)

nasuprot konvencionalnoj ponavljanoj aferezi. Osnovni cilj rada bio je procena primenjenog afereznog protokola (odabir najpogodnijeg termina prikupljanja, volumena procesirane krvi i prinosa ćelija) na klinički ishod transplantacije. **Metode.** Analizirani su rezultati dobijeni izvođenjem LVL (76 bolesnika) i ponavljanih konvencionalnih afereza (PKA) MĆ (20 bolesnika – kontrolna grupa). Volumen procesirane krvi bio je oko 3,5 veći u poređenju sa volumenom bolesnikove cirkulišuće krvi (kretao se od 12,7 l do 37,8 l). Protokol mobilizacije MĆ je podrazumevao primenu ciclofosfamida (4–7 g/m²) ili polihemioterapije sa rHuG-CSF 10–16 µg/kg telesne mase

dnevno. Prikupljanje ćelija je izvedeno pomoću COBE-Spectra (Caridian-BCT, USA). Volumen procesirane krvi prilikom LVL je bio $\geq 3,5$ puta od bolesnikove cirkulišuće krvi (opseg od 12,7 l do 37,8 l). Intenzivni aferezni tretman bolesnici su podneli dobro, bez pojave sporednih ili nepovoljnih efekata. Sopstvena originalna kompjuterski kontrolisana kriokonzervacija je obavljena sa 10% dimetil sulfoksidom (DMSO) uz upotrebu aparata Planer R203/200R ili Planer 560-16 (Planer Products Ltd, UK). Broj ukupnih nukleusnih ćelija (TNC) i mononukleusnih ćelija (MNC) je determinisan pomoću protočne citometrije (Advia-2120 Bayer, Germany; Technicon H-3 System, USA). Ekspresija CD34 ćelijskog antigena je ispitivana pomoću EPICS XL-MCL uređaja (Coulter, Germany). **Rezultati.** Izvođenjem LVL afereze, postignuti su visoki prinosi MNC i CD34+ ćelija ($7,6 \pm 4,6 \times 10^8$ /kg telesne mase, odnosno $11,8 \pm 6,5 \times 10^6$ /kg telesne mase). Samim tim, postignuta je

brza rekonstitucija hematopoeze ("oporavak kalema") – za leukocite i trombocite: prosečno 9,4 odnosno 12,4 dana. Izvođenjem ponavljanih konvencionalnih afereza (2–3 procedure), ukupni broj MNC bio je veći ($8,2 \pm 7,0 \times 10^8$ /kg telesne mase), ali apsolutni prinos CD34+ ćelija bio je niži ($10,8 \pm 9,9 \times 10^6$ /kg telesne mase), shodno manjoj procentnoj zastupljenosti CD34+ u odnosu na MNC. **Zaključak.** Pravovremeno izvođenje LVL-afereze rezultuje većim prinosom MNC u afereznom produktu, bržom repopulacijom kostne srži i rekonstitucijom hematopoeze, kao i povoljnijim kliničkim ishodom transplantacije. Ovi rezultati zahtevaju dodatna ispitivanja proporcionalne zastupljenosti podtipova CD34+ ćelija u afereznom produktu.

Ključne reči:
hemocitoblasti; kostna srž; transplantacija;
kriokonzervacija; hematološke bolesti

Introduction

Stem cells (SCs) are capable to provide complete and long-term reconstitution of hematopoiesis that is the basis for their clinical use in allogeneic and autologous SC-transplantation settings^{1,2}. Stem cells-transplantations involve the use of high-dose chemotherapy and reinfusion of cells collected in order to obtain a marrow repopulation and consecutive hematopoietic reconstitution. Historically, bone marrow was the primary source of SC for transplantations – however peripheral or umbilical cord blood were also used as the source of stem cells¹⁻⁶.

Stem cells could be collected by multiple bone marrow aspirations or apheresis of peripheral blood after mobilization, using chemotherapy and recombinant granulocyte colony stimulating factor (rHuG-CSF) in autologous setting, or just rHuG-CSF in allogeneic setting. Peripheral blood SC transplantation can be characterized by the absence of the risk of bone infections, complications of general or epidural anesthesia and earlier hematopoietic reconstruction. Currently, peripheral blood SC harvests are even more applied in both: allogeneic and autologous transplantation settings.

The intensification of pre-transplantation myeloablative therapy and the increase of the clinical use of SC, as well as the introduction of novel cell-mediated curative approaches (such as adoptive cell therapy and regenerative medicine) resulted in increased needs for SC and improved practical cell processing and other extracorporeal operating procedures⁵⁻⁸. Thus, successful performance of SC transplantations requires both efficient collection and cryopreservation procedures for obtaining an acceptable cell yield and post-thawing recovery^{6,9-13}.

Transfusion practitioners have an important role in SC harvesting, processing or purging, cryopreservation, as well as immunohematological and engraftment monitoring. Since conditioning regimens involve myeloablation – with following severe neutropenia and thrombocytopenia before hematopoietic reconstitution – transfusion support has to be an integral constituent of posttransplantation treatment. The patients needs in blood components differ in dependance on the relationship between donor and recipient, as well as on the

source and count of SC. The primary and most difficult demand for blood banks is platelet support to prevent/control the bleeding³⁻⁶.

The purpose of recent investigation was to determine harvesting protocol with optimized SC collection timing, processed blood volume (large volume vs. repetitive conventional apheresis) and CD34 threshold dose of harvest. We postulated that the use of optimal collection time point and large volume apheresis, as well as controlled rate cryopreservation will result in improved CD34+ yield or recovery and cell viability, improved long term marrow repopulation and engraftment ability with faster hematopoietic reconstitution.

Methods

Autologous peripheral blood SC transplantations applied in the treatment of 76 patients. They were aged 14–52 years, with a female/male ratio of 1.45 : 1. Clinical and laboratory data confirmed existence of hematological diseases: acute myeloid leukemia (AML – 16 patients), acute lymphoblastic leukemia (ALL – 3 patients), chronic myelogenous leukemia (CML – one patient), multiple myeloma (MM – 22 patients), Hodgkin's disease (HD – 16 patients) and non-Hodgkin's lymphoma (NHL – 18 patients). In this study, homogeneous series (such as pre-transplantation chemotherapy, mobilization pretreatment and conditioning regimen) of patients for particular diseases were included. In the treatment of additional 20 patients (control group) conventional apheresis with two to three repetitive SC harvests were performed.

The SC mobilizing regimen used was cyclophosphamide ($4-7 \text{ g/m}^2$) or polychemotherapy and rHuG-CSF $10-16 \mu\text{g/kg}$ body mass (bm) per day. Cell harvesting was performed using COBE-Spectra (Caridian-BCT, USA). The first conventional apheresis (control group) or solely autologous SC collection (large volume apheresis – LVL) was accomplished when the leukocyte and CD34+ count in patient's peripheral blood was $\geq 5-10 \times 10^9/\text{L}$ and $\geq 30-50/\mu\text{L}$, respectively^{8,14}.

Central venous catheters applied across subclavian or jugular veins and occasionally peripheral (antecubital) veins were used as vascular access. During apheresis, patients were anticoagulated with acid citrate dextrose solution (ACD formula B, USP – with 1.8% citrate concentration). There was a variable ratio between volume of anticoagulant solution and processed whole blood (ranging from 1/12 to 1/14) during LVL⁸. The processed blood volume during one apheresis procedure was 12.7 to 37.8 L. Additional patients systemic or SC harvest heparinization was not performed. The patients pulse and blood pressure were monitored before each SC harvest. These parameters were also explored at 30–45 minutes intervals during the procedure. The changes in mental or general status were constantly monitored. Permanent monitoring of pulse, blood pressure and cardiac rhythm was indicated only in medically unstable patients.

Our original controlled rate cryopreservation was carried out with optimized dimethyl sulfoxide (DMSO) solution as cryoprotectant using Planer R203/200R or Planer 560–16 equipments (Planer Products Ltd, UK)^{10–13} (Figure 1).

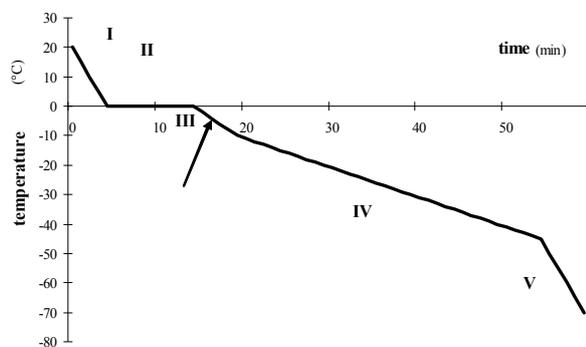


Fig. 1 – Our original controlled–rate stem cell freezing protocol

Note step III, corresponding to release of the fusion heat compensation (arrow).

This procedure consists of next steps: I = -5 °C/min, to 0 °C; II = 0 °C/min, for 5 min (equilibration); III = -2 °C/min, for 5 min; IV = -1° C/min, for 30 min and V= -

5 °C/min, for 5 min¹¹. During the liquid to solid phase transition period an intensified cooling rate (step III) was used due to the compensation of the released fusion heat. For treatment of some patients (with MM) CD34⁺ cell positive selection by immunomagnetic device Isolex 300i (Baxter, USA) was accomplished.

Patients hematological data, total nucleated cell (NC) and mononuclear cell (MNC) count were examined by flow cytometry (Advia–2120 Bayer, Germany; Technicon H–3 System, USA). The cell viability, precisely cell "membrane integrity" of unfrozen and thawed MNCs was determined by trypan blue exclusion test. The CD34 cell surface antigen was investigated by the EPICS XL–MCL device (Coulter, Germany). MNC were incubated with specific monoclonal antibodies and the results were shown as a percentage of CD34⁺ cells/MNCs. CD34⁺ number was calculated (in %) by the following formula:

$$CD34^+ (\%) = (\text{total MNC number} / 100) \times CD34^+ \text{count}^{8,14}.$$

The results of cell quantifications were presented as a mean value ± standard deviation (SD). Statistical analyses were performed by ANOVA-test. Differences were considered as statistically significant if *p* value was less than 0.05.

Results

Patients and disease data, technical and practical aspects of aphereses and results obtained by application of different apheretic procedures (large volume vs. repetitive conventional apheresis) are presented in Table 1 and 2.

Using a minimal target dose (4–5 × 10⁶/kg bm) of CD34⁺ cell count, doing one large volume SC harvesting, for all recipients sufficient number of cells were collected. Namely, adequate MNC quantity could be obtained using both, large volume and repetitive conventional apheresis. Performing one LVL, the MNC and CD34⁺ yields were 7.5±3.5 × 10⁸/kg bm and 12.7±6.8 × 10⁶/kg bm, respectively. Using repetitive conventional apheresis (two or three procedures on 2–3 consecutive days), the MNCs count was higher 8.2±7.0 × 10⁸/kg bm, but the total CD34⁺ yield was lower 10.8±9.9 due to inferior CD34⁺ vs. MNC ratio.

Table 1

Characteristics of patients subjected to the stem cell harvesting-apheresis procedures

Patients	Apheresis type	
	LVL	RCA
Total number	76	20
Sex		
female	45	12
male	31	8
Age (yrs)	14–52	18–54
Type of disease		
acute myeloid leukemia	16	5
acute lymphoblastic leukemia	3	1
chronic myelogenous leukemia	1	/
multiple myeloma	22	8
Hodgkin's disease	16	/
non–Hodgkin's lymphoma	18	6

LVL – large volume apheresis; RCA – repetitive conventional apheresis

Table 2

Procedures	Apheresis type	
	LVL	RCA
Per whole SC-treatment	1	2.35±0.49
Processed blood volume (l)		
per one treatment ($\bar{x}\pm SD$)	22.45±5.2	9,5±1.4
range	12.7–37.8	7.2–12.4
total ($\bar{x}\pm SD$)	/	21.3±3.2
Cell suspension volume (ml), ($\bar{x}\pm SD$)	234.6±55.3	354.6±64.2
MNC yield ($\times 10^8/kg$ bm)		
per one treatment ($\bar{x}\pm SD$)	7.5±3.5	3.2±1.4
total ($\bar{x}\pm SD$)	/	8.2±7.0
CD34 ⁺ yield ($\times 10^6/kg$ bm)		
per one treatment ($\bar{x}\pm SD$)	12.7±4.8* [†]	3.1±1.6*
total ($\bar{x}\pm SD$)	/	10.8±3.9 [†]

LVL = large volume apheresis; RCA = repetitive conventional apheresis; * $p < 0.05$; [†] $p = 0.068$

Significant differences were obtained only for SC quantity between single units of LVL vs. repetitive conventional harvests in accordance with different CD34⁺ percentage.

An apparent example of superior CD34⁺/MNC ratio in LVL-harvest is illustrated using flow cytometry in figure 2.

It is apparent that the application of larger SC doses results in a lowered needs for transfusion support using leukoreduced platelet and/or red blood cell concentrates (Table 2).

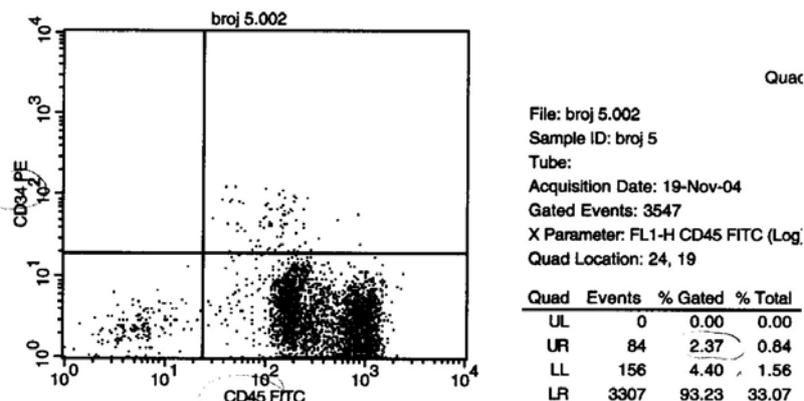


Fig. 2 – CD34⁺ quantification using flow cytometry in large volume apheresis (LVL) setting. The obtained percentage of CD45⁺/CD34⁺ cells in presented sample was 2.37%.

The presented large total CD34⁺ count ($12.7\pm 4.8 \times 10^8/kg$ bm) and their high percentage resulted in rapid hematopoietic reconstitution in group of patients treated with LVL-derived SC (figure 3).

As presented, the differences investigated vs. control group were evident, but not found to be significant ($p = 0.062$).

Apheresis-derived platelets (“single-donor platelets”) collected by cell separators vs. platelet concentrates (“random-donor platelets”) are more effective in the treatment/prevention of bleeding events, maintaining patients’ platelet counts at higher level, with significantly reduced risk of different immediate or delayed side effects.

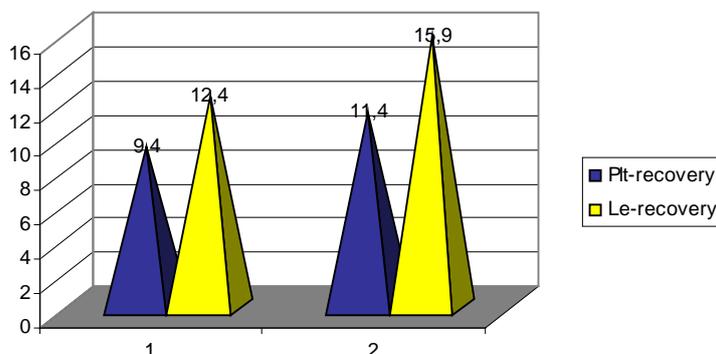


Fig. 3 – Large volume (1) vs. repetitive conventional (2) apheresis hematopoietic reconstitution
Plt - platelets; Le - leukocytes

Table 3.

Transfusion support of patients underwent autologous stem cells (SC) transplantation

Diseases (number of patients)	Transfused PCs (units) ($\bar{x} \pm SD$)	Transfused RCCs (units) ($\bar{x} \pm SD$)	PCs/PRBCs (n/n)
Acute lymphoblastic leukemia (3)	4.47 \pm 1.85	3.93 \pm 3.35	3/3
Acute myeloid leukemia (16)	2.37 \pm 1.41	3.33 \pm 2.82	14/12
Chronic myelogenous leukemia (1)	3	0	1/0
Non-Hodgkin's lymphoma (18)	0.78 \pm 1.06	1.33 \pm 1.68	12/9
Multiple myeloma (22)	2.4 \pm 2.19	2.8 \pm 3.15	20/18
Hodgkin's disease (16)	2 \pm 1.29	1.92 \pm 2.84	11/6

PCs – platelet concentrates; RCCS – red cell concentrates; PRBCs – packed red blood cells.

Discussion

We have previously analyzed our results obtained in cryobiological experimental and clinical settings^{10-13, 15-17}. Microprocessor-controlled (controlled-rate) freezing is more efficient than uncontrolled-rate (without programmed cooling) procedure due to better cell recovery⁹. Our previous findings obtained for cryopreserved bone marrow cells and peripheral or umbilical blood SC was in agreement with these findings¹⁰⁻¹³. These cryoinvestigations pointed out the importance of the specific kinetics of the programmed cooling for the efficacy of cryopreservation. We confirmed that the best survival rate of very primitive hematopoietic cells (Marrow Repopulate Ability – MRA cells) was achieved when 10% DMSO was combined with controlled rate freezing by compensation of released heat of fusion¹¹.

The use of the selection reduces T-cell and/or tumor cell counts with $\geq 3 \text{ Log}_{10}$ or more. At the same time, CD34⁺ cell count should be at least 70% of the total cell number present before purging^{18, 19}. Our results acquired by the using of “positive” SC selection are in accordance with these findings: the CD34⁺ recovery and purity was 62.4 \pm 4.2% and 82.4 \pm 6.3%, respectively^{8, 20}. Even though, these techniques are firstly designed to purify the SC; their use also removes red blood cells and residual plasma, as well. Recently, an another antigen, CD133 (previously named as AC133), present in very primitive SC population has been described. The results obtained suggest that CD133⁺ cell selection – not only for “cell-therapy”, but also for SC transplantation perhaps could be a better option than CD34⁺ selection for some diseases²¹. As demonstrated, the reinfusion of highly purified CD133⁺ SC allowed the rapid and sustained recovery of hematopoiesis after myeloablative treatment in resistant/relapsed chronic lymphocytic leukemia (CLL) patients. However, the purging potential of positive selection of CD133⁺ cells is not adequate to achieve tumor-free autografts²¹.

The questions when is the optimal timing for autologous SC transplantation, and what factors have influence on it open wide area for discussion. Monitoring the timing of leukapheresis in peripheral blood SC mobilization is an important clinical decision that requires an accurate analytical tool. The optimal time for the harvesting of peripheral blood stem cells following chemotherapy and growth factors for autologous transplantation is based on the CD34⁺ cell count. Postchemotherapy mobilization results were reviewed in pa-

tients undergoing apheresis before planned autologous SC transplantation to improve the timing of collection procedures²². Apheresis can be initiated when the blood CD34⁺ content is more than 20 per μL . Mostly, CD34⁺ cells were not harvestable, when CD34⁺ cell count is $< 15 \text{ cells}/\mu\text{L}$. However, association between some laboratory parameters and the number of CD34⁺ cell were observed. Namely, correlation between elevated serum lactate dehydrogenase (LDH) and good stem cell collections (CD34⁺ cells) was strong at the very first day of mobilization. Accordingly, it is reasonable to assume good stem cell mobilization and start apheresis if the LDH is elevated²³. When the CD34⁺ cells were not harvestable, defined as a CD34⁺ cell count of $< 15 \text{ cells}/\mu\text{L}$ – an immature reticulocyte fraction (IRF) was always $\leq 0.2 \text{ cells}/\mu\text{L}$. The IRF is therefore, the negative predictor of the timing of autologous stem cell harvesting²⁴.

In the majority of patients, the first day of apheresis occurred 11 to 13 days after the last dose of chemotherapy with a variety of different chemotherapy regimens.

In our earlier clinical studies repetitive conventional peripheral blood vs. bone marrow transplantations (in allogeneic and autologous settings) on the basis of cell yields and hematopoietic reconstitution were compared^{6-8, 15-17}. Using SC transplantations, patients with acute lymphoblastic leukemia, acute non-lymphocytic leukemia, chronic myelogenous leukemia, multiple myeloma, Hodgkin disease, non Hodgkin's lymphoma, breast and ovarian cancer, extragonadal non-seminal germ cell tumor, and severe multiple sclerosis were treated^{8, 15-17}. Hematopoietic reconstitution was achieved at the 11.4th vs. 15.9th day (for granulocytes) and the 14.1th vs. 17.5th day (for platelets) when blood vs. bone marrow SC were compared, respectively^{6, 8}. In the present study, earlier hematopoietic reconstitution was obtained when a good timing for LVL harvesting was used: at the 9.4th and 12.4th days in average for leukocytes and platelets, respectively. All patients tolerated the use of intensive cell therapy well, without any adverse effects. The results imply that the CD34⁺ cell dose is the most important predictor of hematopoietic engraftment.

As the indication of the most concern for autologous SC transplantation combined with high dose chemotherapy, the most common is MM, where it is the standard treatment. Otherwise, the allogeneic transplantation remains the only curative procedure for these patients. The use of autologous SC transplantation prolongs overall survival (OS) in the results of some study groups, whiles Barlogie in the US study

confirmed the benefit of autologous SC transplantation only for response rate, but not for OS^{25,26}.

In AML which is the most common adult leukemia, harvest due to SC transplantation should be done after second or third course of chemotherapy which improves the outcome compared to the harvest after the completion of consolidation treatment. Otherwise, in ALL the use of autologous SC transplantation vs. conventional chemotherapy showed no benefit of named procedure.

In chronic lymphoid leukemia (CLL) such in MM, autologous SC transplantation could give a substantial therapeutic benefit, without being curative. Patients with advanced CLL who had no matched donor for allogeneic SC transplantation could receive myeloablative therapy followed by reinfusion of autologous bone marrow.

Refractory/relapsed HD is the absolute indication for high dose therapy (BEAM) followed by autologous support with SC transplantation. Therefore, high-dose therapy and autologous SC transplantation represents standard procedures as the first-line therapy of aggressive NHL improving OS. On the contrary, the use of myeloablative therapy combined with autologous SC transplantation in the treatment of low grade NHL has not yet been fully established.

Chronic myelogenous leukemia as a myeloproliferative disease originating from hematopoietic SC starts with a chronic phase, but as a result of genomic instability, it progresses over time to accelerated phase, and then climaxes to the blast crisis, becoming increasingly resistant to therapy²⁷.

Imatinib – CML patients can experience long term disease-free survival with myeloablative therapy and allogeneic hematopoietic cell transplantation; however, associated complications carry a significant risk of mortality. Transplantation of autologous hematopoietic cells has a reduced risk of

complications, but residual tumor cells in the autograft may contribute to relapse²⁸.

In the previous few years, the treatment of CML, using medication therapy with imatinib mesylate, dasatinib or nilotinib were preferred. However, for patients resistant for this therapeutic modality, the use of allogeneic SC transplantation is the treatment of choice. Finally, if for allogeneic SC transplantation family or unrelated donor is unavailable, during the period of major cytogenetic response (molecular remission) autologous SC can be collected for eventual further SC transplantation in the disease progression, which we have already done in one patient.

Autologous SC transplantation has become a standard treatment for a wide variety of malignancies. Several commercial platforms are available to enumerate the circulating levels of CD34⁺ SC. These values can then be used to guide the timing of leukapheresis as well as to measure the success of daily collections. Most mobilization regimens consist of chemotherapy followed by one or more growth factors such as granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor. Despite, a subset of patients will prove unable to mobilize effectively enough to collect at least 2×10^6 CD34⁺ cells/kg, – the number of SC transplantation currently considered to be appropriate for proper timely engraftment and recovery of hematopoiesis^{29,30}.

Conclusion

The optimized time-point of autologous SC harvesting and the use of LVL resulted in higher yields of CD34⁺ cells for transplantation and have influenced better bone marrow repopulative potential and faster hematopoietic reconstitution. These results deserve further examination of CD34⁺ subsets in large cohorts of patients during prospective studies.

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