Influence of applied CD34\(^+\) cell dose on the survival of Hodgkin's lymphoma and multiple myeloma patients following autologous stem cell transplants

Uticaj primenjene doze CD34\(^+\) ćelija na preživljavanje bolesnika sa Hodgkin-ovim limfomom i multiplim mijelomom nakon autologne transplantacije matičnih ćelija

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Abstract

Background/Aim. Autologous stem cell transplants (ASCTs) improve the rate of overall survival (OS) in patients with hematological malignancies such as multiple myeloma (MM) after induction chemotherapy, aggressive non-Hodgkin’s lymphomas (NHL), and relapsed, chemotherapy-sensitive Hodgkin’s lymphoma (HL). The study aim was to evaluate influence of applied CD34\(^+\) cell quantity on clinical outcome, as well as early post-transplant and overall survival (OS) of HL and MM patients following ASCT.

Methods. This study included a total of 210 patients (90 HL/120 MM) who underwent ASCT. Stem cell (SC) mobilization was accomplished by granulocyte-colony stimulating factor (G-CSF) 10–16 µg/kg body mass (bm) following chemotherapy. For proven poor mobilizers, mobilization with G-CSF (16 µg/kgbm) and Plerixafor (24 or 48 mg) was performed. To our best knowledge, it was the first usage of the Plerixafor in our country in the ASCT-setting. Harvesting was initiated merely at "cut-off-value" of CD34\(^+\) cells ≥ 20 × 10\(^6\)/L in peripheral blood with "target-dose" of CD34\(^+\) cells ≥ 5 × 10\(^6\)/kgbm in harvest. The CD34\(^+\) cell count and viability was determined using flow cytometry.

Results. The majority of HL patients (76.7%) were infused with > 5.0 × 10\(^6\)/kgbm CD34\(^+\) cells, while 68.3% of MM patients were treated by approximately 4.0–5.4 × 10\(^6\)/kgbm CD34\(^+\) dose, respectively. Beneficial response (complete/partial remission) was achieved in 83.3% (HL) and 94.2% (MM) patients. Among parameters that influenced survival of HL patients with positive response to the therapy, multivariate analysis (pre-ASCT performance status, CD34\(^+\) cell quantity applied, rapid hematopoietic, i.e. lymphocyte and platelet recovery) indicated that higher CD34\(^+\) cell dose used, along with pre-ASCT performance status correlated with superior event-free survival (EFS) and OS following ASCT. In MM patients, multivariate analysis (renal impairment, infused CD34\(^+\) cell quantity, early platelet recovery) indicated that the number of CD34\(^+\) cells infused was the most important parameter that influenced both EFS and OS after ASCT.

Conclusion. Data obtained in this study undoubtedly confirmed that CD34\(^+\) cell dose applied is an independent factor that may contribute to superior clinical outcome and OS of HL and MM patients following ASCT.

Key words: hematologic neoplasms; stem cells; transplantation, autologous; survival; flow cytometry.

Apstrakt

Uvod/Cilj. Autologna transplantacija matičnih ćelija (ATMĆ) poboljšava učestalost ukupnog preživljavanja (UP) kod bolesnika sa hematoškim malignitetima kao što su multipli mijelom (MM) nakon indukcionе hemoterapije, agresivni non-Hodgkin-ovi limfomi (NHL) i recidivanti hemosenzitivni Hodgkin-ov limfom (HL). Cilj ove studije je bila procena uticaja primenjene doze CD34\(^+\) ćelija na klinički ishod, kao i na rano post-transplantacijsko i UP bo-
lesnika sa Hodgkin-ovim limfomom i multiplnim mijelomom posle ATMČ. Metod. Ova studija obuhvaćala je ukupno 210 bolesnika (90 HL/120 MM) koji su bili lečeni primenom ATMČ. Mobilizacija matičnih čelija (MČ) izvedena je pomoću stimulacijskog faktora granulocitnih kolonija (G-CSF) [10–16 μg/kg telesne mase (tm)] posle hemoterapije. Za dokazane "slabe-mobilizatore" izvedena je dodatna mobilizacija upotrebom G-CSF (16 μg/kgtm) uz dodatak Plerixafora (24 ili 48 mg).

Pored tajnosti, ovo je bila prva primena Plerixafora u našoj zemlji u okvirima ATMČ. Prikupljanje čelija je započelo jedno pri grančnoj, odnosno "cut-off" vrednosti CD34+ ≥ 20 /μL u perifernoj krvi, sa "celijnom dozom" CD34+ ≥ 5 × 10⁶/kg telesne mase (tm) čelija u aferentnom produktu (harvest). Broj CD34+ čelija i vijabilnost bili su određivani primenom protočne citometrije.

Rezultati. Većini bolesnika sa HL (76,7%) infundovano je > 5,0 × 10⁶/kgtm CD34+ čelija, dok je 68,3% MM bolesnika tretrano dozom od 4,0–5,4 × 10⁶/kg tm CD34+ čelija. Po- voljan terapijski odgovor (potpuna/parcijalna remissija) po- stignut je kod 83,3% (HL) i 94,2% bolesnika (MM). Od parametara koji su individualno uticali na preživljanje bolesnika sa HL i povoljan odgovor na terapiju, multivarijantna analiza (status pre ATMČ, primenjena doza CD34+ čelija, rani oporavak hematopoeze, tj. oporavak limfocita i trombocita) ukazali su na to da primena većih CD34+ čelija, zajedno sa karakteristikama pre ATMČ, pozitivno korelira sa boljim preživljanjem i izostanakom neželjenih događaja (IND), kao i UP posle ATMČ. Kod bolesnika sa MM, multivarijantna analiza (oštećenje bubrega, doza primenjenih CD34+ čelija, rani oporavak trombocita) pokazala je da je broj infundovanih CD34+ čelija najznačajniji parametar koji ima uticaja na IND i UP bolesnika posle ATMČ. Zaključak. Podaci dobijeni u ovoj studiji neosporno ukazuju na to da je infundovana doza CD34+ čelija nezavisni faktor koji može doprineti boljem kliničkom ishodu i UP bolesnika sa HL i MM posle ATMČ.

Ključne reči: hematolesko neoplazme; matične čelije; transplantacija, autologna; preživljanje; citometrija, protočna.

Introduction

Autologous stem cell transplants (ASCTs) improve the rate of overall survival (OS) in patients with hematological malignancies such as multiple myeloma (MM) after induction chemotherapy, aggressive non-Hodgkin's lymphomas (NHL) 1, and relapsed, chemotherapy-sensitive Hodgkin's lymphoma (HL) 2. As a result, ASCT has become the standard therapeutic option for these malignancies 3, 4. In order to identify patients benefiting from ASCT, several clinical parameters were reported to be of prognostic importance in HL 5, 6, and MM 7. Of particular importance is the number of CD34 + cells received by patients 7. Of particular importance is the number of CD34 + cells received by patients, which is a common predictor of the potential engraftment 8. Moreover, there may be a correlation between the number of given CD34 + cells, and disease relapse, transplant-related mortality and OS. However, the role of an infused autograft CD34+ cell dose and early lymphocyte, neutrophil, and platelet recovery following ASCT has not been firmly established as standard procedure 1, 2, 7.

The present study aimed to evaluate the influence of applied CD34+ cell dose and various clinical parameters that might influence early post-ASCT and OS of HL or MM patients following transplants.

Methods

This retrospective study included a total of 210 patients who underwent ASCT between November of 2005 and January of 2017. Ninety patients were diagnosed with HL and 120 with MM.

Each patient with HL went through an initial standard staging according to the Ann Arbor classification evaluation before treatment 8, with calculation of the International Prognostic Score (IPS) for risk stratification 9. MM patients were, after initial evaluation, staged according to the Durie and Salmon clinical staging system, and risk groups were determined according to the International Scoring System (ISS) 10. Chromosomal abnormalities were revealed using interphase fluorescence in situ hybridization (iFISH) 11.

All HL patients were initially treated according to ABVD protocol (adriamycin, bleomycin, vinblastine and dacarbazine) and were evaluated according to current response criteria 12. Platinum-based salvage chemotherapy was given at relapse.

Stem cell (SC) mobilization was completed by granulocyte-colony stimulating factor (G-CSF) at standard dose of 10–16 μg per kg of body mass (kgbm) in all patients with previously application of chemotherapy [salvage regimen in HL and cyclophosphamide, adriamycin and dexamethasone (CAD) or high dose (HD)-cyclophosphamide in MM].

Collections of autologous SCs – using Cobe-Spectra and Spectra-Optia (Terumo-BCT, USA) – were initiated merely at "cut-off-value" of CD34+ cells ≥ 20 × 10⁶/L in peripheral blood. The "target-value" of harvested CD34+ cells was ≥ 5 × 10⁹/kgbm. Among of all patients, 6 (2.9%) were described previously 13, 14.

Finaly, cells were cryopreserved using our original controlled-rate freezing procedure by optimized dimethyl sulfoxide (10% DMSO) and stored at -140 ± 5 °C (mechanical freezer) or at -196 °C (liquid nitrogen) and thawed immediately prior clinical use in a water bath at 37 ± 3 °C, as described previously 13, 14.
The CD34+ cell quantity in harvest was determined with a flow cytometer (Beckman-Coulter, USA). Cell viability (i.e. the ratio of "non-apoptotic" CD34+ cells) was also estimated on the basis of the 7-aminoactinomycin D (7-AAD) flow cytometry assay (Immunotech, France), as earlier described 15. The BEAM [total dose (TD) – carmustine 300 mg/m², etoposide 800 mg/m², cytarabine 1600 mg/m² and melphalan 140 mg/m²] conditioning-protocol was given in 76 HL patients (84.4%), while 14 patients (15.6%) received the CBV (TD-cyclophosphamide 6,000 mg/m², carmustine 300 mg/m², etoposide 750 mg/m²). G-CSF was administered after autologous SCs infusion and was continued until the absolute neutrophil count (ANC) was at least 1.0 × 10⁹/L on two consecutive days. Platelet (PLT) transfusions were administered empirically for patients with PLT counts of 20 × 10⁹/L or lower, or in patients who experienced bleeding. Mediastinal radiation was applied after ASCT on initially bulky mediastinal mass, if post ASCT positron emission tomography / computed tomography (PET/CT) was positive. Within post-transplant relapse, five patients received bortenuximab-vedotin (anti-CD30 antibody), and two more cases, as post-transplant consolidation due to high risk of relapse.

Regarding MM patients, a historical VAD regimen, as initial treatment was given in 36 patients (30.0%), Thalidomide-based combinations in 80 (66.7%) patients, and bortezomib-based regimes in 4 (3.3%) patients. Peripheral blood SCs were collected during 1–2 consecutive aphereses following mobilization protocol CAD. In poor mobilizers (6 patients), second mobilization was conducted with addition of Plerixafor with a sufficient number of CD34+ cells for transplant (≥ 4 × 10⁹/kgbm). In 5 patients, who underwent "tandem" ASCT, a target CD34+ cell dose of 8.0 × 10⁹/kgbm was collected. The conditioning regimen consisted of high dose melphalan, as a single agent at a dose of 200 mg/m², and at reduced dose of 100 or 140 mg/m² for patients with reduced creatinine clearance (30–60 mL/min) or with a high comorbidity index. Patient therapeutic response was evaluated according to criteria of the International Myeloma Working Group 16. Relapsed patients were treated with bortezomib-based combinations if they did not receive bortezomib initially.

The study was performed according to the guidelines of the Declaration of Helsinki and was approved by the local Ethics board.

Following ASCT, the OS was measured from the date of ASCT until the last follow-up or until death from any cause, while event free survival (EFS) was measured from the date of ASCT until the disease progression/relapse or the last follow-up. OS functions were calculated using the Kaplan-Mayer approach, while a log-rank test was used to compare statistical differences between curves. The cutoff points for recovery of absolute lymphocyte count (ALC) of 500 × 10⁹/L or greater (ALC500), ANC ≥ 500 × 10⁹/L (ANC500), and PLT count ≥ 20 × 10⁹/L (PLT20), by Day +20, Day +11, and Day +13, respectively, were calculated according to previously published data 7. The Spearman’s correlation coefficient was used to analyze correlations among variables of interest. In order to predict OS after ASCT, cutoff values of CD34+ cells for HL and MM, were determined as 25th and 75th percentile values of its distribution, respectively. Statistical analyses were done using IBM SPSS statistical package (Version 21). All statistical tests were two-sided. The level of significance (alpha level) in all analyses was set at p < 0.05.

**Results**

**Patient characteristics and cellular research**

The clinical and laboratory characteristics of HL and MM patients are summarized in Tables 1 and 2. A total of 90 patients with HL, and 120 patients with MM were analyzed.

The mean dose of transplanted CD34+ cells in HL patients was 7.1 × 10⁹/kgbm (range 2.5–8.0 × 10⁹/kgbm) in 250 mL harvest volume in average (range 100–650 mL). Twenty one patients (23.3%) had CD34+ cell doses of ≤ 5.0 × 10⁹/kgbm (25th percentile value). After administration of a conditioning regimen, the aplasia duration was 11 days in average (range 4–28 days). The median time for ALC500 recovery was 16 days (range 9–31 days), ANC500 was 12 days (range 6–26 days), and PLT20 was 12 days (range 5–44 days). After ASCT, 12 patients (13.3%) had progressive disease (PD), 3 had developed signs of stable disease (SD) (3.3%), 31 had a partial response (PR) (34.4%), and 44 had a complete response (CR) (48.9%) to therapy. There was not a strong correlation between achievement of CR and CD34+ cell doses, nor with recovery of ALC500, ANC500 and PLT20, or disease relapse. There was no difference regarding the clinical characteristics of patients who had received ≤ 5.0 × 10⁹/kgbm CD34+ cell dose compared to those who had received > 5.0 × 10⁹/kgbm CD34+ cells.

Regarding MM patients, the mean CD34+ cell dose administered was 5.0 × 10⁹/kgbm (range 2.5–7.73 × 10⁹/kgbm) in 300 mL harvest volume of (range 100–660 mL). Eighty two patients (68.3%) had CD34+ cell doses of 4.0–5.4 × 10⁹/kgbm (75th percentile value). After applying a conditioning regimen, the average aplasia duration was 8 days (range 4–21 days). The median time until ALC500 recovery was 15 days (range 7–23 days), until ANC500 was 12 days (range 7–24 days) and until PLT20 was 11 days (range 5–26 days). Following ASCT, five (4.2%) patients had PD, two (1.7%) had SD, 32 (26.7%) had PR, 52 (43.3%) patients had very good partial remission (VGPR), and 29 (24.2%) patients had CR. The number of infused cells was not predictive for the time required for lymphocyte, neutrophil or PLT engraftment. Disease relapse was confirmed in 62/113 (54.9%) patients. Bortezomib-based combinations in relapsed disease received 23/59 (40.0%) patients who were not initially treated with proteasome inhibitors.

Finally, the use of original cryopreservation protocol resulted with an acceptable CD34+ recovery (74.2 ± 12%) and cell viability. Namely, the mean fraction of non-viable harvested (fresh) and cryopreserved (post-thawed) 7-AAD positive cells was 2.58 ± 1.2% and 4.58 ± 2.9%, respectively.
### Table 1

**Clinical and laboratory characteristics of 90 patients with Hodgkin’s lymphoma**

| Clinical characteristics | Patients, n (%) |
|--------------------------|-----------------|
| Age at diagnosis (years), median [range] | 28 [18–46] |
| Age at ASCT (years), median [range] | 31 [20–52] |
| Male/female ratio, n | 50/40 (56/44) |
| Ann Arbor stage, n (%) | 57 (63.3) |
| III–IV | 77 (85.6) |
| Bulky disease, n (%) | 44 (48.9) |
| BM infiltration, n (%) | 4 (4.4) |
| IPS, n (%) | 28 (31.1) |
| low | 62 (68.9) |
| Pre-ASCT ECOG PS ≤ 1, n (%) | 73 (81.1) |
| Initial therapy, n (%) | 90 (100.0) |
| Conditioning regimen, n (%) | 76 (84.4) |
| BEAM | 14 (15.5) |
| CD34+ cell dose (mean ± SD = 7.1 ± 3.8 × 10⁶/kgbm), n (%) | | |
| < 5 × 10⁶/kgbm | 21 (23.3) |
| > 5 × 10⁶/kgbm | 69 (76.7) |
| ALC, n (%) | 24 (26.7) |
| ≥ 20 × 10⁹/L | 63 (70.0) |
| ANC, n (%) | 32 (35.6) |
| ≥ 11 × 10⁹/L | 75 (100.0) |
| PLT, n (%) | 71 (83.3) |
| ≥ 13 × 10⁹/L | 19 (21.1) |
| Pre/after ASCT treatment response, n (%) | | |
| CR/PR | 31 (34.4) |
| SD/PD | 54 (60.1) |
| Relapse after ASCT, n (%) | 60 (66.7) |
| Vital status, n (%) | 30 (33.3) |
| alive | 12 (10.0) |
| dead | 75 (83.3) |
| ASCT – autologous stem cell transplant; BM – bone marrow; IPS – International Prognostic Score; ECOG PS – Eastern Cooperative Oncology Group Performance Status; ABVD – adriamycin, bleomycin, vinblastine and dacarbazine; BEAM – carmustine, etoposide, cytarabine and melphalan; CBV – cyclophosphamide, carmustine and etoposide; ALC – absolute lymphocyte count; ANC – absolute neutrophil count; PLT – platelets; CR – complete remission; PR – partial remission; PD – progressive disease; SD – stable disease

### Table 2

**Clinical and laboratory characteristics of 120 patients with multiple myeloma**

| Clinical characteristics | Patients, n (%) |
|--------------------------|-----------------|
| Age at diagnosis (years), median [range] | 54 [22–65] |
| Age at ASCT (years), median [range] | 55.5 [23–65] |
| Male/female ratio, n | 66/54 (55.6/45.4) |
| Type of multiple myeloma, n (%) | | |
| IgG | 75 (62.5) |
| IgA | 23 (19.2) |
| Light chains | 16 (13.3) |
| IgD | 3 (2.5) |
| non-secretory | 3 (2.5) |
| Clinical Stage (Salmon and Durie), n (%) | | |
| I/II | 26 (21.6) |
| III | 94 (78.3) |
| Renal impairment, n (%) | 12 (10.0) |
| (serum creatinine ≥ 2 mg/dL; eGFR < 60 mL/min/1.73m²) | 9 (7.5) |
| at diagnosis | | |
| pre-ASCT | | |
| ISS | 70 (61.4) |
| 1+ 2 | 3 (27.2) |
| 3 | 7/34 (20.6) |
| High risk cytogenetics [del17p or t(4;14) or t(14;16)], n (%) | | |

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Table 2 (continued)

| Clinical characteristics | Patients, n (%) |
|--------------------------|-----------------|
| Initial therapy, n (%)   |                 |
| VAD                      | 36 (30.0)       |
| thalidomide-based        | 80 (66.7)       |
| bortezomib-based in induction | 4 (3.3)       |
| Conditioning regimen, n (%) |              |
| HD-melphalan             | 120 (100.0)     |
| CD34⁺ cell dose (mean ± SD = 5.0 ± 2.8 × 10⁶/kgbm), n (%) | |
| 2.5-4.0 × 10⁶/kgbm       | 18 (31.7)       |
| 4.0-5.4 × 10⁶/kgbm       | 82 (68.3)       |
| ALC500, n (%)            |                 |
| ≥ 20 × 10⁶/L             | 21 (17.5)       |
| ANC500, n (%)            | 68 (56.7)       |
| ≥ 11 × 10⁹/L             | 24 (20.0)       |
| Pre/after ASCT treatment response, n (%) |                 |
| CR/VGPR/PR               | 111 (92.5)/113(94.2) |
| SD/PD                    | 9 (7.5)/7(5.9)  |
| Relapse after ASCT, n (%) | 62/113 (54.9)   |
| Vital status, n (%)      |                 |
| alive                    | 76 (63.3)       |
| dead                     | 44 (36.7)       |

ASCT – autologous stem cell transplant; eGFR – estimated Glomerular Filtration Rate; ISS – International Scoring System; VAD – vincristine, adriamycin, dexamethasone; CAD – cyclophosphamide, adriamycin, dexamethasone; HD – high dose; ALC – absolute lymphocyte count; ANC – absolute neutrophil count; PLT – platelets; CR – complete remission; VGPR – very good partial remission; PR – partial remission; PD – progressive disease; SD – stable disease.

Transplant-related mortality of the patients was less than 1.0% (2/210), and was caused by parainfluenza viral infections. No high grade (III–IV) organ toxicity (cardiac, pulmonary, renal, or liver) was recorded.

Analysis of patients' survival

The median follow-up time for patients with HL was 67 months (range 12–192 months). Median EFS after ASCT was 20 months (range 1–119 months), and median OS after ASCT was 38 months (3–119 months). OS after ASCT wasn't influenced by gender, presence of B symptoms, bulky disease and Ann Arbor clinical stage at diagnosis (p > 0.05). Initial IPS influenced EFS (p = 0.015), but not OS (p = 0.062). Pre-ASCT Eastern Cooperative Oncology Group Performance Status (ECOG PS) influenced both EFS and OS (p < 0.0001). Favorable pre-ASCT treatment response, as well as after-ASCT, strongly influenced EFS and OS (p < 0.0001). OS of the patients with an unfavorable treatment response (PD, SD) was very poor with a median survival of less than 12 months.

The patients with a favorable pre-ASCT treatment response (CR or PR), who had received a lower dose of CD34⁺ cells (≤ 5 × 10⁶/kgbm) experienced inferior EFS (Log Rank = 5.84; p = 0.016; median 50 months vs. median not reached) and OS (Log Rank = 8.076; p = 0.004; median 50 months vs. median not reached) (Figure 1 A, B).

Fig. 1 – Event free survival (A) and overall survival (B) following autologous stem cell transplant (ASCT) according to applied CD34⁺ cell dose in Hodgkin's lymphoma patients.
In these patients, OS was influenced by a prolonged recovery of ALC500 ≥ 20 days (Log Rank = 6.44; p = 0.011) as well as EFS (Log Rank = 5.76; p = 0.016). Early cell recovery of ANC500 by Day +11 was associated with improved OS (Log Rank = 4.03; p = 0.045), but was of borderline significance regarding EFS (Log Rank = 3.59; p = 0.058). Different conditioning regimens (BEAM vs. CBV) influenced neither EFS nor OS (p > 0.05). Multivariate analysis was done for the following variables: pre-ASCT ECOG PS, CD34+ cell dose (> 5 × 10^6/kgbm vs. ≤ 5 × 10^6/kgbm), ALC500 recovery by Day +20, and PLT20 recovery by Day +13. The analysis concluded that CD34+ cell dose was the most important parameter that influenced OS [hazard ratio (HR) = 6.67; 95% CI; 1.64–2.73; p = 0.008], along with ECOG PS [HR = 9.64; 95% CI; 2.39–38.94; p = 0.001]. Regarding parameters that influenced EFS (pre-ASCT ECOG PS, CD34+ cell dose > 5 × 10^6/kgbm vs. ≤ 5 × 10^6/kgbm; and ALC500 recovery by Day +20), again CD34+ cell dose [(HR) = 4.35; 95% CI; 1.165–16.13; p = 0.029], along with ECOG PS [(HR) = 10.0; 95% CI; 2.47–40.73; p = 0.001] significantly influenced OS.

The median follow-up time of MM patients was 52 months (range 10–190 months). The median EFS after ASCT was 25 months (range 1–106 months), and OS after ASCT was 34 months (1–114 months). Variables of gender, age, type of M protein, clinical stage, and ISS, didn’t have influence on EFS or OS after ASCT (p > 0.05). However, the presence of renal impairment at diagnosis and pre-ASCT influenced EFS and OS (p < 0.05). Any favorable treatment response (CR, PR, VGPR) before and after ASCT strongly influenced both EFS and OS (p < 0.0001). OS of the patients with an unfavorable treatment response was very poor with a median of 14 months. Different induction regimens (VAD vs. Thalidomide-based combinations vs. bortezomib-based combinations) influenced neither EFS nor OS (p > 0.05).

Regarding patients who achieved a pre-ASCT favorable treatment response, with respect to clinical parameters, only the presence of pre-transplant renal impairment affected EFS (p = 0.009) and OS (p = 0.005). Furthermore, patients who had an inferior CD34+ cell dose applied (< 4 × 10^6/kgbm) had diminished EFS (Log Rank = 8.61; p = 0.003; median 48 months vs. median not reached) and OS (Log Rank = 10.67; p = 0.001; median 55 months vs. not reached) (Figure 2 A, B).

Discussion

Previous reports showed that many clinical and laboratory variables after ASCT in hematological malignancies were associated with better OS 7, 17–19. However, there is no firm evidence as to which parameter represents the best OS predictor.

Early lymphocyte, neutrophil and PLT recovery were reported to influence OS and EFS after ASCT in patients with HL 7, NHL 7, and MM 17. Our results suggest that a delayed recovery of ALC500 after Day +20, and PLT after Day +13 were associated with inferior OS and EFS in HL, while prolonged PLT20 recovery in MM patients correlated with inferior OS.

Although CD34+ cell dose was investigated as a potential factor that might affect early recovery of ALC500,
ANC500 and PLT20 after ASCT, this was not the case as determined by our study. The absence of any strong correlation between blood cell recovery and CD34+ cell dose in our study might be the result of additional variables such as different pre-transplant conditioning protocols that were administered to patients. Our data support the results of some previous studies that have suggested there might be an OS benefit from receiving higher CD34+ cell dose. The patients with HL who received lower CD34+ cell dose had shorter EFS as well as OS. The administration of CD34+ cell dose remained an independent predictive factor of OS in multi-variate models, which is in accordance with the study of Gordan et al., who have suggested the predictive role of CD34+ cell dose on OS in a mixed population of patients with HL and NHL undergoing ASCT. Additionally, it was demonstrated that ALC by Day +15 was an independent prognostic marker for the progression free survival (but not prognostic for OS), indicating faster overall recovery caused by CD34+ cell dose. Delayed ALC recovery and lower CD34+ cell dose may allow minimal residual disease to outgrow and overcome immunologic activity. Furthermore, current investigations in this field suggest the potential role of lymphocyte subsets that contribute to early immune reconstitution, and may have a protective role against residual disease progression, as well as possibility to better and safer mobilize lymphocyte subsets.

Since in both HL and MM patients the higher CD34+ cell doses correlated with an improved chance for OS, it is possible that receiving higher CD34+ dose indicates a "healthy" marrow which could mobilize more CD34+ cells, since better mobilization is not only represented by number of collected SCs. Furthermore, in the present study, MM patients had lower median collected and infused CD34+ cell dose compared to HL ones, which might be the consequence of age-related factors and poorer mobilization potential, since MM patients are more older compared to HL patients. Moreover, the bone marrow microenvironment likely has an additional, still unknown stimulating role in engraftment, especially in younger patients. In HL patients, not only did CD34+ cell dose independently influenced OS, but pre-transplant disease status showed prognostic significance on EFS and OS after ASCT. This may suggest that high dose chemotherapy followed by ASCT improves treatment response. Of particular interest in MM patients is the presence of renal impairment, which was of borderline significance regarding OS, and correlated with unfavorable EFS, possibly due to disease aggressiveness and reduced-dose melphalan for conditioning. However, some previous studies reported that MM patients with impaired renal function may have outcomes comparable to those with normal renal function, despite the use of conditioning dose reduction. This is mainly due to the usage of proteasome inhibitors in induction treatment, whose proportion in our study is rather small.

Although the current study has a few limitations including its retrospective nature, the fact that different conditioning regimens were used, the relatively limited number of patients and the inability to determine lymphocyte subsets, it points out the prognostic role of CD34+ cell dose as an easy detectable parameter that correlates with OS after ASCT.

Conclusion

Although SC transplant represents standard procedure in relapsed/refractory HL and MM patients, there is no variable that might help in identifying high-risk patients who underwent ASCT. The results obtained in this study confirm that advanced response through pre-ASCT treatment, early recovery of ALC500 and PLT20 (HL patients), as well as PLT20 (MM patients) could influence the patients' OS. Also, superior CD34+ cell dose could be a useful predictive factor for treatment efficacy. More precise evaluation of overall treatment effectiveness by ASCT required prospective CD34+ cell and some lymphocyte subsets investigations using randomized, controlled and larger clinical studies.

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