



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 $\geq 20 \times 10^6/L$ in peripheral blood with "target-dose" of CD34⁺ cells $\geq 5 \times 10^6/kgbm$ in harvest. The CD34⁺ cell count and viability was determined using flow cytometry. **Results.** The majority of HL patients (76.7%) were infused

with $> 5.0 \times 10^6/kgbm$ CD34⁺ cells, while 68.3% of MM patients were treated by approximately $4.0\text{--}5.4 \times 10^6/kgbm$ 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 hematološkim malignitetima kao što su

multipli mijelom (MM) nakon indukciono hemoterapije, agresivni non-Hodgkin-ovi limfomi (NHL) i recidivantni hemiosenzitivni 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 multiplim mijelomom posle ATMĆ. **Metode.** Ova studija obuhvatila je ukupno 210 bolesnika (90 HL/120 MM) koji su bili lečeni primenom ATMĆ. Mobilizacija matičnih ćelija (MĆ) izvedena je pomoću stimulujućeg faktora granulocitnih kolonija (G-CSF) [10–16 µg/kg telesne mase (tm)] posle hemioterapije. Za dokazane "slabe-mobilizatore" izvedena je dodatna mobilizacija upotrebom G-CSF (16 µg/kgtm) uz dodatak *Plerixafora* (24 ili 48 mg). Po našem saznanju, ovo je bila prva primena *Plerixafora* u našoj zemlji u okvirima ATMĆ. Priklupljanje ćelija je započeto jedino pri graničnoj, odnosno "cut-off" vrednosti $CD34^+ \geq 20 /\mu L$ u perifernoj krvi, sa "ciljnom dozom" $CD34^+ \geq 5 \times 10^6/kg$ telesne mase (tm) ćelija u afereznom produktu (*harvest*). Broj $CD34^+$ ćelija i vijabilnost bili su određivani primenom protočne citometrije. **Rezultati.** Većini bolesnika sa HL (76,7%) infundovano je $> 5,0 \times 10^6/kgtm$ $CD34^+$ ćelija, dok je 68,3% MM bolesnika tretirano dozom od $4,0-5,4 \times 10^6/kg$ tm $CD34^+$ ćelija. Povoljan terapijski odgovor (potpuna/parcijalna remisija) postignut je kod 83,3% (HL) i 94,2% bolesnika (MM). Od pa-

rametara koji su individualno uticali na preživljavanje bolesnika sa HL i povoljan odgovor na terapiju, multivarijantna analiza (status pre ATMĆ, primenjena doza $CD34^+$ ćelija, rani oporavak hematopoeze, tj. oporavak limfocita i trombocita) ukazali su na to da primena većih doza $CD34^+$ ćelija, zajedno sa karakteristikama pre ATMĆ, pozitivno korelira sa boljim preživljavanjem i izostanakom neželjenih događaja (IND), kao i UP posle ATMĆ. Kod bolesnika sa MM, multivarijantna analiza (oštećenje bubrega, doza primenjenih $CD34^+$ ćelija, rani oporavak trombocita) pokazala je da je broj infundovanih $CD34^+$ ćelija najznačajniji parametar koji ima uticaja na IND i UP bolesnika posle ATMĆ. **Zaključak.** Podaci dobijeni u ovoj studiji neosporno ukazuju na to da je infundovana doza $CD34^+$ ćelija nezavisan faktor koji može doprineti boljem kliničkom ishodu i UP bolesnika sa HL i MM posle ATMĆ.

Ključne reči:

hematološke neoplazme; matične ćelije; transplantacija, autologna; preživljavanje; 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 HL⁵, and MM⁶. Moreover, some ASCT parameters may also influence OS of transplanted patients including early lymphocyte, neutrophil and platelet recovery, infused lymphocyte dose, and the number of infused $CD34^+$ cells⁷. Of particular importance is the number of $CD34^+$ cells received by patients, which is a common predictor of the potential engraftment⁸. 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 treatment⁸, with calculation of the International Prognostic Score (IPS) for risk stratification⁹.

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 criteria¹². 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 $\geq 20 \times 10^6/L$ in peripheral blood. The "target-value" of harvested $CD34^+$ cells was $\geq 5 \times 10^6/kgbm$. Among of all patients, 6 (2.9%) were proven poor mobilizers. The second mobilization using G-CSF (16 µg/kgbm) and with Plerixafor [24 or 48 mg (one or two doses/bottles), approximately 6–11 hours prior to harvesting] was performed. For all of these patients, $\geq 4 \times 10^6/kgbm$ $CD34^+$ cells were collected. To our best knowledge, it was the first usage of the Plerixafor in the ASCT setting in our country.

Finally, 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 described¹⁵.

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 1.0×10^9 /L on two consecutive days. Platelet (PLT) transfusions were administered empirically for patients with PLT counts of 20×10^9 /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 brentuximab-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 regimens 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 ($\geq 4 \times 10^6$ /kgbm). In 5 patients, who underwent "tandem" ASCT, a target CD34⁺ cell dose of 8.0×10^6 /kgbm was collected. The conditioning regiment 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 Group¹⁶. 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 500×10^6 /L or greater (ALC500), ANC $\geq 500 \times 10^6$ /L (ANC500), and PLT count $\geq 20 \times 10^9$ /L (PLT20), by Day +20, Day +11, and Day +13, respectively, were calculated according to previously published data⁷. 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 7.1×10^6 /kgbm (range 2.5 – 8.0×10^6 /kgbm) in 250 mL harvest volume in average (range 100–650 mL). Twenty one patients (23.3%) had CD34⁺ cell doses of $\leq 5.0 \times 10^6$ /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 $\leq 5.0 \times 10^6$ /kgbm CD34⁺ cell dose compared to those who had received $> 5.0 \times 10^6$ /kgbm CD34⁺ cells.

Regarding MM patients, the mean CD34⁺ cell dose administered was 5.0×10^6 /kgbm (range 2.5 – 7.73×10^6 /kgbm) in 300 mL harvest volume of (range 100–660 mL). Eighty two patients (68.3%) had CD34⁺ cell doses of 4.0 – 5.4×10^6 /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 ($74.2 \pm 12\%$) and cell viability. Namely, the mean fraction of non-viable harvested (fresh) and cryopreserved (post-thawed) 7-AAD positive cells was $2.58 \pm 1.2\%$ and $4.58 \pm 2.9\%$, 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 (%)	
III–IV	57 (63.3)
B symptoms, n (%)	77 (85.6)
Bulky disease, n (%)	44 (48.9)
BM infiltration, n (%)	4 (4.4)
IPS, n (%)	
low	28 (31.1)
high	62 (68.9)
Pre-ASCT ECOG PS \leq 1, n (%)	73 (81.1)
Initial therapy, n (%)	
ABVD	90 (100.0)
Conditioning regimen, n (%)	
BEAM	76 (84.4)
CBV	14 (15.5)
CD34 ⁺ cell dose (mean \pm SD = $7.1 \pm 3.8 \times 10^6$ /kgbm), n (%)	
$< 5 \times 10^6$ /kgbm	21 (23.3)
$> 5 \times 10^6$ /kgbm	69 (76.7)
ALC, n (%)	
$\geq 20 \times 10^9$ /L	24 (26.7)
ANC, n (%)	
$\geq 11 \times 10^9$ /L	63 (70.0)
PLT, n (%)	
$\geq 13 \times 10^9$ /L	32 (35.6)
Pre/after ASCT treatment response, n (%)	
CR/PR	71 (78.9)/ 75 (83.3)
SD/PD	19 (21.1)/ 15 (16.7)
Relapse after ASCT, n (%)	31/75 (41.3)
Vital status, n (%)	
alive	60 (66.7)
dead	30 (33.3)

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 (%)	
(serum creatinine \geq 2 mg/dL; eGFR $<$ 60 mL/min/1.73m ²)	12 (10.0)
at diagnosis	9 (7.5)
pre-ASCT	
ISS	
1+2	70 (61.4)
3	31 (27.2)
High risk cytogenetics [del17p or t(4;14) or t(14;16)], n (%)	7/34 (20.6)

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 × 10 ⁶ /kgbm), n (%)	
2.5-4.0 × 10 ⁶ /kgbm	18 (31.7)
4.0-5.4 × 10 ⁶ /kgbm	82 (68.3)
ALC500, n (%)	
≥ 20 × 10 ⁹ /L	21 (17.5)
ANC500, n (%)	
≥ 11 × 10 ⁹ /L	68 (56.7)
PLT20, n (%)	
≥ 13 × 10 ⁹ /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 ($\leq 5 \times 10^6$ /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).

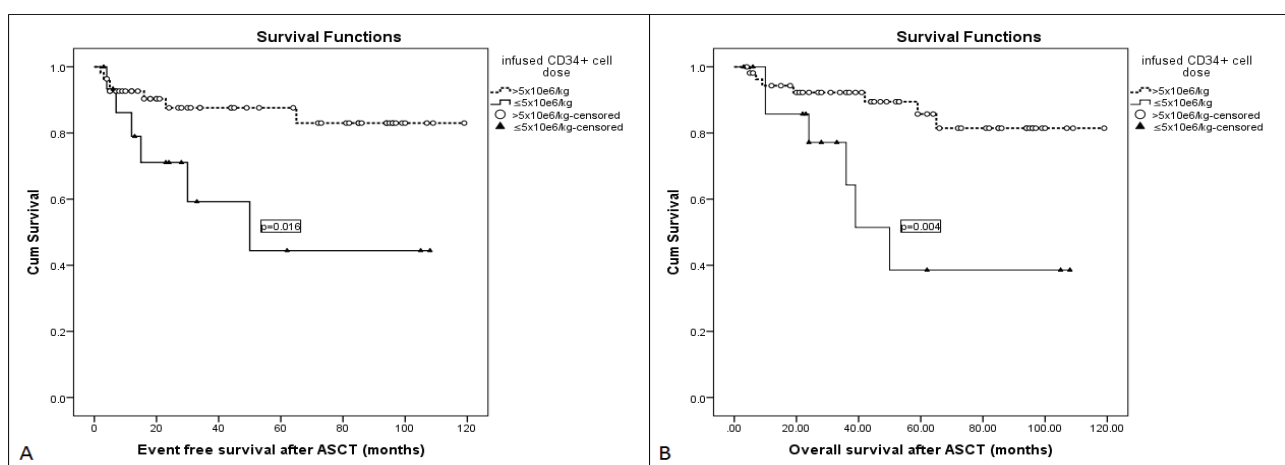


Fig. 1 – Event free survival (A) and overall survival (B) following autologous stem cell transplant (ASCT) according to applied CD34⁺ cell dose in Hodgkin's lymphoma patients.

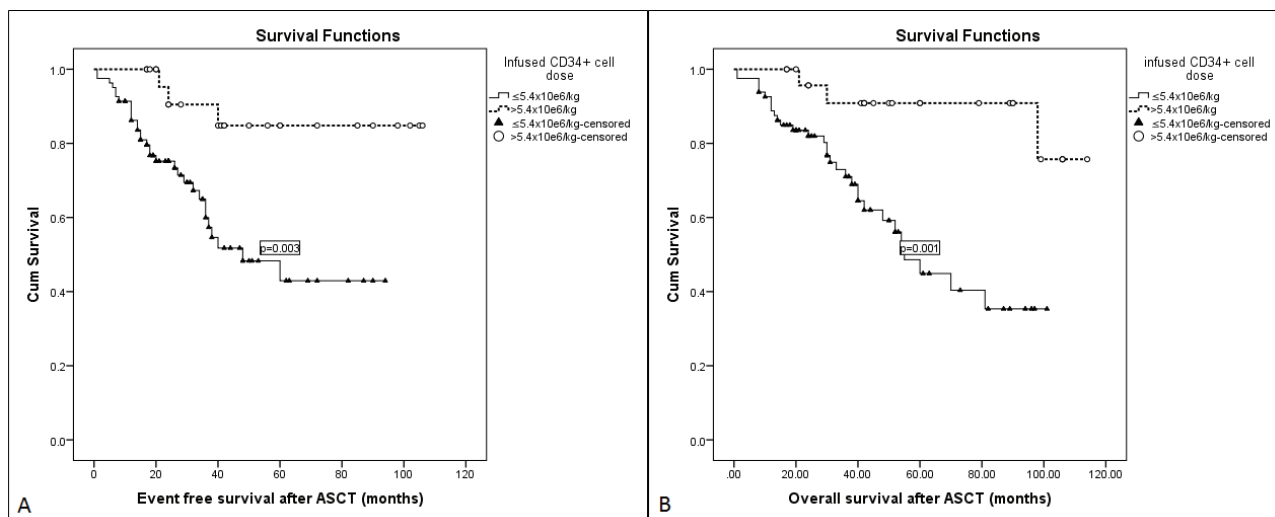


Fig. 2 – Event free survival (A) and overall survival (B) following autologous stem cell transplant (ASCT) according to applied CD34⁺ cell dose in multiple myeloma patients.

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 not in correlation with OS or EFS ($p > 0.05$). However, early PLT recovery by Day +13 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 ($> 5 \times 10^6/\text{kgbm}$ vs. $\leq 5 \times 10^6/\text{kgbm}$), ALC500 recovery by Day +20, and PLT20 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 $> 5 \times 10^6/\text{kgbm}$ vs. $\leq 5 \times 10^6/\text{kgbm}$; 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. Tthalidomide-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 ($< 4 \times 10^6/\text{kgbm}$) 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).

Early PLT recovery by Day +13 was associated with improvement in both OS (Log Rank = 6.98; $p = 0.008$), and EFS (Log Rank = 9.01; $p = 0.003$). Other parameters (ALC and ANC) weren't of OS significance.

Regarding OS, multivariate analysis was done concerning the following variables: infused CD34⁺ cells (> 5.4 vs. $\leq 5.4 \times 10^6/\text{kgbm}$), PLT20 recovery by Day +13, and presence of the pre-ASCT renal impairment. The results of the analysis showed that a CD34⁺ cell dose was the most important parameter that influenced OS (HR = 4.59; 95% CI; 1.314–16.057; $p = 0.017$) and EFS (HR = 3.55; 95% CI; 1.069–11.780; $p = 0.038$), while the presence of renal impairment correlated with inferior EFS (HR = 0.39; 95% CI; 0.167–0.953; $p = 0.039$), and was of borderline influence on OS (HR = 0.39; 95% CI; 0.159–0.999; $p = 0.05$).

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 HL², NHL⁷, and MM¹⁷. 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^{7,20}, 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^{7,21,22}. 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 multivariate models, which is in accordance with the study of Gordan et al.²³, 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 activity²⁴. 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^{25,26}.

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 SCs⁷. 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 response⁵. 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 reduction²⁷. 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 correlate 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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