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The efficacy and safety of autologous stem cell transplantation in relapsed chemosensitive and chemoresistant patients with diffuse large B-cell lymphoma

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High-dose chemotherapy followed by autologous hematopoietic stem cell transplantation (ASCT) remains a valuable therapeutic approach for relapsed and refractory (R/R) patients with diffuse large B-cell lymphoma (DLBCL). The aim of the study was to evaluate the safety and clinical outcome of ASCT for R/R DLBCL. We present a retrospective series of ASCT for 53 DLBCL patients (30 males and 23 females) at the median age of 51 years. Patients were eligible for transplantation if they achieved partial, second, or subsequent response or remained stable to at least 2 prior treatments. Median overall (OS) and progression-free (PFS) survivals were 9 and 6.3 years, respectively. The estimated 4-year OS and PFS were found to be 75% and 69%, respectively. In univariate analysis liver involvement, clinical stage at diagnosis, lymphocyte/monocyte count, and status of clinical response at ASCT were found to influence OS, however, only absolute lymphocyte count remained significant in multivariate analysis (HR 1.42 [95% CI: 1.08-1.87]; p=0.01). Median follow-up from ASCT to the last contact was 4.4 years (range 0.03-18.7). In total, 26 patients died from disease progression and subsequent resistance to chemotherapy. At the last contact, 27 patients were alive in remission. Only a single patient died shortly after ASCT due to infectious complications. Grade 3 or 4 non-hematological side effects were not observed in the remaining patients. ASCT for RR DLBCL is a safe procedure with a high probability of overall and progression-free survival.

Key words: diffuse large B-cell lymphoma, autologous hematopoietic stem cell transplantation, relapse, refractoriness, outcome

Diffuse large B-cell lymphoma (DLBCL) remains a common type of lymphoid neoplasm accounting for about 30–40% of all cases of non-Hodgkin lymphoma (NHL) [1, 2]. It occurs primarily in older individuals, with a median age at diagnosis of approximately 60–70 years, although it can also be seen in children and young adults [2]. DLBCL can be classified based on gene expression profiling (GEP) of tumor tissue into three subgroups: germinal centre B-cell subtype (GCB), activated B-cell subtype (ABC), and unclassifiable. Despite presenting with an aggressive clinical course, patients within the GCB category have a significantly better overall survival than those with ABC following first-line treatment with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) regimen [3, 4]. Approximately 10–15% of all DLBCL patients treated with R-CHOP regimen will eventually relapse within a year from diagnosis and have dismal prognosis [4]. Relapses usually arise at sites, which differ from those of the original location [5]. High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) remains the standard of care for chemosensitive relapsed disease. Chemo-resistant relapse prior to ASCT is associated with particularly poor prognosis [3, 4, 6]. It was demonstrated that 3-year progression-free (PFS) and overall survivals (OS) for DLBCL patients undergoing ASCT after different salvage regimens are about 50% [3].

Herein we report on prognostic factors and safety of ASCT in 53 relapsed/refractory (R/R) DLBCL patients transplanted in our center during the last 20 years.

Patients and methods

Fifty-three patients (30 males and 23 females) at the median age of 51 years at diagnosis (range 16–66) underwent ASCT between 2000 and 2018. The management of patients after diagnosis followed common standards. Histological diagnosis was established by a local pathologist using immunochemistry. The disease stage was evaluated according to the Ann Arbor staging system and (age-adjusted) International Prognostic Index (IPI) score was calculated as published elsewhere [7, 8]. The diagnostic work-up included physical examination, complete blood count with differential and biochemistry tests. Imaging studies including computed

tomography (CT) of the neck, chest, abdomen, and pelvis, and/or positron tomography scan (PET) were performed at diagnosis and for response assessment. Bone marrow biopsy was performed at diagnosis and as needed. Patients were eligible for ASCT if they met the following criteria: 1) partial response (PR) or second or higher complete (CR) remission after conventional immuno-chemotherapy; 2) stable disease (SD); 3) ECOG status 0 to 2; 4) age <70 years; and 5) adequate hepatic, renal, and cardiac function. All patients signed informed consent. The clinical characteristics of patients are presented in Table 1. The distribution of patients entering the study is shown in Figure 1.

Treatment. Induction chemotherapy was unified and consisted of CHOP+/– R (rituximab, cyclophosphamide, vincristine, adriamycin, prednisone). The second and third-line therapeutic options included R-ESHAP (rituximab, cisplatin, methylprednisolone, etoposide, and cytarabine), R-DHAP (rituximab, cisplatin, cytarabine, and dexamethasone), and RB (rituximab, bendamustine) regimens. Subsequent lines included different combined regimens. Forty-four patients (83%) received at least one rituximab-containing therapeutic protocols. The median number of treatment lines before ASCT was 2 (range 2–6). Overall, 8 patients achieved second or higher CR at ASCT, 31 were transplanted in PR whereas 14 remained chemo-resistant (SD). Peripheral blood was the source of stem cells for ASCT in all patients. IVE regimen (ifosfamide, etoposide, epirubicin) was used for stem cell mobilization. G-CSF (granulocyte colony stimulating factor) at 10 μg/kg/day was started from day +5 until the last day of apheresis. More than 2×10^6 CD34-positive cells/kg were found to be sufficient for transplant; however, that was not a case in one patient. The apheresis product was processed, frozen to –150 °C, stored, and re-infused after completion of conditioning. The conditioning consisted of CBV (cyclophosphamide, carmustine, etoposide) in 24 patients, BEAM (carmustine, cytarabine, etoposide, melphalan) in 19 and 10 patients received BeEAM (bendamustine, cytarabine, etoposide, melphalan).

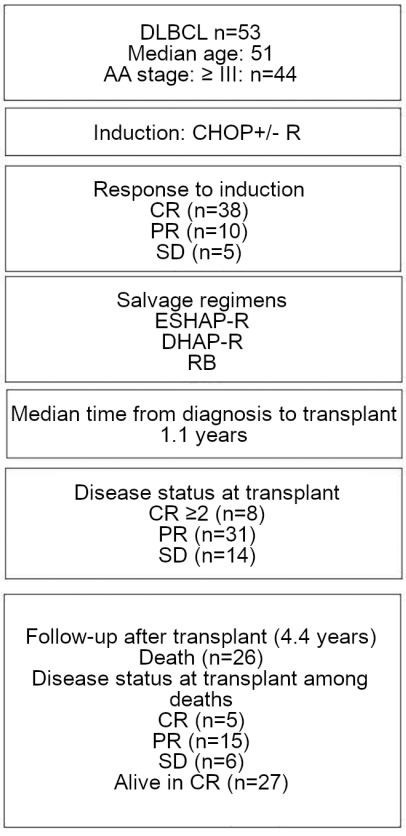

Figure 1. Distribution of patients entering the study.

Table 1. Patients characteristics.

Parameter	DLBCL (n=53)
male/female; no	30/23
median age at diagnosis; (years, range)	51 (16–66)
age >60 years; no; %	8 (15)
median hemoglobin level (g/dl; range)	12.1 (5.2–16.3)
median platelet count ($\times10^9$/l; range)	241 (54–655)
median leukocyte count ($\times10^9$/l; range)	7.8 (0.7–15.9)
median lymphocyte count ($\times10^9$/l; range)	1.5 (0.01–10.5)
median monocyte count ($\times10^9$/l; range)	0.5 (0.0–3.02)
median LDH (IU/l)	234 (99–1298)
>normal range, n; %	22 (42)
median B2M (mg/l)	2.98 (1.3–12.7)
>normal range, n; %	15 (28)
median number of involved lymph node regions	3 (0–7)
extra-nodal involvement	19 (36)
hepatomegaly, n; %	37 (70)
splenomegaly, n; %	31 (58)
bone marrow involvement at diagnosis; no, %	13 (24)
central nervous system involvement at diagnosis; no, %	0 (0)
clinical stage; no, %	
I	1 (2)
II	8 (15)
III	10 (19)
IV	34 (64)
age-adjusted IPI risk; no, %	45 (100)
low	2 (4)
low-intermediate	15 (33)
high-intermediate	21 (47)
ND	7 (16)
IPI risk; no, %	8 (100)
low-intermediate	1 (13)
high-intermediate	4 (50)
high risk	1 (13)
ND	2 (14)
B symptoms; no, %	41 (77)
median number of treatment lines	2 (2–6)
median number of treatment cycles; range	10 (3–20)
rituximab containing regimen pre-ASCT	44 (83)
radiotherapy prior ASCT; no, %	25 (47)

Abbreviations: ASCT – autologous hematopoietic stem cell transplantation; B2M – beta2microglobulin; DLBCL – diffuse large B-cell lymphoma; LDH – lactate dehydrogenase; ND – no data

Response criteria. The response to therapy was evaluated at 3 and 6 months after ASCT and 6 months thereafter using CT+/–PET. CR was defined as the disappearance of all disease-related symptoms and measurable lesions for at least 4 weeks; PR was defined as a >50% decrease in the size of the tumor for at least 4 weeks. The stable disease was defined as <50% reduction in the size of the tumor with chemotherapy preceding ASCT.

Statistical methods. The probability OS and PFS were calculated according to the Kaplan-Meier method. All calculations were made from the date of transplantation. Comparisons between the variables were carried out by the log-rank test. Statistical significance was defined at a p-value <0.05. Transplant-related mortality (TRM) was defined as death within 30 days of high-dose therapy not related to the disease, relapse, and progression. Proportional hazards models (Cox regression) were fitted to investigate the effects of prognostic factors for OS. The following factors were entered into model 1) patient-related: age, clinical stage, liver, spleen, bone marrow involvements, blood parameters, number of chemotherapy lines, use of rituximab and radiotherapy, disease status at transplant and 2) transplant-related: age, type of conditioning, and date of transplant. All computations were performed with StatSoft Poland analysis software (version 12.0).

Table 2. Transplant data.

Parameter	DLBCL (n=53)
median time from diagnosis to ASCT; years, range	1.1 (0.5–5.1)
median age at transplant; years, range	51 (18–67)
disease status at ASCT; no, %	
CR ≥ 2	8 (15)
PR	31 (58)
SD	14 (27)
type of conditioning; no, %	
CBV	24 (45)
BEAM	19 (36)
BeEAM	10 (19)
median number of transplanted CD34-positive cells ($\times 10^6$/kg)	5.6 (1.3–29.3)
median ANC >0.5 ($\times 10^9$/l); days, range	11 (9–18)
median PLT >20 ($\times 10^9$/l); days, range	12 (4–20)
median follow-up post ASCT; years, range	4.4 (0.03–18.7)

Abbreviations: ANC – absolute neutrophil count; ASCT – autologous hematopoietic stem cell transplantation; BEAM – carmustine, etoposide, cytarabine, melphalan; BeEAM – bendamustine, etoposide, cytarabine, melphalan; CR – complete response; CBV – cyclophosphamide, carmustine, etoposide; DLBCL – diffuse large B-cell lymphoma; SD – stable disease; PLT – platelets; PR – partial response

Results

Cell dose and engraftment. The median number of CD34-positive cells was 5.6×10^6/kg (range 1.3–29.3). All patients engrafted. The median time to neutrophil recovery was 11 days (range 9–18) and platelet count $>20\times10^9$/l occurred after median of 12 days (range 4–20).

Adverse events. Thirty-five patients manifested side effects in aplasia period after ASCT. The most common complaints included mucositis, diarrhea, and infections within the upper respiratory tract. No patient had bacteremia within the first 100 days after ASCT. Grade 3 or 4 non-hematological adverse events were not observed except severe bilateral pneumonia which led to respiratory insufficiency and death in one patient. All patients required G-CSF support at the early post-transplant period.

Outcome and prognostic factors. The transplant-related mortality was 2% at day +30 after ASCT. Median OS was 9 years whereas PFS reached 6.3 years. The estimated 4-year OS and PFS were found to be 75% and 69%, respectively (Figure 2). Interestingly, patients who were transplanted in PR fared much better than those in CR or SD in terms of OS and PFS; 88% vs. 62% vs. 58%; p=0.01 and 78% vs. 58% and 57%; p=0.005, respectively (Figure 3). The rituximab-containing regimen before ASCT did not have an impact on OS. In univariate analysis, liver involvement, clinical stage, and disease status at ASCT as well as lymphocytosis and monocytosis were found to influence OS, however, only lymphocytosis remained significant in multivariate analysis (HR 1.42; 95% CI: 1.08–1.87; p=0.01).

Median follow-up from ASCT to the last contact was 4.4 years (range 0.03–18.7). In total, 26 patients died from disease progression and subsequent resistance to chemotherapy. There were 15 deaths in patients transplanted with PR, 5 in CR, and 6 in SD. The single patient died shortly after ASCT due to infectious complications. At the last contact, 27 patients are alive in remission.

Discussion

Standard therapy for relapsed or refractory patients with DLBCL encompasses non-cross-resistant chemotherapy with monoclonal antibody followed by high-dose chemotherapy and ASCT for those with the chemo-sensitive disease. Patients who did not respond to salvage chemotherapies were found to have the worst prognosis with a median survival of less than 6 months. Of note is that only a few of them are candidates for ASCT [9]. We retrospectively collected data of chemo-sensitive and chemo-refractory DLBCL patients who were auto-transplanted in our center during the last 20 years. More than 80% of them received at least one rituximab-containing regimen and the median number of treatment lines was 2. Thirty-nine patients (73%) were sensitive to chemotherapy at transplant (8 CR and 31 PR) whereas the remaining 14 (27%) remained primary resistant.

To date, there is no evidence on the superiority of one salvage regimen over another in R/R DLBCL patients. ESHAP and DHAP plus rituximab remained the most common

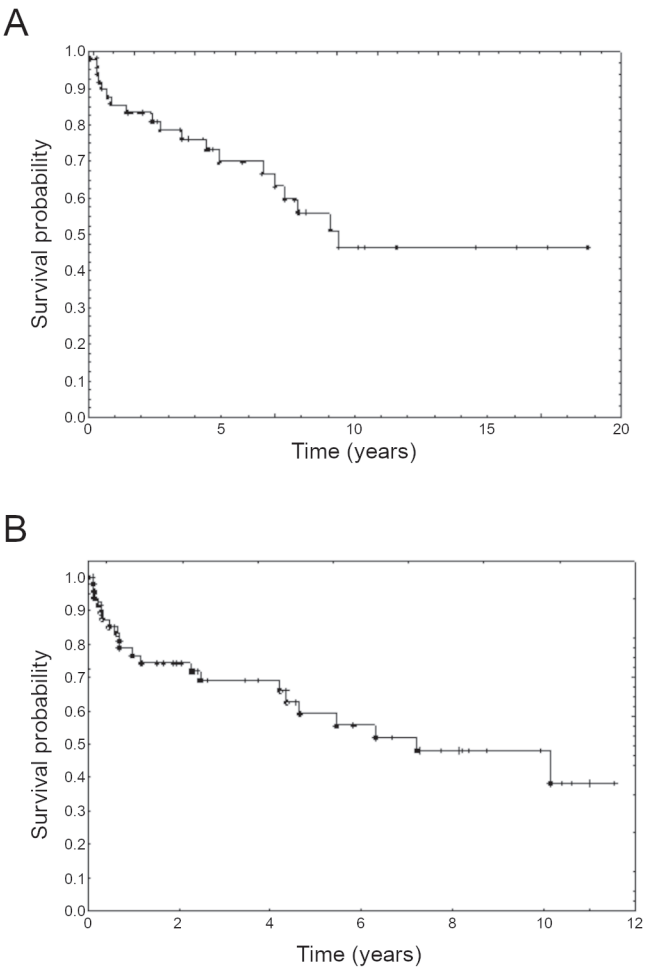

Figure 2. Overall (A) and progression-free survival (B) curves for relapsed/refractory diffuse large B-cell lymphoma after autologous hematopoietic stem cell transplantation.

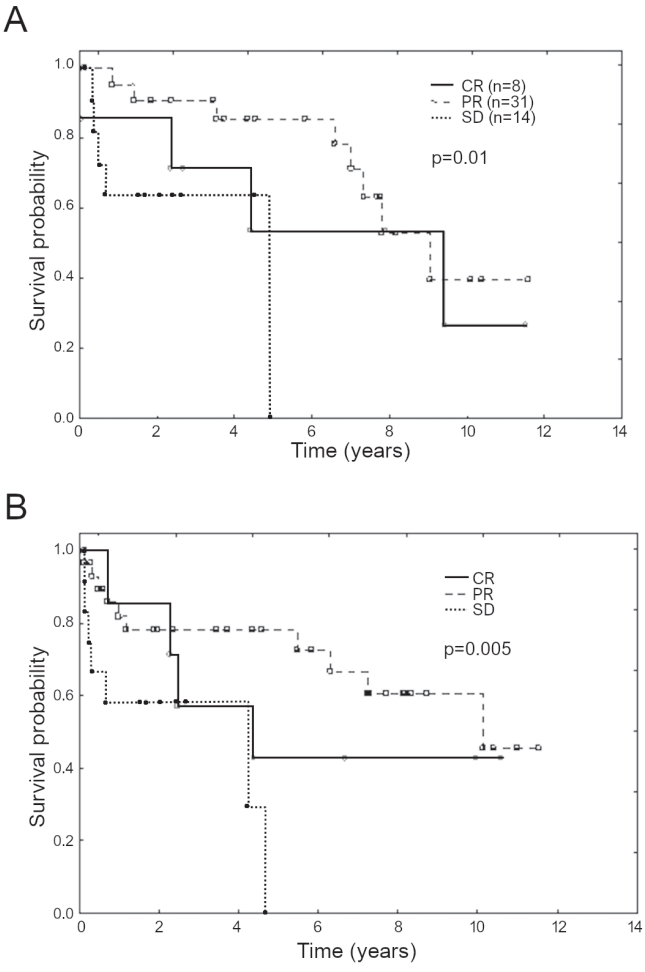

Figure 3. Overall (A) and progression-free survival (B) curves for relapsed/refractory diffuse large B-cell lymphoma depending on disease status at transplant.

regimens used in our patients. In the CORAL study [9], the patients were randomized to 3 cycles of R-ICE or R-DHAP followed by ASCT when responded. No significant difference was demonstrated between those two arms. Several other regimens have been attempted with no clear advantage of one over another [10]. It should however be mentioned that the outcome was much better in those who did not receive rituximab during the first-line therapy [9, 11]. Most of our patients were exposed to rituximab either during induction and salvage treatments thus its impact on clinical outcome was difficult to elucidate. Nevertheless, there was no difference in OS between patients with or without prior rituximab. Most patients who proceeded to ASCT after salvage regimens demonstrate a chemo-sensitive disease. In the CORAL study [9], the patients who responded to the salvage regimen proceeded to ASCT and then were randomized to rituximab maintenance for 1 year or observation. The median follow-up was 44 months and PFS reached 52% and 53% for rituximab and no-rituximab arm. The following factors had an impact on post ASCT outcome: 1) early relapse (less than 12 months) after first-line treatment 2) secondary aaIPI > 1 and 3) prior rituximab administration. There was no benefit of rituximab maintenance after ASCT. The efficacy of ASCT in R/R DLBCL setting has also been demonstrated by EBMT (European Blood and Marrow Transplantation Registry) analysis. The 5-year PFS and OS after ASCT were 48% and 63%, respectively. A significant increase in PFS after transplantation was observed for patients with longer duration of CR1 but not in those with prior exposure to rituximab and early relapse [12]. Of note is that PFS and OS in our chemosensitive transplanted patients were much better than those presented by others [9, 12, 13]. PFS and OS at 4 years after ASCT were 69% and 75%, respectively. Surprisingly, patients transplanted in PR fared much better in terms of OS than those in CR (88% vs. 62%). However, one should bear in mind that the groups were small and the data interpretation might be confused by the fact that CT was only used for response assessment in patients transplanted before 2014.

The results of ASCT for DLBCL patients who remained resistant to the salvage regimens are highly unsatisfactory. There are single reports on the results of ASCT for patients being in stable (SD) or progressive disease (PD) at transplantation. Patients with the chemo-sensitive disease at ASCT had a median PFS of 23 months whereas those with SD less than 4 months [14]. A similar dismal outcome was demonstrated by Gutman et al. [15]. Forty DLBCL patients were auto-transplanted while chemo-resistant. At the last contact, thirty-three patients died with an estimated 3-year OS of 21%. The patients, who failed the second-line salvage regimen in the CORAL study [16], were then treated with the third-line of chemotherapy. Among the 145 included patients, 56 had a response and received ASCT. Median OS was 11 months with a 2-year OS of 34%. One may conclude that some patients may benefit from subsequent treatments even when insensitive to the second salvage regimen.

On the contrary, we observed quite satisfactory results in 14 chemo-resistant patients (SD) who received ASCT in our study with a 4-year OS of 58%. It may indicate that ASCT can overcome chemo-resistance and increases survival. However, these results should be treated with caution as the number of included patients in particular groups was low. Moreover, PET scan has only been used for disease assessment in recent years and this may also affect the interpretation of the results.

Nevertheless, ASCT is not recommended in a chemo-resistant disease. These patients should be offered participation in the clinical trial or receive CAR-T therapy [3].

We looked also at the preparative regimens. The conditioning varied during the years of observation; however, the CBV regimen was the commonest. There was a single report that CBV was found to be less favorable in terms of TRM and OS over BEAM [17], but this finding was not confirmed by others [18].

Several factors have influenced OS in univariate analysis, however, only lymphocytosis remained significant in multivariate analysis. It was demonstrated that absolute lymphocyte count (ALC) at diagnosis and at the time of recovery is an independent predictor of survival in DLBCL patients [19, 20]. It seems likely that host immunocompetence plays a crucial role in response to therapy and survival and ALC remains a helpful surrogate marker. Nevertheless, the predicting role of ALC on the outcome of ASCT for DLBCL has not been reported so far.

Of note is that ASCT remained a safe procedure despite the fact that all patients received several lines of proceeding chemotherapy. Only one death was observed within the first 30 days after transplantation. The other adverse events were manageable.

In conclusion, ASCT for R/R DLBCL is a feasible and safe procedure with a high proportion of patients achieving long-term response. ASCT can overcome the chemo-resistance and be effective in some patients with DLBCL and unsuccessful prior salvage regimens.

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