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Original Article

J Hematol. 2021;10(6):246-254

Blood Graft and Outcome After Autologous Stem Cell Transplantation in Patients With Primary Central Nervous System Lymphoma

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Abstract

Background: Autologous stem cell transplantation (auto-SCT) is a treatment option for patients with primary central nervous system lymphoma (PCNSL).

Methods: In this prospective multicenter study, the effects of blood graft cellular content on hematologic recovery and outcome were analyzed in 17 PCNSL patients receiving auto-SCT upfront.

Results: The infused viable $CD34^+$ cell count > 1.7×10^6/kg correlated with more rapid platelet engraftment (10 vs. 31 days, P = 0.027) and with early neutrophil recovery (day + 15) (5.4 vs. 1.6×10^9/L, P = 0.047). A higher number of total collected $CD34^+$ cells > 3.3×10^6/kg infused predicted worse 5-year progression-free survival (PFS) (33% vs. 100%, P = 0.028). In addition, $CD3^+CD8^+$ T cells > 78×10^6/kg in the infused graft impacted negatively on the 5-year PFS (0% vs. 88%, P = 0.016).

Conclusion: The cellular composition of infused graft seems to impact on the hematologic recovery and PFS post-transplant. Further studies are needed to verify the optimal autograft cellular content in PCNSL.

Keywords: $CD34^+$ cell mobilization; Primary central nervous lymphoma; Autologous stem cell transplantation; Autograft cellular composition; Outcome

Manuscript submitted October 21, 2021, accepted November 30, 2021
Published online December 13, 2021

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doi: https://doi.org/10.14740/jh939

Introduction

Primary central nervous system lymphoma (PCNSL) accounts only about 1-2% of all non-Hodgkin lymphomas. Diffuse large B-cell lymphoma (DLBCL) is the most common histopathological entity of this challenging extranodal disease entity with increasing incidence [1-3]. Autologous stem cell transplantation (auto-SCT) is a widely used treatment option in transplant-eligible patients with PCNSL [4-8], although the superiority of auto-SCT after methotrexate-based induction therapy has not been validated due to a paucity of randomized studies [4, 5, 9-11]. A randomized PRECIS study [8] concluded that upfront auto-SCT is an effective treatment option in PCNSL in line with a previous phase II study [12] and a more recent retrospective analysis [13]. In addition, a prospective German study suggested that auto-SCT as the first-line consolidation is a feasible treatment also for selected elderly patients [14].

Until now, the optimal thresholds of autograft cellular composition to be infused for lymphoma patients have not been established even if the most important parameter of graft quality has suggested to be the graft $CD34^+$ cell content [15-17]. In the most recent prospective study of DLBCL patients including also some PCNSL patients [18], a cut-off point > 2.65×10^6/kg of $CD34^+$ cells infused was associated with not only more rapid hematologic recovery but in line with previous retrospective observations [19, 20] also with better overall survival (OS). Higher $CD34^+CD133^+CD38^-$ cell content of the graft has been shown to hasten hematologic recovery after auto-SCT [21] and in DLBCL patients also to correlate with better 5-year OS [18]. In previous studies, a higher amount of natural killer (NK) cells in the grafts has been linked with earlier lymphocyte recovery and also with better outcome [22-24]. In addition, the most recent prospective study showed a positive correlation between the number of infused $CD3^+$ cells and outcome in DLBCL patients mostly with systemic disease [18].

At present, the more detailed data on the significance of $CD34^+$ cell mobilization and blood grafts cellular composition

on outcome in PCNSL patients are lacking. The aim of this prospective study was to analyze the impact of mobilization characteristics and cellular composition of blood grafts on hematologic recovery and outcome in PCNSL patients with an upfront auto-SCT as a part of a prospective multicenter graft and outcome in autologous stem cell transplantation (GOA) study.

Materials and Methods

Patients

The present study population consisted of 17 patients with PCNSL who received an upfront auto-SCT between May 2012 and December 2018 at the University Hospitals of Kuopio and Oulu in Finland. As an induction treatment (median four cycles), nine patients (53%) received therapy according to Bonn-protocol [25] consisting of systemic and intrathecal methotrexate- and cytarabine-based chemotherapy added to blood-brain barrier disruption (BBBD) treatment [26], four patients (23%) MATRix treatment (methotrexate, cytarabine, thiotepa and rituximab) combined to BBBD therapy, two patients (12%) MATRix-induction alone and two patients (12%) Bonn-protocol therapy alone. The patients participated from the time point of apheresis in the prospective, non-interventional multicenter GOA study. The main aims in the GOA study were to evaluate the impact of different mobilization methods on the cellular composition of blood grafts collected and possible correlations of graft cellular composition with hematologic and immune recovery as well as outcome after auto-SCT. The main patient and mobilization characteristics of the patients are shown in Table 1.

Mobilization and collection of blood grafts

All the patients received chemotherapy combined with granulocyte colony-stimulating factor (G-CSF) in order to mobilize $CD34^+$ cells (Table 1). The mobilizing chemotherapy was chosen according to the institutional standards of care. The majority of patients received BBBD treatment as a mobilization therapy [27]. BBBD treatment composed of intra-arterial mannitol infusion followed by methotrexate and carboplatin with intravenous rituximab, etoposide and cyclophosphamide. Blood $CD34^+$ cell counts were measured with a flow cytometry using an International Society of Hemotherapy and Graft Engineering (ISHAGE) protocol [28]. Aphereses were initiated when blood $CD34^+$ cell counts rose over 10×10^6/L, and the minimal collection target of 2.0×10^6/kg $CD34^+$ cells to proceed to auto-SCT was used. In hard-to-mobilize patients, pre-emptive plerixafor (PLER) was given when blood $CD34^+$ cell counts were still less than 10×10^6/L and simultaneously rising white blood cell counts were over 5×10^9/L [29]. PLER use was also thought of, if the yield of the first apheresis was unsatisfactory ($< 1 \times 10^6$/kg $CD34^+$ cells) or if blood $CD34^+$ cell counts were decreasing before the adequate apheresis yield was achieved.

Table 1. Patient and Mobilization Characteristics in Patients With PCNS Lymphoma

Variables	n = 17
Gender	
Female	10 (59)
Male	7 (41)
Age at auto-SCT, years, median (range)	62 (45 - 73)
LDH serum level at diagnosis[a]	
Normal	10 (59)
Elevated	5 (29)
CSF protein concentration at diagnosis[b]	
Normal	8 (47)
Elevated	6 (35)
Involvement of deep region of the brain at diagnosis	
No	7 (41)
Yes	10 (59)
Mobilization chemotherapy	
BBBD	11 (64)
Bonn block	2 (12)
MATRix	2 (12)
HD-AraC	1 (6)
DHAP	1 (6)
G-CSF used in mobilization	
FIL	11 (65)
PEG	4 (23)
LIPEG	2 (12)
Disease status pre-auto-SCT	
I CR	14 (82)
I PR	3 (18)
PLER use, n (%)	7 (41)

[a]Missing data in two patients. [b]Missing data in three patients. BBBD: blood and brain barrier disruption including therapy with intra-arterial mannitol infusion followed by methotrexate and carboplatin and intravenous rituximab, etoposide and cyclophosphamide; Bonn protocol: methotrexate and cytarabine-based chemotherapy; CR: complete remission; CSF: cerebrospinal fluid; DHAP: dexamethasone-cytarabine-cisplatin; FIL: filgrastim; G-CSF: granulocyte colony-stimulating factor; HD-AraC: high-dose cytarabine; LDH: lactate dehydrogenase; LIPEG: lipegfilgrastim; MATRix: methotrexate-cytarabine-thiotepa-rituximab; PCNS: primary central nervous system; PEG: pegfilgrastim; PLER: plerixafor; PR: partial remission; pre-auto-SCT: before autologous stem cell transplantation.

The aphereses were performed with COBE Spectra (Terumo BCT) AutoPBSC apheresis machine for one patient and since April 2013 with the Spectra Optia leukapheresis system (Spectra Optia, software 7.2., Terumo BCT, Lakewood, USA) at KUH. The Spectra Optia apheresis system was used throughout the study at Oulu University Hospital. The blood volume processed was 2 - 3 times of the estimated total blood volume of each patient. The number of $CD34^+$ cells of each apher-

esis product was measured by flow cytometry at the stem cell laboratory of each hospital using an ISHAGE protocol with a single platform method [28]. In order to protect the cells from stress and death during cryopreservation, dimethylsulfoxide (DMSO) was added to apheresis products at a final concentration of 10%. After freezing the final products were maintained in the vapor phase of a liquid nitrogen freezer.

Graft analysis

After each collection, two additional 0.5 mL specimens were taken from the apheresis product to facilitate the graft cellular composition analyses in the future. DMSO was added to the samples as a final concentration of 10% and they were cryopreserved in a freezer with a controlled-rate freezing program likewise the graft bags.

An experienced flow cytometrist (AR) later analyzed the thawed cryopreserved graft specimens at the Department of Clinical Microbiology, University of Eastern Finland by flow cytometry (FACSCanto, Becton Dickinson, San Jose, CA) using an ISHAGE protocol [28]. In order to determinate both $CD34^+$ cells and subclasses, CD34, CD38, CD45 and CD133 antibodies were used. All antibodies were delivered by Becton Dickinson except for CD133 (Miltenyi Biotec GmbH). Viable $CD34^+$ cells were assorted by using 7-aminoactinomycin (7-AAD) staining. The absolute counts of B, T and NK cells as well as the $CD3^+CD4^+$ and $CD3^+CD8^+$ subpopulations were specified by using both CD3/CD8/CD45/CD4 and CD3/CD16 + CD56/CD45/CD19 reagents (BD Multitest, Becton Dickinson) with tubes (BD Trucount, Becton Dickinson).

High-dose therapy (HDT) and post-transplant course

The response to the induction treatment before HDT was evaluated with magnetic resonance imaging (MRI). All patients received the combination of carmustine (400 mg/m^2 on day -6) and thiotepa (5 mg/kg b.i.d. on days -5 and -4) as an HDT. After the graft infusion, four patients (23%) received filgrastim, three patients (18%) received pegfilgrastim and 10 patients received (59%) lipegfilgrastim, respectively.

The definition of engraftment was composed of the days from the graft infusion (day 0) until absolute neutrophil count was $> 0.5 \times 10^9$/L and platelet (PLT) count was $> 20 \times 10^9$/L without PLT transfusions for previous 3 days, respectively. To assess the hematopoietic recovery complete blood counts were obtained at day +15 and at 1, 3, 6 and 12 months after the graft infusion. Infections and need for intensive care unit (ICU) admission during the early post-transplant period were also recorded.

Statistical analysis

All calculations and analyses were performed with the statistical program package SPSS (IBM SPSS Statistics Version 26, Chicago, USA). Descriptive statistics for continuous variables were described using medians with ranges and categorical variables were presented with frequencies and percentages. In order to determine optimal cut-off points for apheresis parameters and graft cellular components correlating with hematologic recovery, PFS and OS, receiver operating characteristic (ROC) curves were used and Youden's index was applied. Spearman's rank was performed to define associations between variables. In survival analyses, log rank test and Kaplan-Meier's method were used. Two-tailed P values < 0.05 were considered statistically significant.

Ethics

The GOA study protocol was approved by the Research Ethics Committee of the North Savo Hospital District (13/2012) until 12/2016 as well as an amendment of GOA study including non-Hodgkin lymphoma (NHL) patients transplanted in the Kuopio University Hospital (KUH) catchment area in 2017 - 2018, but without analysis of the infused grafts. The study was conducted according to the Declaration of Helsinki.

Results

Mobilization efficiency and collection of $CD34^+$ cells

All patients achieved the minimum collection target $\geq 2 \times 10^6$/kg $CD34^+$ cells. Altogether seven patients (41%) received preemptive PLER to augment $CD34^+$ cell mobilization. The median graft $CD34^+$ cell yield was 3.7×10^6/kg and for majority of the patients, only one apheresis session was needed to reach the adequate graft to proceed to auto-SCT (Table 2).

Graft cellular composition

The detailed data on cellular composition of the infused grafts were available in 10 patients included in the study between 2012 and 2016. The median cryopreservation time before the auto-SCT was 41 days (23 - 91 days). The median loss of $CD34^+$ cells was 48% (range 0-73%) during cryopreservation. The detailed cellular composition of cryopreserved grafts is presented in Table 3.

Hematologic recovery after auto-SCT

A median hospitalization time during auto-SCT was 23 days (range 17 - 80 days). The incidence of febrile neutropenia was 94% (16 patients) and of those only one patient (6%) had positive blood culture. One patient (6%) had a need for ICU admission due to pneumonia.

The neutrophil engraftment took place in a median of 10 days (8 - 18 days) and the median time to reach a PLT count $> 20 \times 10^9$/L without transfusions was 13 days (8 - 98 days). A higher $CD34^+$ cell yield at the first apheresis $> 3.1 \times 10^6$/kg and total collected $CD34^+$ cell yield $> 3.3 \times 10^6$/kg correlated

Table 2. Mobilization and Collection Parameters for $CD34^+$ Cells in Patients With PCNS Lymphoma

Variables	n = 17
WBC count ($\times 10^9$/L) at the time of the first apheresis, median (range)	32.9 (4.8 - 116.2)
Blood $CD34^+$ cell count ($\times 10^6$/L) at the first apheresis, median (range)	43 (7 - 139)
Peak blood $CD34^+$ cell count ($\times 10^6$/L), median (range)	43 (11 - 139)
$CD34^+$ cell yield ($\times 10^6$/kg) of the first apheresis, median (range)	2.5 (0.6 - 6.9)
Total collected $CD34^+$ cell yield ($\times 10^6$/kg), median (range)	3.7 (2.3 - 6.9)
Number of aphereses (%)	
1	9 (53)
2	3 (19)
3	2 (12)
4	3 (19)

PCNS: primary central nervous system; WBC: white blood cell.

with faster platelet engraftment (9 vs. 15 days, P = 0.006 and 9 vs. 23 days, P = 0.009).

Several correlations were observed with the cellular composition of infused graft and the post-transplant hematologic recovery (Table 4). A higher number of infused viable $CD34^+$ cells > 1.7×10^6/kg was linked to more rapid platelet engraftment (10 vs. 31 days, P = 0.027) and neutrophil recovery at day +15 (5.4 vs. 1.6×10^9/L, P = 0.047). A higher amount of cytotoxic $CD3^+CD8^+$ cells infused (> 78×10^6/kg) was correlated with slower neutrophil engraftment (9 vs. 12 days, P = 0.005). $CD3^+$ cell count > 181×10^6/kg in the infused graft was associated with the lower number of neutrophils both at day +15 (1.2 vs. 4.4×10^9/L, P = 0.028), and also at 1 month (0.9 vs. 2.2×10^9/L, P = 0.029) after the graft infusion. In addition, a higher number of infused $CD3^+$ cells was linked with slower platelet engraftment (22 vs. 11 days, P = 0.027) and early platelet recovery (day +15) after the graft infusion (24 vs. 111×10^9/L, P = 0.022). Also, the infused $CD3^+CD8^+$ cell count > 78×10^6/kg was also linked with the slower early post-transplant (day +15) platelet (30 vs. 84×10^9/L, P = 0.004) and leukocyte (2.8 vs. 7.8×10^9/L, P = 0.029) recovery. In addition, the graft $CD3^+CD4^+$ cell count > 94×10^6/kg was associated with lower hemoglobin levels at 1 month (103 vs. 108×10^9/L, P = 0.029) and higher number of lymphocytes at 12 months (2.0 vs. 1.7×10^9/L, P = 0.035) after auto-SCT.

Table 3. Cellular Composition of Infused Grafts in Patients With PCNS Lymphoma

Variables ($\times 10^6$/kg), median (range)	n = 10
$CD34^+$ cells without 7-AAD	3.6 (1.5 - 5.3)
$CD34^+$ cells with 7-AAD	2.1 (0.9 - 5.1)
$CD34^+CD133^+CD38^-$ cells	0.07 (0.011 - 0.17)
$CD3^+$ cells	113.2 (67.5 - 253.4)
$CD3^+CD4^+$ cells	69.6 (39.5 - 164.1)
$CD3^+CD8^+$ cells	41.5 (25.2 - 187.2)
$CD4^+/CD8^+$	1.6 (0.4 - 4.3)
$CD19^+$ cells	0 (0 - 0.3)
NK cells	8.5 (1 - 22.9)

PCNS: primary central nervous system; 7-AAD: 7-aminoactinomycin; NK: natural killer.

Post-transplant outcome

By October 31, 2020, the median follow-up time from auto-SCT was 46 months (range 3 - 95 months). A disease recurrence or progression has been observed in four patients (24%). The median time to relapse was 253 days (range 64 - 1,925 days). Altogether two patients (12%) have died due to disease progression during the follow-up and one of those within 100 days after auto-SCT. In Kaplan-Mayer analysis, 5-year PFS for total patient population was 69% (Fig. 1) and OS was as high as 88% (Fig. 2), respectively.

The first-line therapy before auto-SCT had no meaningful effect on survival (P = 0.130). Disease status complete remission (CR) before auto-SCT was not significantly associated with PFS (P = 0.651) or OS (P = 0.244) either. The better mobilizing capacity of $CD34^+$ cells neither had significant impact on 5-year OS. A higher number of total graft $CD34^+$ yield collected with a cut-off point > 3.3×10^6/kg (AUC 0.444, sensitivity 1.000, specificity 0.444, P = 0.028) was associated with worse 5-year PFS (33% vs. 100%), but the number of viable $CD34^+$ cells after thawing with a cut-off point > 1.7×10^6/kg did not correlate with PFS (P = 0.688) or OS (P = 0.414). Regarding the impact on detailed graft cellular content on outcome, also higher amount of $CD3^+CD4^+$ T cells with a cut-off point > 94×10^6/kg (AUC 0.778, sensitivity 1.000, specificity 0.778, P = 0.069) showed a trend for negative impact on 5-year PFS (30% vs. 100%). In addition, the patients with infused $CD3^+CD8^+$ T cells with a cut-off point > 78×10^6/kg (AUC 0.889, sensitivity 1.000, specificity 0.889, P = 0.016) had poorer PFS (0% vs. 88%) at 5 years.

Discussion

This prospective multicenter study aimed to analyze the cor-

Table 4. Correlation Between Graft Cellular Composition and Hematologic Recovery After Auto-SCT

Variables	$CD34^+$ w 7-AAD		$CD3^+$		$CD3^+CD4^+$		$CD3^+CD8^+$		$CD34^+CD38$		NK	
	r	P	r	P	r	P	r	P	r	P	r	P
Blood count 15 days after auto-SCT												
WBCs ($\times 10^9$/L)	0.491	NS	-0.430	NS	-0.236	NS	-0.685	0.029*	0.624	NS	0.006	NS
Neutrophils ($\times 10^9$/L)	0.714	0.047*	-0.762	0.028	-0.476	NS	-0.905	0.002*	0.643	NS	0.048	NS
Lymphocytes ($\times 10^9$/L)	0.486	NS	-0.429	NS	-0.600	NS	-0.314	NS	0.600	NS	-0.771	NS
PLTs ($\times 10^9$/L)	0.685	0.029*	-0.709	0.022*	-0.503	NS	-0.818	0.004*	0.418	NS	0.067	NS
Blood count 1 month after auto-SCT												
Hb (g/L)	0.455	NS	-0.745	0.013*	-0.685	0.029*	-0.806	0.005*	0.539	NS	-0.212	NS
WBCs ($\times 10^9$/L)	0.164	NS	0.091	NS	-0.115	NS	-0.115	NS	0.139	NS	0.006	NS
Neutrophils ($\times 10^9$/L)	0.576	NS	-0.685	0.029*	-0.600	NS	-0.661	0.038*	0.006	NS	-0.030	NS
Lymphocytes ($\times 10^9$/L)	-0.285	NS	0.628	NS	0.310	NS	0.485	NS	0.033	NS	-0.042	NS
PLTs ($\times 10^9$/L)	0.467	NS	-0.406	NS	-0.430	NS	-0.442	NS	0.091	NS	0.200	NS
Blood count 3 months after auto-SCT												
Hb (g/L)	0.326	NS	-0.603	NS	-0.803	0.009*	-0.494	NS	0.142	NS	-0.452	NS
WBCs ($\times 10^9$/L)	0.418	NS	-0.326	NS	-0.326	NS	-0.176	NS	-0.025	NS	0.159	NS
Neutrophils ($\times 10^9$/L)	0.335	NS	-0.109	NS	0.025	NS	-0.075	NS	0.084	NS	0.385	NS
Lymphocytes ($\times 10^9$/L)	-0.067	NS	0.008	NS	-0.293	NS	0.377	NS	-0.226	NS	-0.151	NS
PLTs ($\times 10^9$/L)	0.533	NS	-0.433	NS	-0.383	NS	-0.583	NS	-0.050	NS	0.317	NS
Blood count 6 months after auto-SCT												
Hb (g/L)	0.217	NS	-0.167	NS	-0.400	NS	-0.317	NS	0.217	NS	-0.433	NS
WBCs ($\times 10^9$/L)	0.217	NS	-0.117	NS	-0.267	NS	-0.217	NS	-0.200	NS	0.083	NS
Neutrophils ($\times 10^9$/L)	0.288	NS	0.198	NS	0.036	NS	0.090	NS	-0.090	NS	0.505	NS
Lymphocytes ($\times 10^9$/L)	0.174	NS	-0.696	NS	-0.464	NS	-0.551	NS	-0.290	NS	-0.058	NS
PLTs ($\times 10^9$/L)	0.183	NS	-0.183	NS	-0.400	NS	-0.217	NS	-0.517	NS	-0.100	NS
Blood count 12 months after auto-SCT												
Hb (g/L)	0.552	NS	-0.494	NS	-0.603	NS	-0.226	NS	0.636	NS	-0.075	NS
WBCs ($\times 10^9$/L)	-0.050	NS	0.133	NS	-0.117	NS	0.017	NS	-0.333	NS	-0.283	NS
Neutrophils ($\times 10^9$/L)	0.195	NS	0.366	NS	0.073	NS	0.342	NS	0.122	NS	0.024	NS
Lymphocytes ($\times 10^9$/L)	-0.539	NS	0.946	< 0.001*	0.743	0.035*	0.431	NS	-0.431	NS	-0.228	NS
PLTs ($\times 10^9$/L)	0.233	NS	0.050	NS	-0.017	NS	-0.167	NS	-0.367	NS	0.333	NS

*P values are statistically significant (< 0.05). $CD34^+$ w 7-AAD: $CD34^+$ with 7-aminoactinomycin; r: Spearman correlation coefficient; PLT: platelet; WBC: white blood cell; auto-SCT: autologous stem cell transplantation; NK: natural killer.

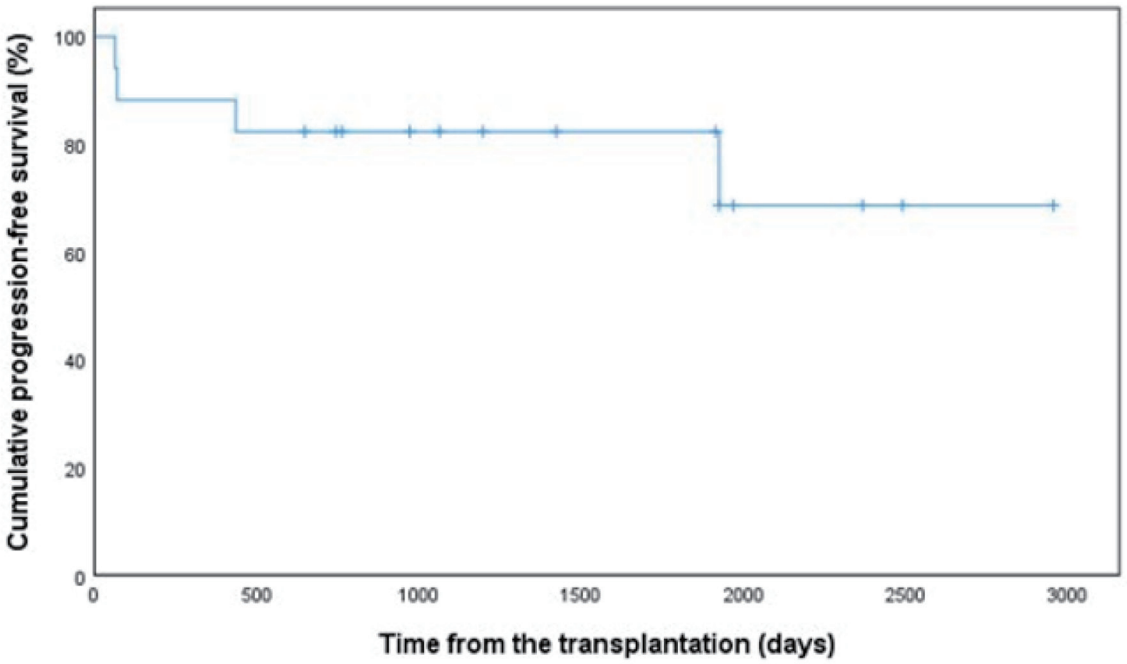

Figure 1. Progression-free survival in 17 patients with PCNSL. PCNSL: primary central nervous system lymphoma.

relations of $CD34^+$ cell mobilization parameters and graft cellular content with hematologic recovery and outcome in PCNSL patients after an upfront auto-SCT. Higher yield of $CD34^+$ cells of the first apheresis and total yield of $CD34^+$ cells harvested correlated with faster platelet engraftment. Instead, higher number of $CD3^+$ lymphocytes infused was associated with slower platelet recovery and higher amount of graft $CD3^+CD8^+$ cells with slower neutrophil engraftment. Surprisingly, higher number of total $CD34^+$ cells in the graft predicted worse 5-year PFS. Also, higher number of $CD3^+CD8^+$ T cells infused affected negatively on the 5-year PFS. However, these associations should be handled with reservation due to small number of patients.

A good response to treatment before auto-SCT has been

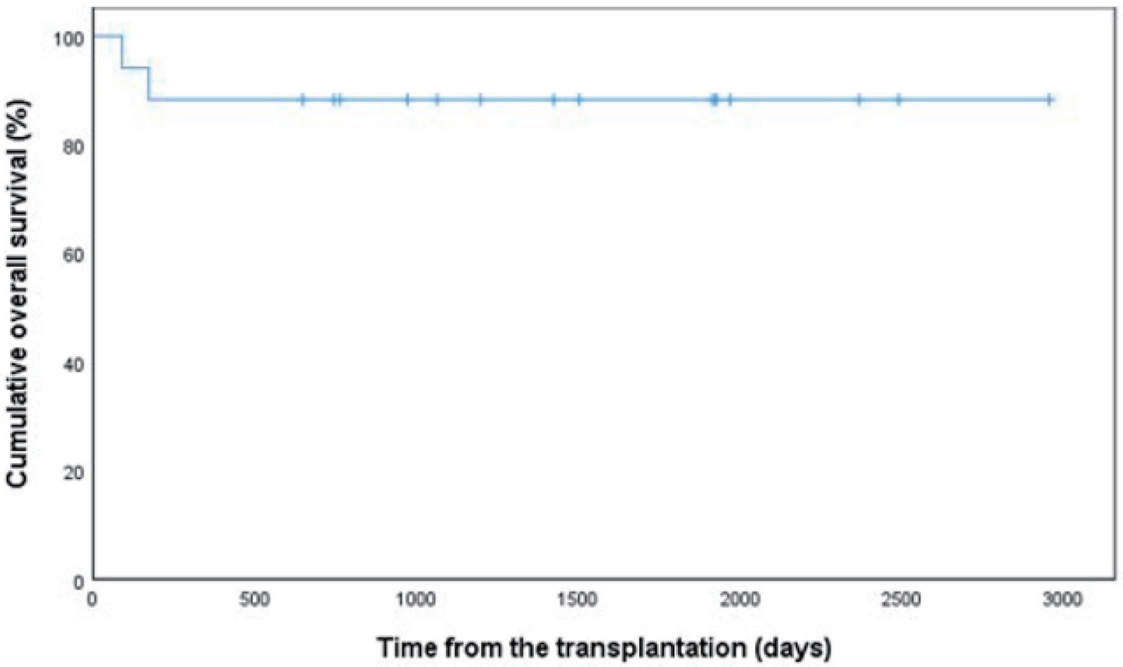

Figure 2. Overall survival in 17 patients with PCNSL. PCNSL: primary central nervous system lymphoma.

suggested to predict a better long-term outcome in a retrospective analysis by the Mayo Clinic [11] and also, in a previous intent-to-treat analysis in the subgroup of the patients with PCNSL [30]. The optimal treatment strategy in order to produce deep remission rates in PCNSL has not been validated. A recent randomized IELSG32 study highlighted MATRix induction therapy as a representative of effective high-dose methotrexate-based treatment [31]. Omuro et al [12] concluded in a prospective study that R-MPV (rituximab, methotrexate, procarbazine, vincristine) before auto-SCT was effective and safe without detectable neurotoxicity. The MATRix combination was used in our study for the majority of the patients before BBBD treatment, which in turn has been proven to result in high remission rates before auto-SCT in a recent retrospective study [32] and also in a successful strategy for disease control during very long follow-up [32]. In the present study, a great majority (82%) of patients achieved CR before auto-SCT, but in contrast to previous report regarding DLBCL patients [18], we did not find any correlations with pretransplant disease status and survival possibly due to small study population.

The variable mobilizing agents were perhaps less effective in the present patient population resulting in an excess need for PLER use compared to the patients with systemic DLBCL in a previous study [18]. Mobilizing therapies used as well as previous treatment history have an impact on the cellular content of collected grafts [33, 34]. Interestingly, the lower mobilizing capacity regarding the cut-off point of the total collected $CD34^+$ cell yield < 3.3×10^6/kg seemed to predict better 5-year PFS in the present analysis. Regarding other graft cellular components, higher number of $CD3^+CD4^+$ T-helper cells and cytotoxic $CD3^+CD8^+$ cells were associated with inferior PFS. Otherwise, we found a median of 48% loss of viable $CD34^+$ cells during the cryopreservation indicating possibly a need for higher goals for collected $CD34^+$ cells. The reasons for these interesting novel observations are unknown.

The long-term outcome of the PCNSL patients has been historically inferior compared to patients with systemic DLBCL. A plausible explanation for divergent outcomes may be the pharmacological differences in therapies used in addition to the different biology of these subtypes. In a recent phase II study, a 2-year OS of up to 81% was observed after auto-SCT consolidation in PCNSL patients [12] in line with a retrospective study with a 71% 2-year OS in patients with either upfront or later auto-SCT [13]. We found a promising 5-year OS of 88% in patients receiving upfront auto-SCT. Of note, 65% of our patients had received BBBD therapy before auto-SCT.

There are obvious limitations in this study. The mobilization chemotherapy varied during the course of this non-interventional study. In addition, small number of enrolled patients hampered more sophisticated statistical analyses. The obvious strengths of this prospective multicenter study are comparable graft collection practices and centrally analyzed graft cellular composition by a single experienced cytometrist.

To conclude, according to this prospective study, the higher mobilizing capacity with a threshold of 3.3×10^6/kg $CD34^+$ cells in the collected graft influenced negatively PFS. Graft cellular composition and especially the number of cytotoxic T cells had an impact on both the hematologic recovery and PFS. Excellent long-term OS rates were observed after an upfront auto-SCT. A larger study is required to verify the optimal autograft cellular content in patients with PCNSL.

Acknowledgments

Antti Ropponen is acknowledged for graft analyses by flow cytometry, Tuomas Selander M.Sc. for the statistical support and David Laaksonen M.D., Ph.D. for linguistic revision.

Financial Disclosure

AP is grateful for the scholarships granted by the Finnish Society of Hematology and the Cancer Foundation of North Savo. A research grant from Sanofi Genzyme for the GOA study is also acknowledged.

Conflict of Interest

AP reports honoraria from Behring and Abbvie and has participated in Scientific Advisory Board meetings organized by Abbvie, Novartis and Takeda. JV reports honoraria from Amgen, Janssen-Cilag and Sanofi. VV reports consultancy fees from Abbvie, Amgen, Celgene, Janssen-Cilag, Roche and Sanofi. EJ reports honoraria from Amgen and Sanofi and has participated in Scientific Advisory Board meetings organized by Amgen, Takeda and TEVA. The other authors declare no conflict of interest.

Informed Consent

Informed consent was obtained from all patients.

Author Contributions

AP, EJ and VV designed the study, obtained patient data, analyzed the data, interpreted the results and wrote the manuscript. AP, OK, AT, JV, MP, HK, KV, TK, PM, JP, EJ and VV participated in the revision and approval of the final version of this manuscript.

Data Availability

Any inquiries regarding supporting data availability of this study should be directed to the corresponding author.

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