# Autologous stem cell transplantation with selected CD34+ cells and unmanipulated peripheral blood stem cells in patients with relapsed and refractory Hodgkin's lymphoma: a single centre experience

V. BALLOVA, M. LADICKA, A. VRANOVSKY, J. LAKOTA*

*Department of Internal Medicine, National Cancer Institute, Klenova 1, 830 10 Bratislava, Slovakia, e-mail: jan.lakota@nou.sk*

**Received December 17, 2007**

With the aim to evaluate the long term outcome after high-dose chemotherapy and autologous stem cell transplantation (HDCT+ASCT) in patients with relapsed or refractory Hodgkin's lymphoma (HL) we performed a retrospective analysis of patients transplanted at our centre. Between January 1993 and December 2005, 126 consecutive patients with relapsed or refractory HL in the age of 16 to 65 years underwent HDCT+ASCT at our centre and were enrolled in this retrospective analysis. Patients were autografted with either $CD34^+$ positively selected or unmanipulated periferal blood stem cells (PBSC). With a median follow up of 69 months (3-162 months), the actuarial 5-y PFS and OS for all patients after HDCT+ASCT were 59% and 72%, respectively. In patients transplanted from 1996 the actuarial 5-y PFS and OS for $CD34^+$ selected group were 64% and 79% and for unmanipulated PBSC group 63% and 66%, respectively**.** A total of 42/126 (33%) patients died. Treatment related mortality (TRM) was 3% (4 patients). In univariate analysis, chemosensitive disease and increased LDH were the strongest prognostic factors for PFS and OS. Our results confirm the efficacy of HDCT+ASCT in relapsed or refractory HL with acceptable toxicity. The use of CD34+ positively selected stem cells for autografting is feasible, safe and effective procedure.

*Key words: Hodgkin's lymphoma, high-dose chemotherapy, autologus transplantation, $CD34^+$ selected stem cells*

High-dose therapy with autologous stem cell transplantation has been tested extensively in the last years in patients with relapsed and refractory HL. The results of two randomized studies have demonstrated significant improvement in event free and progression free survival for patients treated with HDCT+ASCT compared with standard-dose second line chemotherapy. Neither of the trials was powered to show OS advantage [1, 2].

In the recent years PBSC have replaced bone marrow (BM) as the stem cell source, because of faster haematological recovery when the PBSC were reinfused [3]. The EBMT registry data indicated a less favourable outcome in patients supported with PBSC when compared to those supported with BM [4]. It has been shown that tumor cells from various tumors are mobilised together with the CD34+ stem cells [5, 6]. Moreover, the presence of circulating CD30+ clonogenic tumor cells in patients with HL has been demonstrated [5–7]. Contamination of the graft with Hodgkin's tumor cells might be one of the factors contributing to relapse. The positive selection of CD34+ cells from PBSC has been proven to be an effective in-vitro purging method in diverse cancer types. The reduction of 3-4 logs tumor cells in the graft can be achieved without a negative effect on the haematological recovery after HDCT [8, 9]. There have been reported encouraging results in patients with HL, when positively selected CD34+ stem cells were used [10, 24]. However a correlation between the contamination of the graft with the CD30+ tumor cells and the incidence of relapse after transplantation has not been tested in the setting of a randomised trial.

We performed a retrospective analysis of 126 HL patients transplanted at our centre to determine the long term outcome and toxicity of this treatment strategy We assessed also the role of use of CD34+ positively selected stem cells in patients with relapsed or refractory HL.

## Patients and methods

*Patient selection.* Between January 1993 and December 2005, 126 consecutive adult patients with relapsed or refrac-

* Corresponding author

tory HL received HDCT+ASCT at our centre and were included in this retrospective analysis. To be eligible for HDCT+ASCT, patients between the age of 16 and 65 years had to have either failed to enter a complete remission (CR) or relapsed after conventional multiagent chemotherapy. Rebiopsy at the time of disease progression or relapse was recommended. Other criteria for eligibility were Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2; adequate cardiac, pulmonary, renal, and liver function. Patients were required to test negative for antibody against HIV and to be free of active infection. Pre-treatment evaluation included medical history; physical examination; complete blood count; liver and renal function tests; erythrocyte sedimentation rate; computed tomography (CT) of chest, abdomen, and pelvis; bone marrow biopsy. In addition, lung function test and echocardiography were routinely performed before treatment. Each patient signed informed consent.

All patients had received front-line conventional chemotherapy with ABVD (adriamycine, bleomycine, vinblastine, dacarbazine), modified hybrid protocol (lomustine, vincristine, procarbazine, prednisone, adriamycine, bleomycine, vinblastine), COPP/ABVD (cyslophosphamide, vincristine, procarbazine, prednisone, adriamycine, bleomycine, vinblastine, dacarbazine) or similar regimens.

Primary progressive disease was defined as either disease progression during the first-line chemotherapy, or only a transient response lasting ≤ 90 days after completion of the first-line treatment. Progression was defined as either ≥ 25% increase from nadir in the sum of the products of the greatest diameter of any previously identified abnormal lesion for partial responders or non-responders, and/or appearance of any new lesion within ≤ 90 days after the end of therapy. Persistent disease was defined as failure to achieve CR/CRu (complete remission unconfirmed), based on routine radiological assessment, after the first-line treatment. Patients with PR, SD or with biopsy-proven persisting HL after the first line therapy were allocated to this group. Patients with relapsed HL had to have achieved CR/CRu for at least 3 months. Early relapse required a CR lasting 3 to 12 months. Late relapse was defined as relapse after CR lasting >12 months. Multiple relapse was defined as the second or later relapse.

*Treatment procedures.* Before high-dose therapy patients received cytoreductive chemotherapy, usually 2-3 cycles. In not responding patients or, if progression occurred, alternative regimen was instituted. Cytoreductive regimens used were DHAP (cisplatin, cytarabine, dexamethasone), mini-BEAM (carmustine, etoposide, cytarabine, melphalan), IMED (ifosphamide, methotrexate, etoposide, dexamethasone) and ICE (ifosphamide, carboplatin, etoposide).

The conditioning regimen used was BEM (carmustine 300mg/m$^2$ on day-7, melphalan 140mg/m$^2$ on day -3 and etoposide 100mg/m$^2$ every 12 hours on day -6 to -3) or CBV (cyclophosphamide 1.6g/m$^2$ on day -5 to -3, etoposide 100mg/m$^2$ every 12 hours on day -6 to -3, and carmustine 300mg/m$^2$ on day -6). Unmanipulated PBSC or positively selected CD34$^+$ cells were reinfused on day 0 and G-CSF (filgrastim) was administered from the day 0 until haematological recovery. PBSC were mobilized after the second or third cycle of cytoreductive chemotherapy using G-CSF (5μg/kg per day) beginning on day 10–13 and continuing until the completion of leukapheresis. Leukapheresis was performed daily until 1–2x10$^6$ CD34$^+$ cells/kg were collected. From January 1996 protocol of positive CD34$^+$ selection was introduced in patients with HL and the procedure was intended in all patients with sufficient number of CD34$^+$ cells in two apheresis products (at least 3x10$^6$ CD34$^+$ cells/kg). The two apheresis products were positively CD34$^+$ selected according to the manufacturer. The mean purity of the positively selected CD34$^+$ cells was 90% (range 64–99%). The cells were stored in the presence of 7.5% DMSO in the vapour phase of a liquid nitrogen tank at -196°C. The third, unmanipulated apheresis product was stored as a back-up. Patients unable to mobilize sufficient number of CD34$^+$ cells for purging procedure (at least 3x10$^6$ CD34$^+$ cells/kg) were autografted with unmanipulated PBSC.

From the first day of conditioning regimen patients received prophylactic antimicrobial treatment containing ofloxacin, fluconazole and acyclovir. All blood products were irradiated with 30 Gy to prevent transfusion-associated graft-versus-host disease. Prophylactic transfusion of single-donor platelets was given if patient's platelet count was bellow 20x10$^9$/L or in the event of bleeding. Red blood cells were transfused to maintain a haemoglobin level of 80 g/L or more, if clinically indicated. Consolidating involved-field radiotherapy was indicated for residual masses and was delivered after HDCT+ASCT to patients not previously irradiated.

*Response assessment*. Patients were restaged after cytoreductive chemotherapy. All sites of initial disease were reassessed by CT, and bone marrow (BM) biopsy, if BM was involved. Chemoresistant disease was defined as a failure to achieve at least PR. Patients with chemoresistant disease were not excluded from HDCT+ASCT. The final response evaluation was performed 2-3 months after transplantation. Routine follow-up was carried out every 3 months during the first two years, then every 6 months up to 5 years and then yearly or whenever clinically indicated.

*Definition of response.* CR was defined as the complete disappearance of all measurable clinical and radiographic evidence of disease for at least 3 months. CRu was defined by residual lymph-node presence >1.5 cm in greatest transverse dimension that regressed by ≥ 75%. PR was defined as a reduction in tumor mass by ≥ 50%, measured as the sum of the product of the two largest perpendicular diameters of the lesions.

*Statistics.* All statistical analysis and graphic was performed using SPSS 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA). Demographics and disease characteristics were summarized using descriptive statistics. Survival analyses were performed according to the method of Kaplan and Maier and

compared using the log-rank test. PFS was calculated from the date of ASCT until one of the following events occurred: relapse/progression, deaths from any cause, if none of these occurred, to the date of last information on complete remission. OS was calculated from the date of ASCT up to the date of death from any cause, or if no death occurred, to the last documented information on the patient. Cause of death was classified as progressive HL (including death from treatment for relapse after transplantation), toxic death, classified as death from any reason within 100 days after autografting and other causes. A separate survival analysis for patients transplanted from 1996 was performed and the two groups, $CD34^+$ positively selected and unmanipulated PBSC, were compared. The prognostic significance of various factors was tested by univariate Cox proportional hazards model using pre-defined level $\alpha = 0.05$. The following variables were evaluated as potential prognostic factors for PFS and OS (at the time of relapse/progression): age; stage; B-symptoms; extranodal involvement; lung involvement, liver involvement; LDH; autografting with positively $CD34^+$ selected stem cells; number of cytoreductive regimens before HDCT+ASCT; number of cycles of cytoreductive therapy; response to cytoreductive therapy.

**Table 1. Patient characteristics of the whole set of patients at the time of relapse/ progression**

| Patient characteristics | n | % |
|---|---|---|
| **Gender** | | |
| Men | 69 | 55 |
| Women | 57 | 45 |
| **Median age (range)** | 27 (16-64) years | |
| **Duration of first remission** | | |
| Persistent disease | 21 | 17 |
| Primary progression | 32 | 25 |
| Early relapse | 30 | 24 |
| Late relapse | 23 | 18 |
| Multiple relapse | 19 | 15 |
| Unknown | 1 | 1 |
| **Stage** | | |
| I | 3 | 2 |
| II | 44 | 35 |
| III | 11 | 9 |
| IV | 68 | 54 |
| **Extranodal involvement** | 66 | 52 |
| **Lung involvement** | 43 | 34 |
| **Liver involvement** | 15 | 12 |
| **B-symptoms** | 65 | 52 |
| **Chemotherapy** | | |
| ABVD | 38 | 30 |
| Hybrid | 68 | 54 |
| Other | 15 | 12 |
| Unknown | 5 | 4 |
| **Radiotherapy** | 62 | 49 |
| **Primary histology** | | |
| LD | 5 | 4 |
| LP | 5 | 4 |
| MC | 28 | 22 |
| NS | 87 | 69 |
| Unknown | 1 | 1 |
| **Histology at relapse** | 70 | 56 |

ABVD: adriamycine, bleomycine, vinblastine, dacarbazine; LD: lymphocyte depletion; LP: lymphocyte predominance; MC: mixed cellularity; NS: nodular sclerosis

## Results

*Patient characteristics.* One hundred twenty six patients were included in this analysis. Patient characteristics at the time of relapse/ progression are listed in Table 1. The median age was 27 years (range 16–64 years). Twenty one patients (17%) had persistent disease, 32 patients (25%) primary progressive disease, 30 patients (24%) early relapse, 23 patients (18%) late relapse and 19 patients (15%) had multiple relapse. Most of the patients, 79 (63%) had advanced stage (stage III/ IV) at the time of relapse/ progression. Sixty six patients (52%) had extranodal involvement, 43 patients (34%) had lung involvement and 15 patients (12%) had liver involvement. The B-symptoms were present in 65 patients (52%). First-line therapy consisted of modified hybrid regimen in 68 patients (54%), ABVD in 38 patients (30%), other regimen in 15 patients (12%) and unknown therapy received 5 patients (4%). Sixty two patients (49%) had been treated with combined chemo-radiotherapy. Primary histology was as follows: nodular sclerosis (NS) in 87 patients (69%), mixed cellularity (MC) in 28 patients (22%), lymphocyte predominance (LP) in 5 patients (4%) and lymphocyte depletion (LD) in 5 patients (4%). Relapse or progression was biopsy-proven in 70 patients (56 %).

*Cytoreductive therapy.* After documented relapse, progression or persistent disease all, but two patients received cytoreductive therapy. Ninety one patients (72%) received one cytoreductive regimen, 20 patients (16%) 2 regimens and 13 patients (10%) received ≥ 3 regimens before HDCT+ASCT. Fifty seven patients (45%) received 1-2 cycles of cytoreductive therapy, 47 patients (37%) received 3-4 cycles, and 20 patients (16%) received ≥ 5 cycles. The most frequently regimen used was DHAP in 106 patients (84%), thereinafter mini-BEAM in patients 25 (20%), IMED in 11 patients (9%), ICE in 10 patients (8%), and other regimens in 18 patients (14%).

*HDCT+ASCT.* All patients planned for transplantation, underwent HDCT+ASCT irrespective of the effect of cytoreductive therapy. Ninety one patients (72%) received BEM, 21 patients (17%) received CBV and 14 patients (11%) received other conditioning regimen. Eighty eight patients (70%) received positively selected $CD34^+$ cells, 38 patients (30%) received unmanipulated PBSC. A total of 31 patients (25%) had consolidating radiotherapy. At the final evaluation the response rate for the entire group was 86% (109 patients). Sixty three patients (50%) achieved CR/CRu, 46 patients (36%) PR, 14 patients (11%) SD and in 2 patients (2%) PD was seen. Response rates are summarized in Table 2.

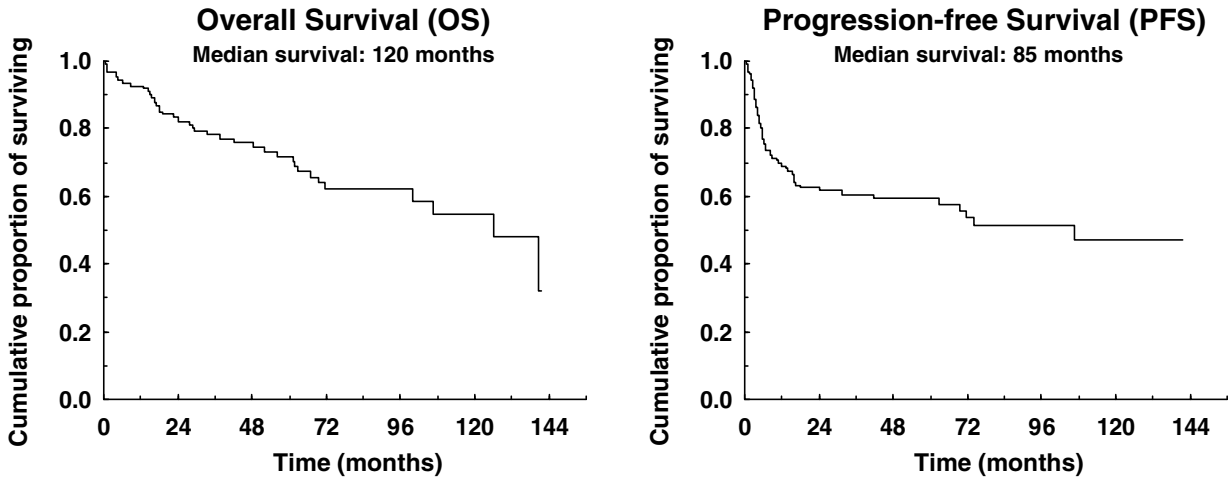


**Figure 1. Overall survival (OS) and progression free survival (PFS) for all patients (n=126)**

*Haematological recovery after transplantation. Pa*tients autografted with unmanipulated PBSC received a median number of 1.3x $10^6$ (range 1.0–2.2) $CD34^+$ cells/kg, the $CD34^+$ positively selected group received a median number of 1.2x$10^6$ (range 0.2-6.8) $CD34^+$ cells/kg. All 126 patients experienced grade 4 neutropenia and thrombocytopenia. Recovery of ANC >0.5x$10^9$/L occurred at a median of 12 days (range 6-63 days). The unsupported platelet count of 30 x$10^9$ /L was reached at a median of 24 days (range 10–220 days). Four patients died of treatment toxicity and failed to achieve a complete engraftment. As a late complication we observed autoimmune thrombocytopenia (ITP) in 3 patients 14, 17 and 18 months after transplantation and Evans syndrome in one patient 17 months after transplantation.

*Survival and causes of death.* With a median follow up of 69 months (3–162 months) from diagnosis, the actuarial 5-y PFS and OS for all patients were 59% (95% CI [confidence intervals], 50–68%) and 72% (95% CI, 63-82%), respectively. The Kaplan-Meier plots for PFS and OS are shown in Figure 1.

There are currently 82/126 (65%) patients alive, of them 70 patients (55%) are alive in durable remission after HDCT+ASCT, 12 patients (10%) are alive after relapse, 2 patients (2%) are lost of follow up. A total of 42/126 (33%) patients died during the observation period. The most frequent cause of death was HL, in 31/42 patients (74%). Secondary malignancy was the cause of death in 6/42 patients (14%) and pneumonia in 1/42 patient (2%). Toxic death occurred in 4/126 patients resulting in 3% TRM. Forty seven patients (37%) relapsed from 1 to 74 months (median 6 months) after transplantation. The majority of patients, 36/47 patients (77%), relapsed within the first year after transplantation. In 7/47 patients (15%) relapse occurred during the second year and in 3/47 patients (7%) relapse occurred more than two years after transplantation. Time to relapse, causes of death and secondary malignancies are summarized in Table 3. Figure 2 shows survival curves with respect to the initial remission duration. The actuarial 5-y PFS and OS for patients with persistent disease were 72% and 81%, for primary progressive disease 55% and 64%, for early relapse

**Table 2. Final response after HDCT+ASCT**

| | n | % |
|---|---|---|
| **Final response** | | |
| Complete remission | 63 | 50 |
| Partial remission | 46 | 36 |
| Stable disease | 14 | 11 |
| Progressive disease | 2 | 2 |
| Unknown | 1 | 1 |
| Total | 126 | 100 |

**Table 3. Time to relapse, secondary malignancies and causes of death**

| | n | % |
|---|---|---|
| **Time to relapse (n=47)** | | |
| ≤ 3 months | 15 | 32 |
| 4 - 12 months | 21 | 45 |
| 13 – 24 months | 7 | 15 |
| > 24 months | 3 | 6 |
| Unknown | 1 | 2 |
| **Secondary malignancies** | 7 | 6 |
| Secondary NHL | 4 | - |
| MDS/sAML | 2 | - |
| Soft tissue sarcoma | 1 | - |
| **Causes of death (n=42)** | | |
| Hodgkin's lymphoma | 31 | 74 |
| Treatment related mortality | 4 | 9 |
| Secondary malignancy | 6 | 14 |
| Pneumonia | 1 | 2 |

NHL: non-hodgkin's lymphoma; MDS/sAML: myelodysplastic syndrome/ secondary acute myeloblastic leukaemia;

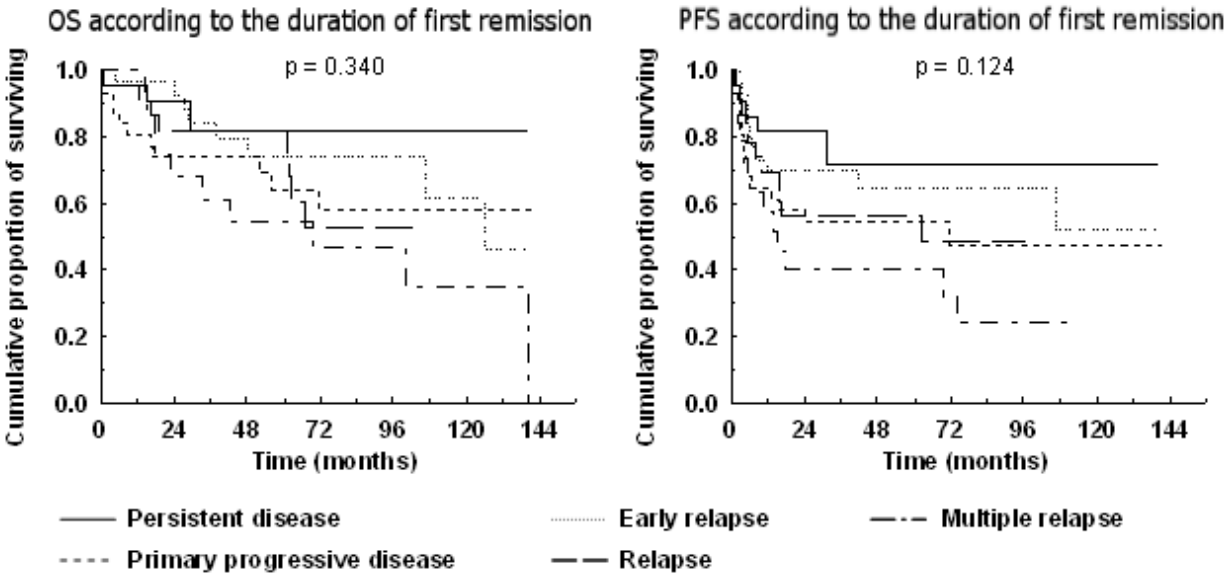


**Figure 2 Overall survival (OS) and progression free survival (PFS) with respect to duration of the first remission**

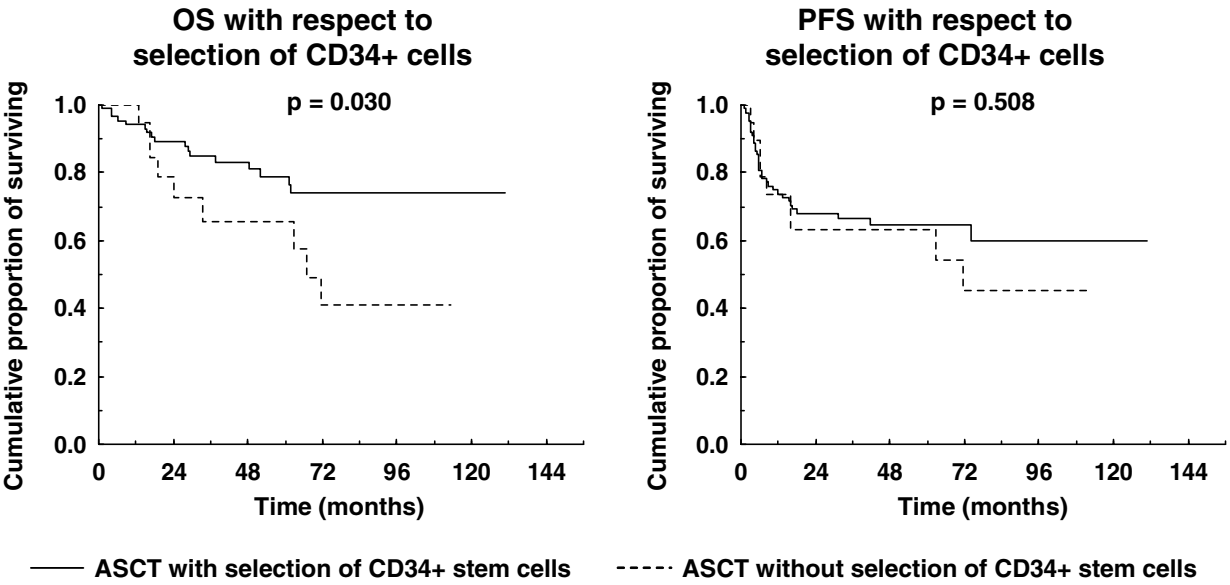


**Figure 3. Overall survival (OS) and progression free survival (PFS) of the patients transplanted after 1996 given either selected CD 34⁺ cells or unmanipulated PBSC.**

65% and 74%, for late relapse 48% and 75% and for multiple relapse 40% and 54%, respectively (Table 4). There was no difference in terms of survival between the patients with early and late relapse. The survival of patients with primary progressive disease is worse compared with patients with persistent disease; however the difference has not reached statistical significance.

Kaplan Mayer curves for PFS and OS of patients transplanted from 1996 supported with CD34⁺ positively selected stem cells (88 patients) and unmanipulated PBSC (19 patients) are shown on Figure 3. Median follow of the entire subgroup is 61 months (3–131 months).The actuarial 5-y PFS and OS for the CD34⁺ selected group were 64% and 79%, and for unmaniplated PBSC group 63% and 66%, respectively (Table 5). Table 6 shows a comparison of risk factors between the CD34⁺ selected and unmanipulated PBSC group. There were more patients with advanced stage of disease in the unmanipulated PBSC group (84%) compared with CD34⁺ se-

**Table 4. Progression free survival (PFS) and overall survival (OS) at 5 years of the whole group of patients and according to duration of the first remission**

| | 5 years PFS (95% CI) | | 5 years OS (95% CI) | |
|---|---|---|---|---|
| | n | | n | |
| **All patients** | 126 | 59% (50 – 68%) | 124 | 72% (63 – 81%) |
| Persistent disease | 22 | 72% (48 – 95%) | 21 | 81% (61 – 100%) |
| Primary progressive disease | 31 | 55% (37 – 72%) | 31 | 64% (46 – 83%) |
| Early relapse | 30 | 65% (46 – 83%) | 30 | 74.% (56 – 92%) |
| Late relapse | 23 | 48% (26 – 71%) | 23 | 75% (56 – 95%) |
| Multiple relapses | 19 | 40% (17 – 63%) | 18 | 54% (29 – 80%) |

**Table 5. Progression free survival (PFS) and overall survival (OS) at 5 years of the patients transplanted from 1996 with CD34⁺selected and unmanipulated stem cells**

| | 5 years PFS (95% CI) | | 5 years OS (95% CI) | |
|---|---|---|---|---|
| | n | | n | |
| CD34⁺ selected group | 87 | 64% (54 – 75%) | 87 | 79% (69 – 89%) |
| PBSC unmanipulated group | 19 | 63% (41 – 84%) | 19 | 66% (43 – 88%) |

PBSC: peripheral blood stem cells

**Table 6. Comparison of factors between the CD34⁺ selected and unmanipulated group (patients transplanted from 1996**

| | **CD34⁺** (n=88) | | **PBSC** (n=19) | | P |
|---|---|---|---|---|---|
| | n | % | n | % | |
| **Duration of first remission** | | | | | 0.931 |
| Persistent/primary progressive disease | 39 | 44% | 8 | 42% | |
| Early/late relapse | 39 | 44% | 9 | 47% | |
| Multiple relapses | 9 | 10% | 2 | 10% | |
| Secondary HL | 1 | 1% | 0 | 0% | |
| **Stage** | | | | | **0.021** |
| I + II | 38 | 43% | 3 | 16% | |
| III + IV | 50 | 57% | 16 | 84% | |
| **Extranodal involvement** | 40 | 45% | 13 | 68% | 0.058 |
| **Lung involvement at the time of relapse** | 24 | 27% | 9 | 47% | 0.077 |
| **Liver involvement at the time of relapse** | 7 | 8% | 4 | 21% | 0.104 |
| **B-symptoms** | 40 | 45% | 12 | 63% | 0.126 |
| **Response at the final evaluation** | | | | | 0.938 |
| Complete remission | 47 | 53% | 11 | 58% | |
| Partial remission | 31 | 35% | 6 | 32% | |
| Stable/persistent disease | 10 | 11% | 2 | 10% | |
| **Relapse after ASCT** | 29 | 33% | 7 | 34% | 0.496 |

HL: Hodgkin's lymphoma; ASCT: autologous stem cell transplantation

lected group (57%; P= 0.021). Other factors did not differ significantly.

*Prognostic factors.* Results of univariate analysis of prognostic factors for PFS and OS using Cox regression model are listed in Table 7. In univariate analysis chemosensitive disease at the time of relapse (P <0.001) and using of CD34+ positively selected stem cells (P=0.010 and P=0.002, respectively) were significant prognostic variables associated with improved PFS and OS. Increased LDH level (P<0.001) and lung involvement (P=0.010 and P=0.005, respectively) at the time of relapse were associated with decreased PFS and OS. Moreover, extranodal involvement was an adverse prognostic factor for OS (P=0.033).

## Discussion

The objective of this analysis was to assess results of HDCT+ASCT in 126 patients with relapsed or progressive HL transplanted at our centre. Our aim was to determine the long term outcome, clinical risk factors, toxicity of the treatment and the role of use of the CD34⁺ positively selected stem cells in patients with relapsed or refractory HL.

The following results emerge from this analysis: (i) The complete program is feasible with acceptable acute toxicity rate and low TRM (3%). (ii) There is no outcome difference in terms of PFS and OS between early and late relapsed HL.

**Table 7. Potential risk factors and associated relative risk for OS and PFS in univariate proportional hazards Cox model (n=126).**

| Factor | PFS | OS |
|---|---|---|
| | p-value | p-value |
| Age at ASCT | 0.926 | 0.647 |
| Stage before transplantation | 0.283 | 0.054 |
| B-symptoms before transplantation | 0.331 | 0.068 |
| Extranodal involvement | 0.077 | **0.033** |
| Lung involvement | **0.010** | **0.005** |
| Liver involvement | 0.489 | 0.173 |
| Increased LDH level | **<0.001** | **<0.001** |
| Selection of $CD34^+$ stem cells | **0.010** | **0.002** |
| Number of cytoreductive regimens before transplantation | 0.059 | 0.164 |
| Number of cycles of cytoreductive chemotherapy | 0.114 | 0.310 |
| Disease status at the time ASCT | **<0.001** | **<0.001** |

ASCT: autologous stem cell transplantation; PFS: progression free survival; OS: overall survival; LDH: lactate dehydrogenasis

The outcome of patients with primary progressive disease is worse than in patients with persistent disease, though the difference is marginal. The outcome of patients with multiple relapses is dismal. (iii) In univariate analysis chemoresistant disease, use of unmanipulated PBSC, increased LDH and lung involvement at the time of relapse/progression were adverse prognostic factors for PFS and OS. Moreover, extranodal involvement was an additional risk factor for OS. (iv) The use of CD34+ positively selected stem cells for autografting is feasible, safe and effective procedure.

HDCT+ASCT has been shown to produce long term OS of 50-80% and PFS of 45–77% in patients with relapsed/refractory HL [2, 11–15]. The outcome of this group of patients transplanted at our centre compares favourably with the reported results in terms of 5-y OS (72%) and 5-y PFS (59%). Moreover the sensitivity to cytoreductive chemotherapy was not the requisition in order to proceed to HDCT+ASCT. In our study we carried out a subgroup analysis according to the duration of the first remission. The results of this subanalysis must be interpreted with caution because of a low number of patients in the cohorts. The outcome of patients with early and late relapse was similar in terms of OS (74% vs. 75%), with trend toward a better PFS in patients with early relapse (65% vs. 48%), though the difference was not statistically significant. This observation is in line with data reported recently by other groups [13, 16]. The outcome results of patients with primary progressive disease indicate that in a substantial proportion of these unfavourable patients a long term OS (64%) and PFS (55%) can be achieved after HDCT+ASCT. Variability in reported survival results in patients with primary refractory disease is obvious. There has been reported OS of 30–50% and PFS of 20-30% [14, 16–19] in these patients. The differences could be explained by a non-uniform definition of primary progressive disease. Unlike others [14, 18, 19], we included into this subgroup also patients with transient CR and relapse within 3 months after the end of first-line treatment. Patients with multiple relapses proved to have the worst outcome in terms of OS (54%) and PFS (40%). Based on the herein presented and previously published results [12, 13, 16, 17, 19], HDCT+ASCT should be performed early in HL patients failing the first-line treatment. The outcome of patients transplanted because of persistent disease after the front line therapy in terms of OS (81%) and PFS (72%) compares favourably with the rest of the group. This subset of patients was frequently affiliated in one group with patients with primary progressive disease as induction failure, whereas we evaluated this subgroup separately. In the past we used for the response assessment routine radiological methods (X-ray and CT scanning). It is therefore possible that some patients with persistent disease according to the CT criteria underwent intensification followed with HDCT+ASCT with already non-viable residual masses. This suggestion confirms the importance of assessment of both, radiological and metabolic response after the initial treatment by means of CT and positron emission tomography (PET).

Several groups have evaluated prognostic factors identifiable before transplantation affecting adversely the outcome after transplantation [12, 13–15, 17, 19], including duration of the first remission, chemoresistance, poor performance status, elevated LDH, extranodal disease, B-symptoms and failure of more than two prior regimens. In our analysis the strongest adverse prognostic factors for OS and PFS were the lack of response to the cytoreductive chemotherapy and elevated LDH at the time of relapse/ progression. Chemosensitivity to the salvage therapy, assessed radiologically, has been previously reported as the most important prognostic factor. Most recently Jabbour et al. confirmed in a retrospective analysis that incomplete metabolic response to cytoreductive chemotherapy as indicated by positive PET appeared to be predictive of poor outcome after HDCT+ASCT [20]. Such chemoresistant patients with positive $^{18}$F-fluorodeoxyglucose (FDG) uptake may benefit from more intensive pre-transplantation chemotherapy or from innovative approaches. Additional risk factor for OS and PFS emerging from our analysis was lung involvement. A speculative explanation for this finding, reported also by Horning and colleagues [11], may be an inferior efficacy of chemotherapy, yet in high doses, in the lung tissue.

We observed a remarkable decline in TRM during the observation period. In the group of 19 patients transplanted within the earlier period (1993–1995), toxic death occurred in 3 patients, while in the group of 107 patients transplanted later (1996–2005) only 1 toxic death occurred. This reflects an improvement in supportive treatment and increased transplantation experience. Considering infections, no difference in the incidence and severity of infections between the CD34+ selected and unmanipulated PBSC groups has been seen (data not shown). Immune haematological disorders occurred in 4 patients. Three patients had ITP and one patient had Evans syndrome. After the standard therapy all the 4 patients completely recovered. Up to now, with the median follow up of 69 months, we have noted 7 secondary malignancies. Of them

4 secondary NHL, 2 sAML/MDS and 1 soft tissue sarcoma in previously irradiated field. The outcome of our patients with secondary malignancies after transplantation was very poor.

Despite of encouraging results, disease relapse/progression remains the major cause of death and occurs in about 40–45% of transplanted HL patients. To reduce the relapse and progression incidence, several strategies have been tested, such as introduction of new cytoreductive regimen [15], sequential HDCT [16, 21], tandem auto-allo SCT [22] and also the use of positively selected CD34+ stem cells [10, 25, 26]. The last mentioned strategy is based on the evidence of the circulating clonogenic CD30+ tumor cells in peripheral blood [5–7] and in harvested PBSC in HL patients [23]. The positive selection of CD 34+ cells has been proven to be an effective purging method without a negative effect on the haematological and immunological recovery [9, 23]. In 1996 we started to use the positive CD34+ selection of stem cells. Based on the encouraging results in the first 10 HL patients [24], the purging became a routine method used in patients with relapsed/refractory HL at our centre. The actuarial 5-y OS and PFS of the 88 patients supported with $CD34^+$ selected stem cells were 79% and 64%, and of the 19 patients supported with unmanipulated PBSC were 66% and 63%, respectively. We observed a trend toward a better OS in CD34+ positively selected group. This is indicative of a better outcome in the case of relapse after HDCT+ ASCT in this group. However, a comparison of the outcome results between the two groups is hampered because of imbalances in patient characteristics, lower number of patients in unmanipulated group and of non-random allocation to a treatment group. Differences in risk factors profile between the two groups may reflect the inability of some patients to mobilise a sufficient number of stem cells to perform the purging procedure and also the retrospective nature of the analysis. The use of CD34+ positively selected stem cells for autografting is feasible, safe and effective. However, to demonstrate a potential survival advantage for patients supported with positively selected CD34+ stem cells over those supported with unmanipulated PBSC, the two strategies should be assessed within a randomised trial.

## References


[1] LINCH DC, WINFIELD D, GOLDSTONE AH et al. Dose intensification with autologous bone marrow transplantation in relapsed and resistant Hodgkin's disease: results of a BNLI randomised trial. Lancet 1993; 341: 1051–1054.

[2] SCHMITZ N, PFISTNER B, SEXTRO M et al. Aggressive conventional chemotherapy compared with high-dose chemotherapy with autologous haematopoietic stem-cell transplantation for relapsed chemosensitive Hodgkin's disease: a randomised trial. Lancet 2002; 359: 2065–2071.

[3] SCHMITZ N, LINCH DC, DREGER P et al. Randomised trial of filgrastim-mobilised peripheral blood progenitor cell transplantation versus autologous bone-marrow transplantation in lymphoma patients. Lancet 1996; 347: 353–357.

[4] MAJOLINO I, PEARCE R, TAGHIPOUR G et al. Peripheral-blood stem-cell transplantation versus autologous bone marrow transplantation in Hodgkin's and non-Hodgkin's lymphomas: a new matched-pair analysis of the European Group for Blood and Marrow transplantation Registry data. Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol 1997; 15: 509–517.

[5] BRUGGER W, BROSS KJ, GLATT M et al. Mobilisation of tumor cells and hematopoietic progenitor cells into peripheral blood of patients with solid tumors. Blood 1994; 83: 636–640.

[6] KVALHEIM G, ERIKSTEIN B, GILEN E et al. The presence of micrometastases in bone marrow and blood in in high-risk stage II breast cancer patients before and after high-dose therapy. Autologous Blood and Marrow transplantation. Proceedings of the Ninth International Symposium, Arlington, Texas. Carden Jennings Publishing: Charlottesville, VA, 1999, pp 247–255.

[7] WOLF J, KAPP U, BOHLEN H et al. Peripheral blood mononuclear cells of patients with advanced Hodgkin's lymphoma give rise to permanently growing Hodgkin-Reed Sternberg cells. Blood 1996; 87: 3418–3428

[8] SCHILLER G, VESCIO R, FREYTES C et al. Transplantation of CD 34+ peripheral blood progenitor cells after high-dose chemotherapy for patients with advanced multiple myeloma. Blood 1995; 86: 390–397.

[9] MAMPARA MY,KORNER IJ, HILDEBRANDT M et al. Monitoring of tumor cell purging after highly efficient immunomagnetic selection of CD34+ cells from leukapheresis products in breast cancer patients: comparison of immunocytochemical tumor cell staining and reverse transcriptase-polymerase chain reaction. Blood 1997; 89: 337–344.

[10] BLYSTARD AK, HOLTE H, KVALOY et al.High-dose therapy in patients with Hodgkin's disease: the use of selected CD34+ cells is as safe as unmanipulated peripheral blood progenitor cells. Bone Marrow Transplant 2001; 3: 295–305.

[11] HORNING SJ, CHAO NJ, NEGRIN RS et al. High dose therapy and autologous hematopoietic progenitor cell transplantation for recurrent and refractory Hodgkin's disease: Analysis of the Stanford University results and prognostic indices. Blood 1997; 89: 801–813.

[12] SUREDA A, ARRANZ R, IRIONDO A et al. Autologous stem-cell transplantation for Hodgkin's disease: Results and prognostic factors in 494 Patients from Grupo Aspanol de Linfomas/Transplantate Autologo de Medula Osea Spanish Cooperative Group. J Clin Oncol 2002; 19: 1395–1404.

[13] LAVOIE JC, CONNORS JM, PHILLIPS GL et al. High dose chemotherapy and autologous stem cell transplantation for primary refractory or relapsed Hodgkin lymphoma: lon-term outcome in the first 100 patients treated in Vancouver. Blood 2005; 106: 1473–1478.

[14] FERMÉ C, MOUNIER N, DIVINE M et al.Intensive salvage therapy with high-dose chemotherapy for patients with advanced Hodgkin's disease in relapse or failure after initial

chemotherapy: results of the Groupe d'Etudes des Lymphomes de l'Adulte H89 trial. J Clin Oncol 2002; 20: 467–475.

[15] MOSKOWITZ CH, NIMER SD, ZELENETZ AD et al. A 2-step comprehensive high-dose chmoradiotherapy second-line program for relapsed and refractory Hodgkin disease: analysis by intent to treat and development of a prognostic model. Blood 2001; 97: 616–623.

[16] JOSTING A, RUDOLPH C, MAPARA M et al. Cologne high-dose sequential chemotherapy in relapsed and refractory Hodgkin lymphoma: results of a large multicenter study of the German Hodgkin Lymphoma Study Group (GHSG). Ann Oncol 2005; 16: 116–123.

[17] REECE DE, CONNORS JM, SPINELLI JJ et al. High-dose therapy with cyclophosphamide, carmustine, etoposide +/- cisplatin, and autologous bone marrow transplantation for Hodgkin's disease in first relapse after combination chemotherapy. Blood 1994; 83:1193–1199.

[18] ANDRÉ M, HENRY-AMAR M, PICO JL et al. Comparison of high-dose therapy and autologous stem-cell transplantation with conventional therapy for Hodgkin's disease induction failure: A case-control study. J Clin Oncol 1999; 17: 222–229.

[19] SWEETENHAM JW, CARELLA AM, TAGHIPOUR G et al. High-dose therapy and autologous stem cell transplantation for adult patients with Hodgkin's disease who do not enter remission after induction chemotherapy: results in 175 patients reported to the European Group for Blood and Marrow Transplantation. J Clin Oncol 1999; 17: 3101–3109.

[20] JABBOUR E, HOSING CH, AYERS G et al. Pretransplant positive positron emission tomography/ gallium scans predict poor outcome in patients with recurrent/refractory Hodgkin lymphoma. Cancer 2007; 109: 2481–2489.

[21] TARELLA C, CUTTICA A, VITOLO U et al. High-dose sequential chemotherapy and peripheral blood progenitor cell autografting in patients with refractory and/or recurrent Hodgkin lymphoma. Cancer 2003; 97: 2748–2759.

[22] CARELLA AM, CAVALIERE M, LERMA E et al. Autografting followed by nonmyeloablative immunosuppressive chemotherapy and allogenic peripheral-blood hematopoietic stem-cell transplantation as treatment of resistent Hodgkin's disease and non-Hodgkin's lymphoma. J Clin Oncol 2000; 18: 3918–3924.

[23] BLYSTAD AK, TORLAKOVIC E, HOLTE H et al. CD34+ cell enrichment depletes atypical CD30+ cells from PBSC grafts in patients with HD. Cytotherapy 2001; 3: 295–305.

[24] LAKOTA J. Use of selected CD34+ cells in the treatment of relapsed Hodgkin's lymphoma. Bone Marrow Transplant 1999; 24: 697–699.

[25] LAKOTA J, BALLOVA V, DRGONA et al. Use od selected CD34+ cells in the treatment of relapsed/progressive HD: experience from a single center. Cytotherapy 2002; 4: 177–180.

[26] SORA F, FEBO AL, PRICCIRILLO N et al. Autologous stem cell transplantation for primary refractory or relapsing Hodgkin's disease: comparison between CD34+ immunoselected and unselected cell graft. Gene Ther Mol Biol 2007; 11: 21–26.