Predictors for Successful Mobilization of Peripheral Blood Progenitor Cells with ESHAP + G-CSF in Patients with Pretreated Non-Hodgkin’s Lymphoma

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Introduction

High-dose chemotherapy followed by autologous hematopoietic progenitor cell transplantation (AHPCT) has become an established modality of treatment for patients with refractory or high-risk non-Hodgkin’s lymphoma (NHL) as well as a wide variety of hematologic malignancies.1–3 With the advantages of a relatively easy collection procedure and short duration to engraftment, peripheral blood progenitor cells (PBPCs) or peripheral blood stem cells (PBSCs) are now preferred over bone marrow progenitor cells as a source of
AHPCT. However, successful engraftment in AHPCT relies much on the cell dose of PBPCs. Clinical trials have shown that engraftment can be accelerated by infusion of large dose of progenitor cells. With respect to a safe cell dose, a number of studies have demonstrated that a CD34+ cell dose of 2 × 10^6/kg or higher in AHPCT is associated with excellent hematopoietic recovery.

A variety of regimens have been used successfully in NHL patients for PBPC mobilization, including hematopoietic growth factors with or without cyclophosphamide and combinations of granulocyte-colony stimulating factor (G-CSF) and chemotherapy used to treat NHL. Nonetheless, the optimal timing for PBPC harvest following mobilizing therapy remains undetermined. Also, since the infused CD34+ cell dose influences the outcome of engraftment, how to maximize CD34+ cell yield has continued to be studied. Efforts have been devoted to use peripheral blood CD34+ cell count, total white blood cell count (WBC) or both as surrogate markers to start leukapheresis for maximizing CD34+ cell collection. The CD34+ cell count seems to reflect more directly the resultant CD34+ cell yield. Other factors such as age, interval between treatment and harvest, preceding chemotherapy and radiotherapy, dose of chemotherapy used for PBPC mobilization, and PB platelet count on the first day of PBPC collection have also been reported to influence PBPC yield. However, some of the reported results are inconsistent, especially among studies using different mobilizing regimens.

ESHAP (etoposide/methylprednisolone/cytarabine/cisplatin) plus G-CSF has been shown to be effective for mobilizing PBPCs in NHL patients. Notwithstanding, factors impacting on maximizing PBPC collection remain to be explored. We conducted an analysis on 20 consecutive advanced NHL patients for whom PBPCs were harvested following ESHAP chemotherapy and G-CSF. The correlation of the pre-apheresis peripheral blood CD34+ cell count on the collection day to the apheresed CD34+ cell yield was analyzed. The predictability of factors for CD34+ cell yield along with the feasibility of this mobilizing regimen are discussed.

### Methods

#### Patients

The patients’ characteristics are listed in Table 1. Twenty NHL patients were recruited between March 2003 and September 2006, underwent ESHAP chemotherapy plus G-CSF to mobilize PBPCs and were analyzed for factors potentially correlated to the PBPC yields. There were 10 males and 10 females, with a median age of 48 years (range, 19–72 years). All patients had high-risk diseases that warranted high-dose chemotherapy rescued by AHPCT. Before PBPC mobilization, all patients had received chemotherapy of 4 or more cycles (range, 4–7 cycles) of CHOP (cyclophosphamide, adriamycin, vincristine, prednisolone), but still had residual tumor. One patient had received 6 additional cycles of high-dose methotrexate for brain lymphoma and 2 other patients had received 1–3 additional cycles of ESHAP chemotherapy in addition to the mobilizing ESHAP chemotherapy.

All the patients were treated in Taipei Veterans General Hospital. The study was conducted in accordance with the institutional regulations and informed consent was obtained from each patient before enrolment in the study.

#### Mobilization, leukapheresis and storage

The mobilizing method and timing of leukapheresis have been described previously. Intravenous ESHAP (methylprednisolone 500 mg/day on days 1–4, etoposide 40 mg/m²/day on days 1–4, cisplatin 25 mg/m²/day continuous infusion on days 1–4, and cytosine...
arabinoside 2 g/m² on day 5) was administered and followed by 5 μg/kg/day subcutaneous injection of G-CSF from day 7 until the day when PBPC harvest was completed.

All 20 patients underwent daily blood cell count examination starting from day 6, with day 1 referring to the day when ESHAP chemotherapy was initiated. Two consecutive daily leukaphereses were started once peripheral blood WBC exceeded 10 × 10⁹/L after a nadir. Leukapheresis was conducted using the COBE Spectra Version 6.1 cell separator (COBE BCT, Lakewood, CO, USA). Anticoagulant citrate dextrose solution was used to prevent clotting. Ten liters of blood were processed for each leukapheresis. The product obtained was mixed with DMSO (Merck, Darmstadt, Germany) in autologous plasma to a final concentration of 10%. By programmed freezing, the cells were subsequently cryopreserved in liquid nitrogen.

**Enumeration of CD34⁺ cells**

The circulating CD34⁺ cell count was determined in peripheral blood sampled in the early morning of each leukapheresis day. Mononuclear cells were stained with phycoerythrin (PE)-conjugated anti-CD34 (anti-HPCA-2) mouse monoclonal antibody and counterstained with fluorescein isothiocyanate (FITC)-conjugated anti-CD45 mouse monoclonal antibodies (Becton Dickinson, San Jose, CA, USA). Simultest™ control γ1/γ2a (Becton Dickinson) was used as a negative control to quantify the non-antigen-specific antibody binding. More than 60,000 cells were detected using a FACS flow cytometer and analyzed with Cellquest software (Becton Dickinson).

**Statistical analysis**

SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Pearson’s correlation analysis was applied for evaluating the relevance of pre-leukapheresis peripheral blood CD34⁺ cell counts and CD34⁺ cell yield per leukapheresis. The Mann-Whitney test was used to test the significance of difference between CD34⁺ cell yields of cohorts with peripheral blood CD34⁺ cell count ≥50 × 10⁶/L and <50 × 10⁹/L. The cut-off value of peripheral blood CD34⁺ cell count was set at 50 × 10⁹/L. The mean CD34⁺ yield of 2 leukaphereses in the cohort with less than the cut-off value in pre-leukapheresis peripheral blood CD34⁺ cells was 1.80 × 10⁹/kg, a value that is less than the safe CD34⁺ cell dose (2.0 × 10⁹/kg) for PBPC autografting.6–8 To evaluate other factors affecting the CD34⁺ cell yield per leukapheresis, a 2 × 2 crosstable with χ² test was used. Statistical significance was considered for all tests when p < 0.05.

**Results**

**Leukapheresis and yield**

For the 20 patients, the first day when leukapheresis started ranged from day 12 to day 18 (median, day 15), with day 1 referring to the day when ESHAP chemotherapy was initiated. Two leukaphereses were performed for each patient. The mean ± standard error of the total mononuclear cells and the total CD34⁺ cells harvested for the 20 patients were 6.48 ± 3.52 × 10⁶/kg and 14.4 ± 16.7 × 10⁶/kg, respectively. Fourteen (70%) patients had their CD34⁺ cell yield on the first leukapheresis day exceeding 2 × 10⁶ cells/kg. Sixteen patients (80%) had CD34⁺ cell yield of 2 leukaphereses above 2 × 10⁶/kg body weight; another 3 (15%) between 1–2 × 10⁶/kg and the remaining 1 (5%) below 1 × 10⁶/kg.

**Correlation of pre-leukapheresis peripheral blood CD34⁺ cell count to PBPC yield**

The pre-leukapheresis peripheral blood CD34⁺ cell counts on the PBPC collection days were available among 28 of the 40 leukaphereses. The mean CD34⁺ cells collected per leukapheresis was 6.70 ± 7.46 × 10⁶/kg. A significant correlation between the pre-leukapheresis peripheral blood CD34⁺ cell count and the CD34⁺ cell yield of each leukapheresis was shown by a linear regression analysis (r² = 0.870, p < 0.001; Figure 1A). The mean ± standard error of CD34⁺ cell yield of patients with pre-leukapheresis peripheral blood CD34⁺ cell count ≥50 × 10⁶/L was 5.60 ± 4.32 × 10⁶/kg/leukapheresis, while that of patients with pre-leukapheresis peripheral blood CD34⁺ cell count <50 × 10⁹/L was 0.96 ± 0.56 × 10⁶/kg/leukapheresis (median, 0.56 × 10⁶/kg/leukapheresis). The CD34⁺ yield in the group with pre-leukapheresis peripheral blood CD34⁺ cell count ≥50 × 10⁶/L had a significantly higher total CD34⁺ cell yield (p < 0.001; Figure 1B).

**Factors affecting CD34⁺ cell yield**

Data were analyzed to determine possible factors affecting the CD34⁺ cell yield (Table 2). Patients who experienced 6 or more courses of preceding chemotherapy had lower CD34⁺ cell yield (p = 0.032). In addition, patients with >3,500/μL of peripheral blood WBC before mobilizing chemotherapy had better CD34⁺ cell yield than those with <3,500/μL. On the other hand, sex, age or whether bone marrow was involved at the initial diagnosis did not significantly affect PBPC yield. Peripheral blood hemoglobin and platelet count on day 1 of mobilization, chemotherapy, severity of neutropenia and thrombocytopenia after ESHAP, along with the time required for white cells
to recover from nadir to number above \(10 \times 10^9/L\), all of which possibly represent marrow-reserving capacity, did not affect PBPC yield either.

**Engraftment after high-dose therapy in NHL patients**

Sixteen of the 20 NHL patients later proceeded to high-dose chemotherapy and AHPCT. Four patients did not proceed to AHPCT due to disease progression shortly after PBPC harvesting (in 1 patient) or complete remission achieved after chemotherapy (in 2 patients) or inadequate PBPC (<\(1.0 \times 10^9/kg\)) harvested (in 1 patient). Among the 16 patients undergoing PBPC autografting, 14 had CD34\(^+\) cell dose ≥\(2.0 \times 10^9/kg\) and the other 2 had CD34\(^+\) cell doses of 1.26 and 1.60 \(\times 10^6/kg\), respectively. The median time to myeloid engraftment (defined as absolute neutrophil count ≥\(0.5 \times 10^9/L\) for 3 consecutive days) was 10 days (range, 9–11 days), while engraftment of platelets (defined as platelet count ≥\(20 \times 10^9/L\) for 7 consecutive days without transfusional support) was seen at a median of 15 days (range, 12–18 days).

**Discussion**

As infused CD34\(^+\) cell dose correlates well with hematopoietic recovery and transplant outcome in PBPC transplantation, adequate PBPC collection has become a prerequisite for successful autograft. Hematopoietic progenitor cells can be mobilized into the circulation by G-CSF, chemotherapy or both. To maximize efficient PBPC yield, the timing for PBPC collection after these mobilizations is critical. Some criteria have been utilized to determine when to initiate collection. Among them, circulating peripheral blood CD34\(^+\) cell count has been a predictor of PBPC yield mobilized with regimens other than the ESHAP+G-CSF used in this study.\(^13,14,19\) Using ESHAP+G-CSF, we hereby proved that circulating peripheral blood CD34\(^+\) cell count remains a predictor of CD34\(^+\) cell yield.

Choosing mobilizing modality has been a field of controversy for years. The jury on the Second International Consensus Conference on High-Dose Therapy with Hematopoietic Stem Cell Transplantation in Aggressive NHL recommended that chemotherapy plus cytokines, rather than either alone, should be used to mobilize hematopoietic stem cells.\(^20\) Based on that recommendation, high-dose cyclophosphamide followed by G-CSF has been traditionally used as a mobilization regimen.\(^20\) Additionally, combinations of NHL treatment regimen and growth factors, which benefit patients with both tumor-killing and PBPC mobilization, were employed in increasing frequency and demonstrated to be effective mobilization regimens.\(^9,11,12,18\) Some of them, including ESHAP+G-CSF, appeared to be superior in PBPC yield.\(^9,11,12\)

In this study, using ESHAP+G-CSF for NHL patients as a PBPC-mobilizing regimen, the mean CD34\(^+\) cells collected per leukapheresis was \(7.2 \pm 8.3 \times 10^6/kg\).
The result is comparable with those in other reports. Among them, Lee et al reported that a mean of $6.0 \times 10^6$ CD34$^+$ cells/kg/leukapheresis was mobilized with ESHAP + G-CSF with a mean of 6 cycles of previous chemotherapy in patients with relapsed or refractory lymphoma. A better CD34$^+$ cell yield was found in patients treated with ESHAP + G-CSF than in those who underwent high-dose cyclophosphamide + G-CSF. We confirmed that ESHAP + G-CSF is not only an active alternative therapy for advanced NHL but is also effective in progenitor cell mobilization. 

To mobilize PBPCs with ESHAP + G-CSF in NHL patients, one of the significant findings of this study is that the pre-leukapheresis peripheral blood CD34$^+$ cell count on the day of PBPC collection reliably predicted CD34$^+$ cell yield. The pre-leukapheresis peripheral blood CD34$^+$ cell count of $50 \times 10^6$/L on the day of PBPC collection could be regarded as a distinctly safe threshold guaranteeing successful PBPC harvesting.

Factors influencing progenitor cell yield have been extensively studied in numerous trials. However, it is still difficult to draw a definite conclusion regarding these factors from these studies owing to the heterogeneity in the patient population in terms of their different disease characteristics. Nonetheless, there still exists the general consensus that drugs with stem cell-toxic properties, such as melphalan, cyclophosphamide, carmustine, lomustine and mechlorethamine, are associated with inferior stem cell yield. In our patients, the only 2 factors with a favorable impact on the harvest were less than 6 preceding chemotherapy cycles and more than 3,500/μL for pre-mobilization WBC, which might reflect bone marrow reserve. Since the CHOP regimen has always been the frontline treatment for our patients in this study, it is unknown

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*p* $\chi^2$ test. BW = body weight; CT = chemotherapy; WBC = white blood cell; Hb = hemoglobin; BM = bone marrow; ANC = absolute neutrophil count.
whether the aggregate effect of all the chemotherapeutic agents or the cumulative dose of cyclophosphamide played the major role in impairing the CD34+ cell yield.

In conclusion, with PBPC mobilization using ESHAP + G-CSF, pre-leukapheresis peripheral blood CD34+ cell count $\geq 50 \times 10^9$/L on the day of collection was a good indicator for initiating stem cell harvesting. We confirmed that ESHAP + G-CSF is an efficient mobilization regimen for NHL patients. High-dose chemotherapy followed by AHPCT has been proven to be superior to conventional chemotherapy in patients with chemosensitive relapse of aggressive NHL.34 Also patients with NHL may benefit from high-dose chemotherapy followed by AHPCCT as part of first-line therapy.5 It is possible to decide before 6 cycles of frontline chemotherapy whether patients with NHL will undergo AHPCCT and have PBPC harvested. From our data, ESHAP + G-CSF is recommended for PBPC harvesting before 6 cycles of chemotherapy with CHOP or equivalent regimen once high-dose chemotherapy followed by AHPCCT is contemplated. However, PBPC mobilization after 6 cycles of frontline chemotherapy may still possibly have suboptimal quantity of CD34+ cells adequate for autograft.

Acknowledgments

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References


Circulating CD34+ cells predict PBSC yield