Peripheral Blood Stem Cell Harvesting in Young Children Weighing Less Than 15 Kilograms: A Single-Institute Experience in Taiwan

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Article

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Abstract

Autologous peripheral blood stem cell (PBSC) transplantation is crucial in pediatric cancer treatment, and tandem transplantation is beneficial in certain malignancies. Collecting PBSCs in small children with low body weight is challenging. We retrospectively analyzed data of pediatric cancer patients weighing < 15 kg who underwent autologous peripheral blood stem cell (PBSC) transplantation in our institute. Collections were performed in the pediatric intensive care unit over 2 or 3 consecutive days, to harvest sufficient stem cells (goal: ≥2 × 10^6 CD34+ cells/kg per apheresis). From April 2006 to August 2021, we performed 129 collections after 50 mobilizations in 40 patients, with a median age of 1.9 (range, 0.6–5.6) years and a body weight of 11.0 (range, 6.6–14.7). The median CD34+ cell collection in each apheresis was 4.2 × 10^6/kg. 78% of mobilizations achieved cell dose for single transplantation, while 56% for tandem transplantation, without additional aliquoting. Age < 2 years, no radiation exposure, and mobilization with chemotherapy were predictors of goal achievement through multivariate analysis (p < 0.05). PBSC collection in 2 or 3 consecutive days is safe and feasible for pediatric patients weighing < 15 kg. Granulocyte colony-stimulating factor alone was not effective for mobilization in children > 2 years, even without radiation exposure.

Introduction

High-dose chemotherapy followed by autologous stem cell transplantation has become a crucial treatment modality for certain pediatric cancers, such as high-risk neuroblastoma, recurrent or refractory lymphoma, embryonal brain tumor, and other solid tumors (1–4). The collection of hematopoietic stem cells is essential for this treatment. Peripheral blood stem cells (PBSCs) are currently the preferred hematopoietic stem cells because of their faster hematopoietic recovery (5). PBSC collection is less invasive than bone marrow harvest, which requires multiple punctures and generalized anesthesia. A growing body of evidence indicates that an intensive chemotherapy regimen combined with tandem transplantation (double or triple transplantation) is feasible in pediatric patients and may improve treatment outcomes in selected malignancies, such as embryonal brain tumor and high-risk neuroblastoma (1, 6).

During PBSC collection, stem cells need be mobilized from the bone marrow into peripheral blood. The most widely used mobilization protocol is the administration of granulocyte colony-stimulating factor (G-CSF) alone or following chemotherapy (5). The minimum number of CD34+ cells required for safe autologous transplantation is 2 × 10^6 per kg (7, 8), and failure to achieve this target cell number during collection is considered as poor mobilization.

Poor mobilization leads to repeated collection, increases the cost and complication risk, and eventually makes patients ineligible for receiving further high-dose chemotherapy followed by stem cell transplantation (5). Studies have identified several factors associated with poor mobilization, such as heavy pretreatment and previous radiation (9–11). In addition, the timing of apheresis is crucial because of the short window of stem cells being in peripheral blood. Some predictive models and indicators have
been developed to determine the optimal time to begin apheresis in adults (9). The application of an automated hematopoietic progenitor cell (HPC) counting system has been evaluated as a guide to start collection (9, 12). However, collecting PBSCs in the pediatric population is difficult. Several obstacles are encountered, particularly in young children with low body weight. The relatively large extracorporeal volume required for apheresis may cause hemodynamic instability in young children, requiring packed red blood cell (RBC) priming. In addition, low body weight increases the risk of metabolic disturbance, especially citrate toxicity, which may cause hypocalcemia and subsequent numbness or seizures. Small body size also causes difficulty in establishing vascular access, and the use of a small catheter caliber may compromise apheresis flow. In addition, children are usually uncooperative during this invasive and time-consuming procedure, and sedation or anesthesia is required occasionally (5, 13, 14). If tandem transplantation is planned, more PBSCs will be required. Some medical centers may adopt different approaches to overcome these challenges in young children with low body weight (11, 13, 15).

In this study, we share our approach and the results of harvesting autologous PBSCs in young children weighing < 15 kg. We planned to conduct 3 rounds of apheresis over 3 consecutive days to harvest PBSCs for at least single or tandem transplantation.

Methods

Patients

From April 2006 to August 2021, all consecutive young children with a body weight of < 15 kg who underwent PBSC harvesting at the Division of Pediatric Hematology/Oncology in our hospital were enrolled.

Mobilization

Supplementary Fig. 1 presents the mobilization protocol. For mobilization, the majority of patients received chemotherapy (88%) followed by G-CSF at a dose of 6–10 µg/kg/day, which was administered either in 2 divided dose every 12 hours, or once daily. Complete blood count (CBC) was examined every other day and then daily after the white blood cell (WBC) count reached nadir; at this time, the HPC count in peripheral blood was measured using the Sysmex hematology analyzer (Sysmex XE-2100, Sysmex Corp., Kobe, Japan). PBSC harvesting was initiated when the WBC count was \( \geq 3000/\mu L \) and the HPC count was \( \geq 20/\mu L \) on the collection date or on the basis of the treating physician's clinical judgement. Patients were admitted to the pediatric intensive care unit, and then an arterial line, a large-bore peripheral line (LBPL), or a central venous catheter (CVC) for hemodialysis was inserted for outflow in the intensive care unit. Inflow access was achieved by inserting another LBPL, CVC, or port-A catheter. The type of blood outflow access used for each patient was determined by treating physician's decision.

Harvesting
All patients stayed in the pediatric intensive care unit for close monitoring during PBSC harvesting until 12 to 24 hours after collection in each mobilization. Extracorporeal circuit priming with 2 units of packed RBCs was applied to all patients. Continuous 10% calcium gluconate infusion was routinely administered through a secure central line. During collection for 2 to 3 hours, oral chloral hydrate or occasional intravenous midazolam bolus were used for sedation if outflow was inadequate due to poor cooperation despite the use of age-appropriate comfort measures and distractions. Collection was performed on 2 or 3 consecutive days to ensure the collection of an adequate number of stem cells for tandem or single transplantation in accordance with the underlying disease of the patient. After each apheresis, the CBC and free calcium level of patients were measured. Platelet and hemoglobin levels were maintained above 50 k/µL and 8 g/dL during apheresis, respectively. Goal achievement was defined as the collection of \( \geq 2 \times 10^6 \) CD34\(^+\) cells/kg during any apheresis.

PBSC Product

All products were sampled for bacterial culture to ensure sterility immediately after collection and before freezing. The CBC and CD34\(^+\) cell count of each apheresis product were calculated. Final products were cryopreserved in 10% dimethyl sulfoxide.

Data Collection

Information on patient characteristics, underlying diseases, previous radiotherapy exposure, mobilization by G-CSF alone or with chemotherapy, CBC prior to apheresis, stem cell count in each apheresis product, vascular access, and complications was collected through a retrospective chart review.

Statistical Analysis

Possible predictors, namely age, sex, body weight during collection, mobilization by G-CSF alone or with chemotherapy, prior radiotherapy exposure, HPC count, underlying diseases, and outflow access, were included in the univariate analysis to evaluate goal achievement in each apheresis.

A logistic regression model was used to identify the predictors of poor goal achievement in the univariate and multivariate analyses. A receiver operating characteristic (ROC) curve was plotted using the significant predictors identified in multivariate analysis. The area under the ROC curve (AUC) was calculated to determine the significance of predictors and is expressed as a number.

A 2-sided \( p \) value of <.05 was defined as statistically significant. Statistical analysis was performed using IBM SPSS statistics software (version 25).

Results

Patient Characteristics
From April 2006 to August 2021, we performed 129 collections after 50 mobilizations in 40 patients weighing <15 kg who were diagnosed as having atypical teratoid/rhabdoid tumor (n = 14), medulloblastoma (n = 6), embryonal tumors with multilayered rosettes (n = 3), neuroblastoma (n = 6), hepatoblastoma (n = 3), germ cell tumor (n = 2), Ewing sarcoma (n = 2), and others (n = 4). Table 1 lists the characteristics of these patients. The median age and weight of the patients were 1.9 (range, 0.6–5.6) years and 11.0 (range, 6.6–14.7) kg, respectively.

Stem Cell Apheresis

Table 1 presents the findings of apheresis products. Most of the apheresis was completed through an arterial line (70%) or a CVC (29%). The median number of CD34+ cells collected in each apheresis was \(4.20 \times 10^6\) (range, 0.01–40.13) per kilogram in a median apheresis volume of 60 (range, 30–76) mL. In 78 (60.5%) of the 129 rounds of apheresis, the goal of collecting \(2 \times 10^6\) CD34+ cells per kilogram was achieved.

Table 2 presents findings regarding goal achievement in any apheresis. Apheresis was performed for 2 and 3 consecutive days in 17 (34.0%) and 31 (62.0%) mobilizations, respectively. The goal of collecting \(2 \times 10^6\) CD34+ cells per kilogram in all 3 rounds of apheresis (sufficient for triple transplantation) and in any 2 rounds of apheresis (sufficient for tandem transplantation) was achieved in 18 (58.1%) and 21 (67.6%) of the 31 mobilizations that involved 3 consecutive collections, respectively. This goal was achieved in 28 (90.3%) mobilizations after the cell dose of each apheresis for single transplantation was totaled. We collected \(2 \times 10^6\) CD34+ cells per kilogram in each apheresis, sufficient for tandem transplantation, in 7 (41.2%) of the 17 mobilizations involving 2 consecutive collections. Among the total 50 mobilizations, 39 (78.0%) and 28 (56%) mobilizations resulted in a sufficient cumulative cell dose for single or tandem transplantation, respectively, without requiring additional aliquoting prior to cryopreservation (Fig. 1A). Table 3 lists the details of cell dose in each apheresis per mobilization.

Predictors of Goal Achievement

The predictors of poor goal achievement were identified (Table 4). The goal was achieved in 75% (60/80) of apheresis initiated with the precollection of \(\geq 20/\mu\text{L}\) peripheral HPCs. The goal was met in 12% (3/25) of apheresis initiated with the precollection of \(< 20/\mu\text{L}\) HPCs \((p < .001)\). The rate of achieving the target cell dose was higher in mobilization performed with chemotherapy and G-CSF than in mobilization performed without chemotherapy (65.8% vs 20.0%, \(p = .003\)). Age older than 2 years \((p < .001)\) and prior radiation exposure \((p = .011)\) were associated with a higher chance of poor goal achievement. In multivariate analyses, factors independently associated with goal achievement included age younger than 2 years, mobilization with chemotherapy, and no radiation exposure (all \(p < .05\)). Figure 1B presents the prediction rates for goal achievement based on the 3 significant factors. The highest prediction rate of goal achievement in each apheresis was up to 83.4% in the patients aged < 2 years who did not receive prior radiotherapy and were mobilized with chemotherapy in addition to G-CSF. The ROC AUC was 0.748 (Fig. 1C). When the preharvesting HPC of \(\geq 20/\mu\text{L}\) was combined, the ROC AUC was 0.787 (Fig. 1D).
Remobilization Results

In 10 patients with poor mobilization, remobilization was performed after another cycle of chemotherapy. One (10%) patient had a sufficient cell dose in each apheresis in the 2nd mobilization after the addition of chemotherapy, whereas 3 patients (30%) did not have the sufficient cell dose despite the 2nd attempt. The other 6 patients had a cumulative cell dose that was adequate for either single or tandem transplantation.

Complications

Table 5 lists all complications that occurred during the apheresis procedure. Re-establishment of outflow vascular accesses was required in 22 (17.1%) of 129 rounds of apheresis. Other complications included muscle cramping and numbness, possibly related to hypocalcemia, in 11 (8.5%) patients, transient nausea/vomiting in 5 (3.9%) patients, fever in 2 (1.6%) patients, and local oozing or hematoma formation in 2 (1.6%) patients. Transient cyanosis/apnea that occurred in 4 (3.1%) patients was recovered soon after oxygen supplementation without requiring additional respiratory support. One (0.8%) patient developed transient sinus arrhythmia that was recorded on a bedside electrocardiogram monitor without symptoms. No other significant complications developed during the apheresis procedure.

Discussion

Studies have reported that PBSC collection can be successful and safe in children, even in those with low body weight (15–17). Tandem transplantation is increasingly being used to treat various pediatric malignancies, such as neuroblastoma (1, 18), embryonal brain tumor (6), Ewing sarcoma (19), and germ cell tumors (20). Thus, the safe and effective collection of stem cells sufficient for tandem transplant during a single mobilization in young children is the cornerstone for performing tandem transplantation.

Our study results indicate that 58.1% of mobilizations resulted in an adequate cell dose for triple transplantation and that 90.3% of mobilizations resulted in an adequate cumulative cell dose for at least a single transplantation when successful apheresis was conducted for 3 consecutive days after one mobilization. If adequate cell doses in each PBSC product for triple/tandem transplantation can be achieved through a single mobilization, additional mobilization, intensive care unit admission, repeated catheterization, sedation, and complications following the procedures can be prevented in young children with low body weight; this would also reduce medical expenditures.

Most of our patients received chemotherapy followed by G-CSF with a dose of 3–5 µg/kg/dose twice daily for mobilization. Among 129 rounds of apheresis, only 18 were performed after G-CSF without chemotherapy, depending on the physician's clinical judgement. Some studies have reported that a significantly higher stem cell count was achieved in mobilization performed with chemotherapy in addition to G-CSF than in mobilization performed with G-CSF alone (21, 22). Previous radiation exposure is a well-described risk factor for poor mobilization because of its potential toxicity to hematopoietic stem cells and the niche environment of the bone marrow (9, 23). Our data indicate the same trend in the
pediatric group ($p = .002$ for mobilization with chemotherapy or not, $p = .017$ for previous radiation exposure or not). However, patients aged $< 2$ years having better goal achievement ($p = .012$) was less described in very young children group in the literatures. The prediction rate for goal achievement was calculated on the basis of 3 significant host factors: age, chemotherapy for mobilization, and radiation exposure. The prediction rate for goal achievement in each apheresis in patients aged $< 2$ years, mobilization with chemotherapy, and no radiation exposure was 83.4%; the older patients without radiation exposure had a lower chance (15.9%) of achieving the goal while being mobilized with G-CSF alone. Our finding may help clinicians optimize mobilization by combining chemotherapy and G-CSF in older (aged $\geq 2$ years) patients to yield a sufficient cell dose for tandem transplant.

Plerixafor, a CXCR4 antagonist, has been used together with G-CSF for the rescue of poor mobilization in adult populations (24, 25). It has been approved by the European Medicines Agency for children from 1 year of age, based on previous studies (26). However, data of the safety and efficacy of plerixafor for children less than 1 year of age is still limited. Further study is required for this young population.

To increase collection efficiency, the timing is crucial, and several predictors for the performance of apheresis have been identified. An Indian study reported that a preprocedure CD34$^+$ count of $\geq 20/\mu$L on the day of collection may result in the successful collection of an adequate stem cell dose (27). Other studies have revealed that preharvest HPC counts were strongly correlated with the total CD34$^+$ cell yield in apheresis (9, 28). Compared with the CD34$^+$ cell count, the HPC count can be determined using an automated analyzer, which is much faster and less labor intensive, and still serve as a satisfactory predictor for the optimal timing of PBSC collection in the pediatric population.

Some challenges are encountered during PBSC apheresis in pediatric patients, especially those related to vascular approach. Studies have reported that CVCs, arterial lines, or LBPLs are feasible to maintain blood outflow during harvesting in pediatric patients (15, 29, 30). Because of the poor compliance and small caliber of blood vessels in children with low body weight, maintaining vascular devices and smooth outflow becomes challenging. In our studies, we found that maintaining an arterial line for harvesting on 3 consecutive days was difficult in children with low body weight, with 16.7% (15 of 90) of the children requiring the re-establishment of outflow access. CVCs may be a superior option if apheresis is expected to be performed on 2 to 3 days consecutively, with only 7.7% (2 of 26) of children requiring line re-establishment in our study. As for the goal achievement rate, there was no statistical difference between A-line or CVC ($p = .215$). Another concern regarding pediatric patients with low body weight is their low blood volume and hemodynamic stability during apheresis. We primed the extracorporeal circuit with packed RBC and monitored all patients weighing $< 30$ kg in an intensive care unit setting. In addition, young children are uncooperative and thus sometimes require sedation during the apheresis procedure. Therefore, close monitoring during apheresis in an intensive care unit is highly recommended to manage possible respiratory events during sedation. Since no extra respiratory support is needed other than oxygen supplement, fewer procedural pain and less blood loss in PBSC harvesting, it is much safer than bone marrow harvest which requires generalized anesthesia, endotracheal intubation in the operation room, blood transfusion and pain management after bone marrow harvesting.
Few studies have investigated the PBSC harvest procedure in pediatric patients with low body weight (13, 16, 17). Ravagnani indicated that PBSC harvesting under anesthesia was feasible and safe in 47 children with weight ranging from 7 to 20 kg. Salazar-Riojas reported that PBSC collection in 22 children weighing ≤ 20 kg was safe and effective, and central venous catheter placement was the most appropriate technique to ensure successful collection (13, 14). Sevilla revealed the safety of the procedure in patients weighing < 20 kg and indicated that 77% of patients achieved the target CD34+ cell dose of $2 \times 10^6$/kg (11). In our study, PBSC collection was safely performed in patients with low body weight. A sufficient cell dose per bag could be achieved through leukapheresis in 2–3 consecutive days during a single mobilization in 56% of mobilizations in young children. Tandem transplantation, if indicated, using autograft collection in one mobilization was feasible. After remobilization, a sufficient cumulative cell dose was collected for at least one transplantation in 92.5% (37/40) of the children.

This study has some limitations that should be addressed. First, because this is a retrospective study and only data from a single institute were included, the results may not be generalizable. Second, various disease types, cumulative doses, and regimens of previous chemotherapy may affect collection results. However, these factors were not analyzed because of an insufficient number of patients.

### Conclusion

In this study, PBSC collection was safely and effectively performed in pediatric patients weighing < 15 kg. A sufficient cell dose per bag for tandem transplantation was collected through leukapheresis in 2–3 consecutive days during each mobilization in 56% of the patients. An arterial line as the outflow access may not be suitable because of the poor maintenance of blood outflow for several days. Because of the small vessel caliber, low blood volume, and poor cooperation of pediatric patients, extra care and monitoring in the intensive care unit are necessary for children with low body weight. Significant risk factors for poor goal achievement were age older than 2 years, mobilization with G-CSF alone, radiation exposure, and preapheresis HPC count < 20/µL. Mobilization with G-CSF alone is not feasible in children aged older than 2 years with or without a history of radiation exposure.

### Abbreviations
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<th>Abbreviations</th>
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<tr>
<td>ATRT</td>
<td>atypical teratoid/rhabdoid tumor</td>
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<td>AUC</td>
<td>area under the curve</td>
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<td>CBC</td>
<td>complete blood count</td>
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<tr>
<td>CVC</td>
<td>central venous catheter</td>
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<td>DMSO</td>
<td>dimethyl sulfoxide</td>
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<td>ETMR</td>
<td>embryonal tumors with multilayered rosettes</td>
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<tr>
<td>G-CSF</td>
<td>granulocyte colony-stimulating factor</td>
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<tr>
<td>HPC</td>
<td>hematopoietic precursor cell</td>
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<tr>
<td>LBPL</td>
<td>large-bore peripheral line</td>
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<tr>
<td>MB</td>
<td>medulloblastoma</td>
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<tr>
<td>PBSC</td>
<td>peripheral blood stem cell</td>
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<tr>
<td>ROC</td>
<td>receiver operating characteristic</td>
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<tr>
<td>TNC</td>
<td>total nucleated cells</td>
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<td>WBC</td>
<td>white blood cells</td>
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**Declarations**

**Conflict of interest:** The authors declare no conflicts of interest.

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**Authors contribution:**

C.Y.L: concept and design, data acquisition, analysis and interpretation of data, and drafting of the manuscript.

T.Y.Y: concept and design, data acquisition, analysis and interpretation of data, and drafting of the manuscript.

F.L.L: concept and design, data acquisition, and critical revision.

G.Y.H: concept and design, data acquisition, and critical revision.

M.H.H: analysis and interpretation of data and critical revision.
C.Y.H: analysis and interpretation of data and critical revision.

C.Y.L: analysis and interpretation of data and critical revision.

T.J.C: analysis and interpretation of data and critical revision.

H.J.Y.: concept and design, analysis and interpretation of data, drafting of the manuscript, and critical revision.

**Ethics statement:** This study was approved by the institutional review board (IRB) of the study hospital (TPVGH-IRB 2023-04-004BC). A waiver of documentation for signed informed consent was also granted by the IRB.

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**References**


Tables

Tables 1 to 5 are available in the Supplementary Files section.

Figures
Figure 1

A. Cumulative CD34+ Cell Doses in Each Mobilization

B. Predictive Rate of Goal Achievement with or without Prior Radiotherapy and Mobilization with or without Chemotherapy in Various Age Groups

C. Receiver Operating Characteristic Curve Analysis of Prior Radiotherapy, Mobilization with Chemotherapy, and Age for Predicting Goal Achievement. Area Under the Curve (AUC), 0.748

D. Receiver Operating Characteristic Curve Analysis of Prior Radiotherapy, Mobilization with Chemotherapy, and Age for Predicting Goal Achievement “PLUS” HPC ≥20/µL. Area Under the Curve (AUC), 0.787

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PBSCharvesttable1.xlsx
- PBSCharvesttable2.xlsx
- PBSCharvesttable3.xlsx
- PBSCharvesttable4.xlsx
- PBSCharvesttable5.xlsx