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Comprehensive technical and patient-care optimization in the management of pediatric apheresis for peripheral blood stem cell harvesting

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ABSTRACT

Background: Pediatric apheresis for peripheral blood stem cell transplantation should be carried out with due concern for low corporeal blood volume and vulnerability to hypocalcemia-related complications, hypovolemic shock, and hypervolemic cardiac overload.
Study Design And Methods: We retrospectively investigated a total of 267 apheresis procedures from 1990 to 2013 on 93 children between 0 and 10 years old, including 89 patients and 4 healthy donors, with body weights of 6.3 to 44.0 kg.
Results: The median CD34+ cell yield per apheresis procedure was 2.3×10^6 CD34+ cells/kg ($0.2–77.9 \times 10^6$ CD34+ cells/kg). Adverse events occurred in 11.6% of procedures (n = 31), including mild perivascular pain (n = 12), emesis (n = 9), hypotension (n = 3), urticaria (n = 2), numbness (n = 2), chest pain (n = 1), facial flush (n = 1), and abdominal pain (n = 1). Among hypotensive events, shock in a 9.6 kg one-year-old boy required emergency treatment in 1996. Thereafter, we adopted continuous injection of calcium gluconate, ionized calcium monitoring, central venous catheter access and circuit priming with albumin in addition to concentrated red cells. Since then we have had fewer complications: 16.4% per apheresis during 1990–1997 versus 5.8% during 1998–2013. No healthy pediatric donors suffered from any late-onset complications related to apheresis or G-CSF administration.
Conclusion: By employing appropriate measures, peripheral blood stem cell apheresis for small children can have an improved safety profile, even for children weighing <10 kg.

1. Introduction

Autologous transplantation of hematopoietic stem cells derived from peripheral blood is an established therapy to restore hematopoiesis impaired by intensive high-dose chemotherapy for pediatric hematologic malignancies and solid

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tumors [1–3]. For diseases in which standard chemotherapy and local treatment are expected to yield a survival prognosis less than 50%, myelotoxic treatment with hematopoietic stem cell rescue is an alternative. For example, in high-risk neuroblastoma, autologous peripheral blood stem cell transplantation has been shown to have significant impact [4] and is now one of the standard therapies. We have provided autologous peripheral blood stem cell transplantation following intensive chemotherapy to pediatric patients since 1990.

Collection of peripheral blood stem cells is accompanied by risks arising from citrate toxicity [5] and extracorporeal volume. In children, apheresis is more likely to provoke hypocalcemia, hypotension, and catheter-related pain, partly due to their narrower vessels and lower circulatory blood volume compared with adults [6–8]. Various collection methods and measures related to priming the extracorporeal circuit, vascular access, and monitoring are employed by different facilities [9–11], although consensus guidelines for pediatric autologous peripheral blood stem cell collection have yet to be established.

In 1996, we encountered a case in which a one-year-old boy required emergency treatment for shock during apheresis collection. Because it was attributed to citrate hypocalcemia and dilutional hypoalbuminemia, over the next two years (1996–1998), we introduced countermeasures such as continuous injection of calcium, supplementation of albumin with packed red cells, and central vein catheter access. Therefore, we analyzed pediatric apheresis over two periods, 1990–1997 and 1998–2013, to assess whether our revised procedures improved the safety, comfort, and efficacy of our peripheral blood stem cell collections.

2. Materials and methods

2.1. Patients and donors

We reviewed a total of 267 peripheral blood stem cell apheresis procedures performed at our hospital between January 1990 and July 2013. These were performed on 93 children, including 89 patients and 4 healthy donors, all between 0 and 10 years of age. This investigation was authorized (#1938) by the Ethics Review Board of Fukushima Medical University, which is guided by local policy, national law, and the World Medical Association Declaration of Helsinki. Data were anonymized and de-identified prior to analysis.

2.2. Granulocyte-colony stimulating factor (G-CSF) administration

For autologous patient-donors, G-CSF (400 μg/m^2/day Filgrastim, Kyowa Hakko Kirin, Tokyo, Japan; or 10 μg/kg/day Lenograstim, Chugai, Tokyo, Japan) was given intravenously following chemotherapy through the last day of apheresis to mobilize hematopoietic stem cells for peripheral collection. For allogeneic donors, generally starting 4 days before the scheduled collection, daily subcutaneous G-CSF (400 μg/m^2/day Filgrastim or 10 μg/kg/day Lenograstim) was administered.

2.3. Apheresis collections

Continuous-flow apheresis was performed using the Spectra system in MNC mode with software version 4.7, 6.0, or 6.1 (Terumo BCT, Tokyo, Japan) [12]. The initial inlet flow rate was 15 to 75 mL per minute with a 12:1 to 18:1 ratio of whole blood to acid citrate dextrose (ACD-A Solution, Terumo). We did not routinely monitor CD34+ cell counts in deciding when to proceed with apheresis. Instead, we proceeded from 4 days after G-CSF administration. Collection efficiencies CE1 and CE2 were not calculated in this study.

2.4. Safety measures

We introduced the following safety measures: (1) monitoring ionized calcium (ICa) in blood; (2) continuous injection of 3–7 mL/h of a calcium preparation (Calcicol Nichi-Iko, Toyama, Japan; calcium gluconate 8.5%, Ca 0.39 mEq/mL); (3) use of albumin (5%, 150 mL) in addition to packed red cells to prime the collection pathway in patients weighing <30 kg; (4) insertion of a double-lumen central venous catheter (Arrow 7Fr, Teleflex Medical, Tokyo, Japan) through the groin of patients weighing <20 kg; and (5) attendance of multidisciplinary staff including a transfusion medicine physician, a pediatrician, a board-certified apheresis nurse (http://yuketsu.jstmct.or.jp/authorization/apheresisns/), and a medical technologist.

ICa was measured every 30 minutes and corrected to a value corresponding to pH 7.4, using a 634 calcium++/pH analyzer (Ciba-Corning, Tokyo, Japan) or EPOC Blood Analysis System (Alere, Waltham, MA, USA).

2.5. Flow cytometry

As described elsewhere [13], CD34+ cell yields were assessed with a Stem-Kit (Beckman Coulter, Brea, CA, USA) using flow cytometry (Cytomics FC 500, Beckman Coulter).

Pre-apheresis peripheral blood white blood cell or collected blood samples containing 1×10^6 cells were incubated with phycoerythrin-conjugated anti-CD34 or, as a negative control, mouse IgG1 (anti-CD34+PE or IgG1PE, Beckman Coulter).

2.6. Statistical analysis

Numerical data are reported as the mean ± SD or the median and range, as appropriate. Statistical analysis included the t-test, Mann-Whitney test and Fisher's exact test using JMP 10.0.2 software (SAS Institute Inc, Cary, NC, USA), with $p < 0.05$ considered to be statistically significant.

3. Results

3.1. Baseline parameters

A total of 267 peripheral stem cell apheresis procedures were performed on 93 children, including 89 patients and 4 healthy donors, 0 to 10 years old, between January 1990 and July 2013 (Table 1). Of the 89 patients, 25 had hematologic tumors while 64 had solid tumors (Table 2). The children's median age was 4 years (0–10 years), and median

Table 1
Summary of patient/donor data and technical data.

Data	Technical data
Number of patients/donors	93
Total number of apheresis collections	267
Collections per patient/donor: median (range)	2 (1–9)
Male:Female	45(51%):44(49%)
Age in years: median (range)	4 (0–10)
Body weight in kg: median (range)	15.5 (6.3–44)
<10 kg	11
≧10 kg	82
Procedure duration in minutes: median (range)	155 (65–323)
Processed volume in milliliters: median (range)	3307 (545–7390)
Processed volume per body weight in mL/kg: median (range)	200 (26–384)

body weight was 15.5 kg (6.3–44 kg), including 10 patients and 1 donor (together 11.8%) weighing <10 kg. The median apheresis run time was 155 minutes (65–323 minutes). The median number of procedures is 2 (1–9 procedures) per child. The median processed volume per procedure was 3660 mL (545–7390 mL).

3.2. Stem cell harvesting

CD34+ cell yield data were available for 134 procedures on 56 patients/donors, because routine measurement of CD34+ cell yield began only in 1996. Among 134 evaluable procedures, the median CD34+ cell yield per apheresis procedure was 2.3×10^6 CD34+ cells/kg ($0.2–77.9 \times 10^6$ CD34+ cells/kg). Median CD34+ cell yields per body weight from 1990 to 1997 and from 1998 to 2013 were 2.0 (0.8–77.9) $\times 10^6$/kg (n = 23) and 2.3 (0.3–65.2) $\times 10^6$/kg (n = 111) respectively, with no statistically significant difference between the two (p = 0.65). Among cases from 1998 onward, we observed no significant difference in the CD34+ yield between children <10 kg and children ≥10 kg (Fig. 1).

3.3. Adverse events and perivascular pain

A total of 31 adverse events were recorded (Table 3a). These consisted of perivascular pain (n = 12), emesis (n = 9), hypotension (n = 3), urticaria (n = 2), numbness (n = 2), chest pain (n = 1), facial flush (n = 1), and abdominal pain (n = 1).

Table 2
Diagnosis of patients.

Diagnosis	Patient count
Acute myeloblastic leukemia	9
Acute lymphoblastic leukemia	8
Lymphoma	5
Chronic myelogenous leukemia	1
Myelodysplastic syndrome	1
Langerhans cell histiocytosis	1
Neuroblastoma	28
Brain tumor	15
Rhabdomyosarcoma	6
Hepatoblastoma	5
Wilms tumor	5
Retinoblastoma	3
Small round cell sarcoma	1
Ewing sarcoma	1

Fig. 1. CD34+ cells/kg yields among children.

The frequency of adverse events per procedure was 16.4% (24/146) until 1997, and dropped to 5.8% (7/121) or less after 1998 (p < 0.05).

In an assessment of perivascular pain associated with blood access in patients with a central venous catheter versus those with a peripheral line, our data show that the use of a central venous catheter reduced perivascular pain associated with peripheral apheresis (p < 0.05, Table 3b).

Table 3
a. Adverse events among 267 apheresis procedures. b. Perivascular pain reported, central venous catheter versus peripheral line

a.

Symptoms	Whole (n = 267)		1990–1997 (n = 146)		1998–2013 (n = 121)		p[a]
	N	Rate (%)	N	Rate (%)	N	Rate (%)	
Perivascular pain	12	4.5	10	6.8	2	1.7	
Emesis	9	3.4	7	4.8	2	1.7	
Hypotension (≧10 mmHg decrease)	3	1.1	1	0.7	2	1.7	
Urticaria	2	0.7	2	1.4	0	0.0	
Numbness	2	0.7	2	1.4	0	0.0	
Chest pain	1	0.4	1	0.7	0	0.0	
Facial flush	1	0.4	0	0.0	1	0.8	
Abdominal pain	1	0.4	1	0.7	0	0.0	
Total	31	11.6	24	16.4	7	5.8	<0.05

b.

	Peri-vascular pain	
	Yes	No
Central venous catheter (n = 35)	0	35
Peripheral line (n = 90)	12	78
Total (n = 125)	12	113
p[a]	<0.05	

[a] Fisher's exact test.
N, number; Rate (%), rate per apheresis.

Table 4
Comparison of ages, body weights, circulation volumes and blood pressures, 1990–1997 vs. 1998–2013.

	1990–1997 (n = 146)	1998–2013 (n = 121)	p[c]
Age[a] (years)	5 (0–10)	4 (0–10)	<0.001
Body weight[a] (kg)	18.5 (7.5–44)	15.4 (6.3–38.5)	<0.001
Processed blood volume[a] (mL)	3660 (545–7390)	3130 (649–6002)	<0.001
Processed blood volume per body weight[b] (mL/kg)	193 (57–345)	206 (26–384)	N.S.
Pre-apheresis systolic blood pressure[b] (mmHg)	113.3 (±16.8)	109.3 (±15.9)	<0.05
Minimum systolic blood pressure during apheresis[b] (mmHg)	93.5 (±11.9)	93.3 (±12.7)	N.S.
Pre-apheresis diastolic blood pressure[b] (mmHg)	60.0 (±12.0)	56.1 (±12.8)	<0.05
Minimum diastolic blood pressure during apheresis[b] (mmHg)	47.3 (±12.5)	42.8 (±9.8)	<0.05

[a] Values are shown as median (range).
[b] Values are shown as mean±SD.
[c] Mann-Whitney test or unpaired t-test, as appropriate.

3.4. Physical parameters

A median age of 5 years over 1990–1997, versus 4 years over 1998–2013, is a little older, with median body weights of 18.5 kg vs. 15.4 kg in the corresponding periods (Table 4). Over time, peripheral apheresis was performed for younger and smaller children.

Significant differences were observed in pre-apheresis systolic blood pressure ($p < 0.05$), with mean values of 113.3 mmHg over 1990–1997, versus 109.3 mmHg over 1998–2013. No significant differences were observed in lowest systolic blood pressure during apheresis. Mean values for pre-apheresis and lowest diastolic blood pressures during apheresis were 60.0 mmHg and 47.3 mmHg, respectively, over 1990–1997, versus 56.1 mmHg and 42.8 mmHg, respectively, over 1998–2013. Thus, diastolic blood pressures decreased significantly ($p < 0.05$) in the second period (Table 4).

3.5. Calcium supplementation

ICa was monitored during 45 peripheral apheresis procedures involving 26 patients weighing <20 kg from 2001 to 2013. Prior to apheresis, these patients were started on continuous infusion (3–7 mL/h) of calcium gluconate. Mean ± SD ICa prior to the start of apheresis was 1.32 ± 0.14 mmol/L. At 30 minutes after starting apheresis, ICa had decreased to 1.19 ± 0.15 mmol/L. However, ICa gradually recovered to nearly pre-apheresis levels; at 60 minutes, 120 minutes, and termination, ICa measured 1.22 ± 0.10 mmol/L, 1.25 ± 0.13 mmol/L, and 1.29 ± 0.14 mmol/L, respectively (Fig. 2).

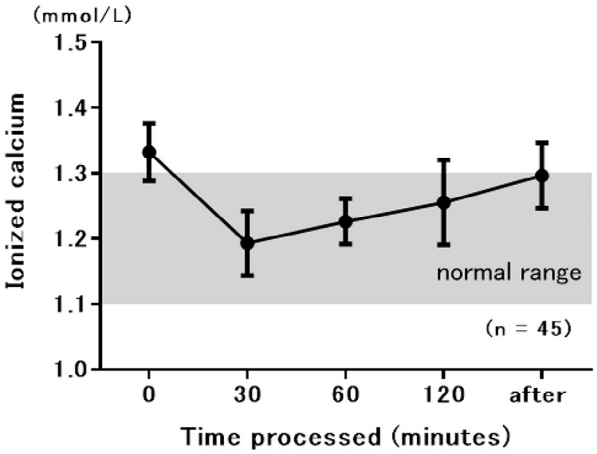

Fig. 2. Ionized calcium levels during apheresis.

3.6. Healthy pediatric donors

Four healthy donors for allogeneic transplantation for their siblings included two 1-year-old boys, one 3-year-old girl and one 8-year-old boy at their apheresis. G-CSF was used on these donors to mobilize hematopoietic stem cells for peripheral collection. The median body weight of four recipients was 25.4 kg (5.9–48.2 kg), and the median CD34+ cell yield per recipient body weight was 3.3×10^6 CD34+ cells/kg ($1.5–6.4 \times 10^6$ CD34+ cells/kg). Four allogeneic donors required 2 apheresis procedures each. Two donors needed two procedures because of insufficient numbers of CD34+ cells in the first procedure. The others required 2 apheresis procedures, because their recipients were not in complete remission. Allogeneic hematopoietic transplantation was successfully performed for two pediatric recipients; however two died from the disease. We confirmed all four healthy donors are well in general health at present 16 to 20 years after their apheresis.

4. Discussion

Due to the relatively low circulatory volume and narrow vessels of pediatric patients, apheresis provokes more adverse events such as hypotension, hypocalcemia and catheter-related pain [6–8].

To address circulatory blood volume reduction and hypoxemia, priming the collection pathway with packed red blood cells before peripheral apheresis has been reported [14–16]. Until 1997, our practice was to draw blood from the radial artery and return it to a peripheral vein, but there were cases of insufficient blood flow and perivascular pain. Consequently, since 1998, we have used a central venous catheter in the femoral vein for children weighing <20 kg. This measure corresponds with fewer complaints of perivascular pain and maintenance of sufficient flow during apheresis.

The patients treated from 1998 onward were younger and smaller than those treated between 1990 and 1997. The pre-apheresis systolic blood pressure and pre-apheresis diastolic blood pressures were lower in patients from 1998

onward than those between 1990 and 1997; these trends may have been due to the younger age and lower body weight of the patients/donors. Patients treated from 1998 onward also had statistically indistinguishable yields of CD34+ cells by body weight. Despite the increase from 1998 onward in peripheral apheresis procedures performed for younger patients, CD34+ yields per kg were maintained.

Anticoagulant solutions containing sodium citrate and citric acid are used in apheresis. Citrate effects, such as numbness, may appear, and in some cases, laryngospasm and arrhythmia may occur, and become fatal [5]. The importance of measuring and managing ICa levels should be emphasized, especially for smaller infants [17]. We have treated patients with a continuous intravenous injection of calcium gluconate from the start of collection [18], and, since 2001, for children weighing <20 kg, calcium gluconate injection was shifted to start prior to peripheral apheresis. Although ICa decreases 30 minutes after starting apheresis, we have been able to reverse the decline and maintain ICa in a normal range, even for small children. To our knowledge, this is the first demonstration of changes in ICa level in children weighing <20 kg who receive continuous injection of calcium throughout the duration of apheresis.

Safety of G-CSF has not been fully established for donors as a potential carcinogenicity on myeloid lineage [19,20]. Bitan and colleagues argue for greater attention to issues of safety, ethics, and legality in cases of healthy juvenile donors mobilized with G-CSF. In common with other donors, they incur risk without personally accruing any health benefits, and as relatives of a recipient, may be enmeshed in complicated social dynamics that focus on the patient [21]. We have engaged juvenile donors in ways consistent with their maturity and intellect, while obtaining and documenting informed consent through their parents or legal guardians. Follow-up of four sibling donors, 16 to 20 years after their G-CSF-mobilized apheresis (at ages 1–8), has confirmed their good health and the absence of enduring adverse effects. Still, four donors are too few to generalize about the safety of G-CSF administration. From an abundance of caution, we shifted to bone marrow rather than apheresis collection for healthy stem cell donors less than 10 years old.

More recently, large volume apheresis in small children has been reported as efficient and safe [22,23]. The average procedure duration is more than four hours, but it ends in one cycle. For multiple days of apheresis with small children, placement of a catheter should be considered.

In conclusion, complications related to apheresis procedures in our pediatric patients/donors were higher than other reports in the literature. Although most complications are mild and transient, severe events can occur. Hypocalcemia prophylaxis, maintaining stable circulatory volume of patients and vascular access are among the main challenges of apheresis for small children. Pediatric apheresis should be performed in facilities with well-trained multidisciplinary teams so as to properly and efficiently mitigate risks.

Author contributions

H.O. organized the study and managed the study protocol. A.K. and H.O. developed measures to improve the safety of apheresis. Y.O. collected, analyzed and interpreted the data. H.O., T.T., K.I., C.O., T.K. and Y.S. operated apheresis. Y.O., H.S., K.M., M.A., S.K., T.W., M.I., M.H. and A.K. treated patients and took care of patients/donors. Y.O., K.E.N. and H.O. wrote the main manuscript text. H.O., T.T., K.E.N. and S.Y. edited the manuscript. All authors reviewed the manuscript and finally approved the conclusion.

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