Received: 18 September 2025 | Revised: 12 December 2025 | Accepted: 23 January 2026

DOI: 10.1111/trf.70102

ORIGINAL RESEARCH

TRANSFUSION

Pediatric autologous peripheral blood stem cell collection without heparin using a highly concentrated sodium citrate anticoagulant: A retrospective comparison with standard ACD-A

Keiko Fujii[1,2] | **Wataru Kitamura**[1] | **Kana Washio**[3] | **Kazuhiro Ikeuchi**[1,4] | **Joji Shimono**[1,4] | **Hiroyuki Murakami**[1,4] | **Fumio Otsuka**[2,5] | **Yoshinobu Maeda**[1] | **Nobuharu Fujii**[1,4]

[1]Department of Hematology, Okayama University Hospital, Okayama, Japan

[2]Division of Clinical Laboratory, Okayama University Hospital, Okayama, Japan

[3]Department of Pediatrics, Okayama University Hospital, Okayama, Japan

[4]Division of Transfusion and Cell Therapy, Okayama University Hospital, Okayama, Japan

[5]Department of General Medicine, Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Correspondence
Keiko Fujii, Department of Hematology, Okayama University Hospital, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan.
Email: pwxc0mv9@s.okayama-u.ac.jp

Abstract

Background: Heparin combined with sodium citrate has been used in leukocytapheresis for pediatric patients. Since 2022, we have performed leukocytapheresis using a highly concentrated sodium citrate solution (HSC, 5.32%) instead of acid citrate dextrose solution A (ACD-A). We conducted this study to determine whether HSC use reduces run time and the total amount of anticoagulant solution in children.

Study Design and Methods: We retrospectively analyzed data from consecutive autologous peripheral blood stem cell harvests (auto-PBSCHs) between June 2012 and May 2025, including patient characteristics, mobilization methods, protocol used, anticoagulant type, run time, total anticoagulant solution volume, and collection efficiency.

Results: Auto-PBSCH was performed using the mononuclear cell collection (MNC) protocol in 28 procedures and the continuous MNC protocol in 20 procedures. ACD-A was used in 35 procedures and HSC in 13. The run time was significantly shorter (204 [range, 117–302] vs. 157 min [range, 103–227], $p = .02$) in the HSC group and also confirmed in multivariable regression analysis (coefficient, −55.6; 95% confidence interval, −106.2 to −5.04; $p = .03$). In a subgroup analysis of cMNC procedures, $CD34^+$ collection efficiency showed a strong negative correlation with the proportion of run time devoted to establishing the initial interface ($r = -.73$, $p = .0003$).

Conclusion: Delays in establishing the initial interface can reduce the duration of the effective MNC collection phase and may negatively affect collection efficiency. Careful attention to the initial interface phase is therefore warranted when using HSC.

KEYWORDS

acid citrate dextrose solution, autologous, continuous mononuclear cell collection, highly concentrated sodium, pediatric, peripheral blood stem cells

1 | INTRODUCTION

Hematopoietic stem cell transplantation is an established therapy for various pediatric disorders, and peripheral blood stem cell collection is a critical component of this process. Apheresis-based cell collection has also expanded to lymphocyte collection for chimeric antigen receptor (CAR) T-cell therapy. These procedures require the efficient separation of mononuclear cells using a centrifugal cell separator, for which sodium citrate is the standard anticoagulant (AC).[1,2]

Although acid citrate dextrose solution A (ACD-A), a 2.2% sodium citrate solution, is commonly used, small blood volume often necessitates high inlet flow rates and increased citrate exposure, for which heparin has been empirically combined[3–5] despite manufacturer non-recommendation. To avoid heparin-associated risks, our institution performs leukocytapheresis without heparin and introduced a highly concentrated sodium citrate (HSC, 5.32%) formulation[6,7] in 2022 to reduce anticoagulant volume. Furthermore, the mononuclear cell collection (MNC) protocol of the Spectra Optia system has been used since 2012 and was transitioned to the continuous MNC (cMNC) protocol in 2020. The MNC protocol on the Spectra Optia uses intermittent mononuclear cell collection with a smaller extracorporeal volume, whereas the cMNC protocol provides continuous collection, enabling more stable interface control. We retrospectively evaluated whether HSC shortens run time and decreases anticoagulant requirements in pediatric auto-PBSCH.

2 | STUDY DESIGN AND METHODS

2.1 | Patient selection

Pediatric patients who underwent leukocytapheresis for the treatment of a primary disease at Okayama University Hospital between June 2012 and May 2025 were included in this study. Patient data, including diagnoses and tests for leukocytapheresis, were retrospectively collected from medical records. The study protocol was approved by the Institutional Review Board of Okayama University Hospital, and participation was conducted using an opt-out approach. This study was conducted in accordance with the ethical standards of the Institutional and National Research Committee and the 1964 Declaration of Helsinki and its later amendments.

2.2 | Peripheral blood stem cell mobilization and transfusion prior to leukocytapheresis

Peripheral blood stem cell (PBSC) mobilization was performed using granulocyte-colony stimulating factor (G-CSF) (filgrastim biosimilar, 400 μg/m^2 [Mochida Pharmaceutical Co., Ltd., Tokyo, Japan] or lenograstim, 10 μg/kg [Chugai Pharmaceutical Co., Ltd., Tokyo, Japan]) in combination with chemotherapy or G-CSF alone for 5 days. In addition, patients with peripheral blood (PB) CD34$^+$ cell counts <20/μL on the day before leukocytapheresis received plerixafor (0.24 mg/kg; Sanofi Co., Ltd., Paris, France), which has been available to children since 2018 at our institution. Leukocytapheresis was initiated 2 h after the final G-CSF injection. If the target CD34$^+$ cell yield was not achieved on the first attempt, additional G-CSF administration and leukocytapheresis were performed the following day and continued until the third day. To ensure safe leukocytapheresis, red blood cell (RBC) and/or platelet (Plt) concentrate transfusions were administered, as described in our previous report.[8]

2.3 | Leukocytapheresis procedures

All leukocytapheresis procedures were performed in the intermittent MNC protocol or the continuous MNC (cMNC) protocol using Spectra Optia (Terumo BCT, Tokyo, Japan) according to local standard operating procedures. The MNC procedures followed the initial protocol set as the default settings provided by the company. In contrast, the cMNC protocols used a collection flow rate of 1 mL/min or a .05-fold initial inlet flow rate (if the

initial inlet flow rate was <20 mL/min) and a packing factor of 4.0, which indicated the centrifugation forces. This protocol has been implemented for pediatric autologous PB stem cell harvesting (auto-PBSCH) using only cMNCs since January 2020. The time taken for establishing initial interface using the cMNC protocol was calculated by subtracting the time recorded when the device display switched from "Establishing Initial Interface in Progress" to "Collecting MNC in Progress" from the start time.

Red blood cell priming was performed in patients weighing <20 kg under the MNC protocol and <25 kg under the cMNC protocol, and additionally when recommended by the device owing to excessive extracorporeal blood volume after patient information input.

According to a previous report,[6] we used a mixed solution of acid citrate dextrose solution A (ACD-A; Terumo BCT) and 10% sodium citrate hydrate (Fuso Pharmaceutical Industries, Osaka, Japan) as the AC in the HSC group, and this protocol has been performed since December 2022. The AC ratios were initially set at 12:1 in the normal concentrated sodium citrate (NSC) group and 24:1 in the HSC group. To prevent hypocalcemia, all patients received a continuous intravenous infusion of 85 mg/mL calcium gluconate hydrate (Nichi-Iko Pharmaceutical Co., Ltd.) during leukocytapheresis (4–15 mL/h depending on the body weight [BW] of the patient). Vascular access was obtained via central venous cannulation (internal jugular or femoral vein) or via arterial access (radial or dorsal foot artery) for blood withdrawal; when arterial access was used, return was performed through a central venous line. Since 2023, we transitioned from arterial to central venous access to enhance procedural safety and circuit stability. This transition coincided with the introduction of the HSC protocol, resulting in a higher proportion of central venous access patients in the HSC group.

Apheresis procedures were performed by a four-member team consisting of two physicians, one nurse, and one clinical engineer. Continuous cardiorespiratory monitoring, including ECG, was implemented in all pediatric patients irrespective of sedation status. Sedation was applied only when clinically indicated and was performed either as intravenous conscious sedation or, in one exceptional case, under general anesthesia with mechanical ventilation.

Electrolytes were generally measured at the start and end of apheresis with ionized calcium and potassium assessed in whole blood and other electrolytes in serum. When signs suggestive of citrate toxicity were noted, additional measurements were obtained. Because pediatric patients may have difficulty reporting subjective symptoms and some underwent intravenous sedation, citrate reactions were screened through continuous observation of agitation, discomfort, abnormal limb movements, transient weakness, tetany, heart rate fluctuations, and ECG abnormalities. These assessments were conducted collaboratively by the multidisciplinary apheresis team.

The total blood volume (TBV) was calculated using the Spectra Optia device for patients weighting ≥25 kg. For patients weighting <25 kg, TBV was manually calculated as 80 mL/kg for those <1 year and 70 mL/kg for those ≥1 year. $CD34^+$ collection efficiency 2 ($CD34^+$ CE2) was calculated as follows: $CD34^+$ CE2 (%) = ([product $CD34^+$ cell counts × product volume]/[pre-PB $CD34^+$ cell counts × processing blood volume {PBV}]) × 100.[9] Pre-PB $CD34^+$ cells have only been available since January 2017. For patients undergoing auto-PBSCH for two consecutive days, only data from the first day were analyzed in this study.

2.4 | Sample evaluation

Complete blood cell counts were determined using an ADVIA 2120i hematology analyzer (Siemens Healthineers, Erlangen, Germany). Circulating immature cell counts, morphologically identified as myeloblasts, promyelocytes, myelocytes, metamyelocytes, and erythroblasts, were assessed by clinical laboratory technicians. Potassium and magnesium levels were measured using a JCA-BM8040 automatic biochemical analyzer (Japan Electron Optics Laboratory Co., Ltd., Tokyo, Japan), and ionized calcium levels were measured using an ABL800 FLEX Radiometer (Copenhagen, Denmark). $CD34^+$ cell counts were determined in pre-leukocytapheresis PB samples collected immediately before each procedure and in the final product using Stem-Kit Reagents (Beckman Coulter, Brea, CA, USA) and were analyzed using a NAVIOS EX flow cytometer (Beckman Coulter). The electrolyte (potassium, ionized calcium, and magnesium) or Plt loss rates were calculated using the following equation: loss rate (%) = ([pre-leukocytapheresis level − post-leukocytapheresis level]/pre-leukocytapheresis level) × 100.

2.5 | Statistical analyses

The Mann–Whitney U test was used to compare continuous variables, and Fisher's exact test was used to compare categorical variables. Spearman's rank correlation was used to determine the association between continuous variables, and the strength of the correlation was determined using the absolute *r*-value at each evaluation. To aid interpretation of the scatter plots, simple linear regression lines were superimposed.

Because vascular access (arterial vs. central venous) could potentially affect run time and anticoagulant solution volume, it was included as a covariate in the regression analyses. To address potential confounding that affects run time, anticoagulant solution volume, or collection efficiency, we additionally performed regression analyses incorporating factors including apheresis protocol (MNC or cMNC), vascular access type (arterial vs. central venous), anticoagulation type (NSC or HSC), pre-PB $CD34^+$ cell count, and mobilization method (G-CSF alone, chemotherapy plus G-CSF, or plerixafor use). To identify independent predictors of run time, anticoagulant solution volume, and collection efficiency, multivariable regression analysis was performed based on factors with $p < 0.10$ on univariate regression analysis as independent variables.

All statistical tests were two-tailed, and statistical significance was set at $p < .05$. All statistical analyses were performed using GraphPad Prism version 9 software (GraphPad Software, San Diego, CA).

3 | RESULTS

3.1 | Patient characteristics

Patient backgrounds during leukocytapheresis are summarized in Table 1. Forty-eight patients were included in

TABLE 1 Patient characteristics.

	NSC ($n = 35$)	HSC ($n = 13$)	p-value (95% CI)
Age (years), median (range)	5 (1–15)	6 (0–11)	.62 (−4 to 3)
Sex, *n* (%)			
Male	21 (60.0)	9 (69.2)	.74 (.33 to 7.94)
Female	14 (40.0)	4 (30.8)	
BW (kg), median (range)	16.6 (6.8–54.1)	18.5 (7.9–36.9)	.57 (−7.6 to 4.3)
Disease, *n* (%)			
Hematopoietic malignancy	4 (11.4)	1 (7.7)	1.00 (.13 to 82.5)
B-NHL	1	1	
BL	2		
HL	1		
Solid tumors	31 (88.6)	12 (92.3)	
Sarcoma[a]	6	2	
Brain tumor[b]	14	5	
Kidney tumor[c]	1	3	
Neuroblastoma	9	1	
Retinoblastoma	1	1	
WBC ($\times 10^3/\mu L$), median (range)	6.65 (1.95–51.22)	32.27 (4.75–85.95)	**.001 (−33880 to −5290)**
CIC ($\times 10^3/\mu L$), median (range)	0.58 (0.00–8.92)	1.59 (0.26–10.31)	.06 (−1508 to 67)
$CD34^+$ cell (/μL), median (range)	51 (1–267)	87 (1–428)	.20 (−81 to 26)
Hct (%), median (range)	28.0 (22.5–43.3)	32.1 (25.4–40.4)	.06 (−5.4 to 0.1)
Plt ($\times 10^3/\mu L$), median (range)	98 (45–387)	219 (65–684)	**.02 (−175 to −9)**
Mobilization, *n* (%)			
G-CSF	4 (11.4)	6 (46.2)	**.02 (.03 to .87)**
G-CSF + chemotherapy	31 (88.6)	7 (53.8)	
Use of plerixafor, *n* (%)	4 (11.4)	5 (38.5)	**.048 (.80 to 29.6)**

Note: Bold values indicate statistical significance ($p < .05$).

[a]Sarcoma include Ewing sarcoma, rhabdomyosarcoma, and undifferentiated sarcoma.

[b]Brain tumor include medulloblastoma, ependymoma, germ cell tumor.

[c]Kidney tumors include clear cell sarcoma of the kidney, malignant rhabdoid tumor of the kidney, Wilms tumor.

Abbreviations: AC, anticoagulant; B-NHL, B cell non-Hodgkin lymphoma; BW, body weight; CIC, circulating immature cell; CE2, collection efficacy 2; CNS, central nervous system; G-CSF, granulocyte-colony stimulating factor; Hct, hematocrit; HL, Hodgkin lymphoma; HSC, highly concentrated sodium citrate; NSC, normal concentrated sodium citrate; PBV, processing blood volume; Plt, platelet; RBC, red blood cell; TBV, total blood volume; WBC, white blood cell.

this study. There was a significant difference in the two groups in the mobilization method and also in using plerixafor. Consequently, the median white blood cell (WBC) tended to be higher in the HSC group than in the NSC group (32.27 [range, 4.75–85.95] vs. 6.65 × 10^3/μL [1.95–51.22], $p = .001$), similarly with the Plt count (219 [range, 65–684] vs. 98 × 10^3/μL [45–387], $p = .02$). However, there was no significant difference between the two groups in $CD34^+$ cell and median other cell counts in the PB at leukocytapheresis (Table 1).

3.2 | Peripheral blood stem cell harvest characteristics

Leukocytapheresis variables are summarized in Table 2. A total of 19 patients (39.6%) in both groups underwent leukocytapheresis under sedation. Vascular access for leukocytapheresis was achieved via peripheral venous, central venous, or arterial puncture, with a significant difference between the two groups. There was no significant difference in the median TBV or PBV/TBV. The median maximum inlet flow rate was significantly higher in the HSC group than in the NSC group, and there were no cases of inability to maintain the inlet flow rate for physical reasons related to vascular access. In the HSC group, the total amount of AC solution was significantly lower (290 mL [range, 77–1118] vs. 137 mL [range, 64–360], $p = .003$) and the run time was significantly shorter (204 [range, 117–302] vs. 157 min [103–227], $p = .02$) than in the NSC group. The procedure was not interrupted because of extracorporeal circuit obstruction. However, in two cases within the HSC group, the procedure was interrupted due to occlusion of the return-side catheter, but it was quickly resolved and resumed. The $CD34^+$ CE2 in the HSC group was significantly lower (51.6 [range, 13.6–74.7] vs. 35.2% [4.4–70.1], $p = .04$) than in the NSC group. There was no significant difference

TABLE 2 Leukocytapheresis variables.

	NSC ($n = 35$)	HSC ($n = 13$)	p-value (95%CI)
Procedure, *n* (%)			
MNC protocol	28 (80)	0 (0)	**<.001 (8.8 to NE)**
cMNC protocol	7 (20)	13 (100)	
Use of anesthetics, *n* (%)	16 (45.7)	3 (23.1)	.20 (.05 to 1.8)
Vascular access[a], *n* (%)			
Peripheral venous	5 (14.3)	1 (7.7)	<.001 (NE)
Central venous	2 (5.7)	10 (76.9)	
Arterial	28 (80.0)	2 (15.4)	
RBC priming, *n* (%)	25 (71.4)	12 (92.3)	.25 (.54 to 225.1)
TBV (mL), median (range)	1162 (475–3382)	1295 (635–2828)	.51 (−602 to 301)
PBV/TBV, median (range)	2.89 (1.43–4.29)	2.86 (1.79–4.29)	.66 (−.37 to .68)
Time to establishing initial interface[b] (min), median (range)	40 (15–65)	36 (12–82)	1.00 (−20 to 18)
Time to establishing initial interface[b] run time (%), median (range)	17.0 (6.7–36.8)	25.0 (7.6–36.8)	.29 (−.20 to .056)
Inlet flow rate (mL/min), median (range)	20.0 (9.5–75.0)	32.0 (10.0–67.4)	**.02 (−24.8 to 2.0)**
AC solution volume (mL), median (range)	290 (77–1118)	137 (64–360)	**.003 (59 to 275)**
Run time (min), median (range)	204 (117–302)	157 (103–227)	**.02 (7 to 71)**
$CD34^+$ CE2[c] (%), median (range)	51.6 (13.6–74.7)	35.2 (4.4–70.1)	**.04 (.38 to 29.9)**
$CD34^+$ cell/patient BW (×10^6/kg), median (range)	4.69 (0.02–59.5)	7.14 (0.14–12.78)	.86 (−3.2 to 5.5)
Number of leukocytapheresis (days), *n* (%)			
1 day	26 (74.3)	12 (92.3)	.25 (.005 to 2.2)
≥2 days	9 (25.7)	1 (7.7)	

Note: Bold values indicate statistical significance ($p < .05$).

Abbreviations: AC, anticoagulant; CE2, collection efficiency 2; cMNC, continuous mononuclear cell collection; HSC, highly concentrated sodium citrate; MNC, mononuclear cell collection; NSC, normal concentrated sodium citrate.

[a]Vascular access defines the vascular route used as the inlet line. Arterial include radial artery and dorsal artery of the foot.

[b]Time to establishing initial interface (min) is evaluated only when the cMNC protocol ($n = 20$) is adopted.

[c]$CD34^+$ CE2 is only evaluated in the NSC group ($n = 14$) and the HSC group ($n = 13$).

between the two groups in the CD34$^+$ cell yield per patient BW or in the proportion of patients requiring leukocytapheresis on subsequent days.

We evaluated the clinical tolerability of this novel method. The median ionized calcium loss rates were significantly higher in the HSC group than in the NSC group (Figure 1B; 0.0 [range, −32.0 to 23.6] vs. 11.1% [range, −11.9 to 23.0], $p = .005$). However, no citrate reactions occurred in any of the 10 evaluable cases in the HSC group, and only one occurred in the 19 evaluable cases in the NSC group. In contrast, the median potassium and magnesium loss rates, Plt loss rates were similar in both groups (Figure 1A,C,D). Temporary hypotension was observed in one case in the HSC group.

To explore whether baseline hematologic parameters were associated with collection performance, we examined correlations between pre-apheresis values and CD34$^+$ CE2. As shown in Figure 2, pre-apheresis WBC count demonstrated a weak negative association with CE2 (Figure 2A; $r = -.20$, $p = .40$), whereas

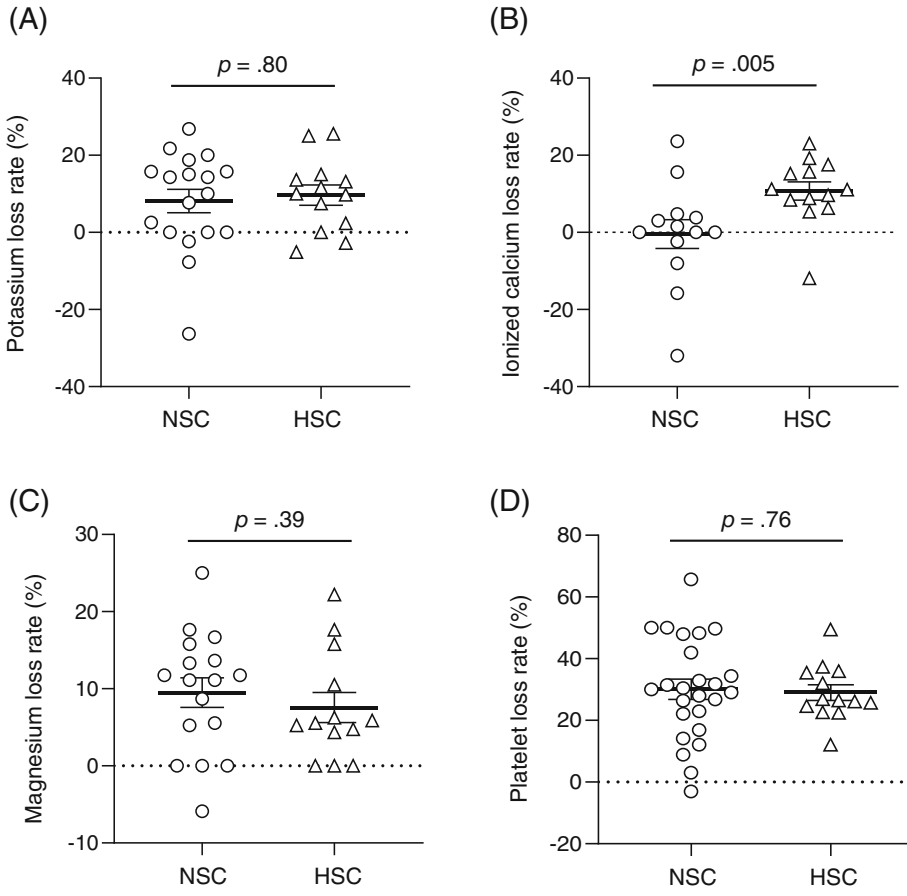

FIGURE 1 Analysis of apheresis-related adverse events. Comparison of the potassium (A), ionized calcium (B), magnesium (C), and platelet (D) loss rates between the normal concentrated sodium citrate (NSC) and highly concentrated sodium citrate (HSC) groups. Data are presented as the means ± SE of the means (A–D).

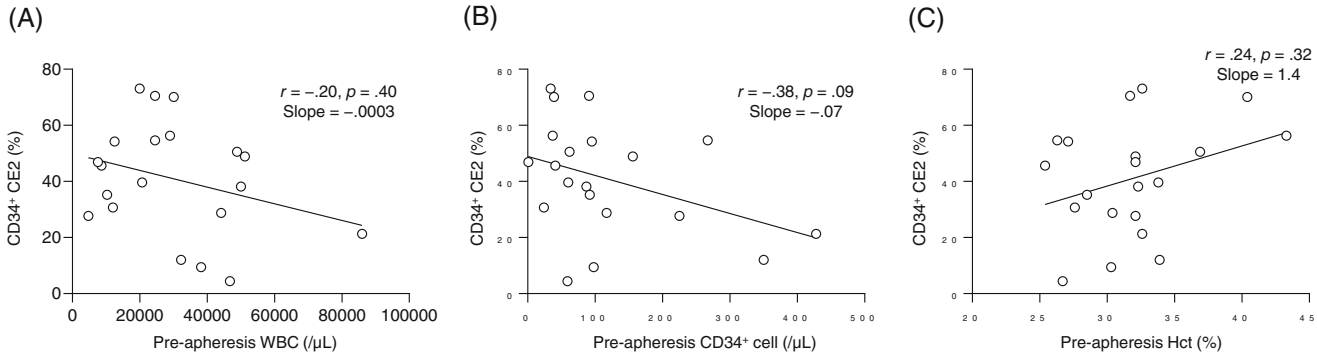

FIGURE 2 Correlations among the CD34$^+$ cell collection efficiency 2 (CE2), clinical variables before apheresis. Correlations between CD34$^+$ CE2 and (A) pre-apheresis white blood cell (WBC), (B) pre-apheresis CD34$^+$ cell, and (C) pre-apheresis hematocrit (Hct).

TABLE 3 Comparison of patient variables among the NSC with MNC protocol, NSC with cMNC protocol, and HSC with cMNC protocol group.

	NSC + MNC ($n = 28$)	NSC + cMNC ($n = 7$)	HSC + cMNC ($n = 13$)	*p*-value
Pre-leukocytapheresis variables				
Age (years), median (range)	5 (1–15)	5 (1–15)	6 (0–11)	.59
Sex, *n* (%)				
Male	16 (57.1)	5 (71.4)	9 (69.2)	.66
Female	12 (42.9)	2 (28.6)	4 (30.8)	
BW (kg), median (range)	17.2 (6.8–44.0)	15.4 (9.0–54.1)	18.5 (7.9–36.9)	.72
Disease, *n* (%)				
Hematopoietic tumors	3 (10.7)	1 (14.3)	1 (7.7)	.90
Solid tumors	25 (89.3)	6 (85.7)	12 (92.3)	
WBC ($\times 10^3$/μL), median (range)	4.67 (1.95–46.19)	24.52 (12.00–51.22)	32.27 (4.75–85.95)	**<.001**
CIC ($\times 10^3$/μL), median (range)	0.55 (0.00–8.92)	1.30 (0.20–4.56)	1.59 (0.26–10.31)	.07
$CD34^+$ cell (/μL), median (range)	26 (1–206)	91 (24–267)	87 (1–428)	.21
Hct (%), median (range)	28.0 (22.5–34.6)	31.7 (26.3–43.3)	32.1 (10.0–67.4)	**.04**
Plt ($\times 10^3$/μL), median (range)	94 (45–238)	98 (66–387)	219 (65–684)	**.04**
Mobilization, *n* (%)				
G-CSF	1 (3.6)	3 (42.9)	6 (46.2)	**.002**
G-CSF + chemotherapy	27 (96.4)	4 (57.1)	7 (53.8)	
Use of plerixafor, *n* (%)	2 (7.1)	2 (28.6)	5 (38.5)	**.04**
Leukocytapheresis variables				
Use of anesthetics, *n* (%)	10 (35.7)	6 (85.7)	3 (23.1)	**.02**
Vascular access, *n* (%)				
Peripheral venous	5 (14.3)	1 (14.3)	1 (7.7)	**<.001**
Central venous	2 (5.7)	0 (0.0)	10 (76.9)	
Arterial	28 (80.0)	6 (85.7)	2 (15.4)	
RBC priming, *n* (%)	19 (67.9)	6 (85.7)	12 (92.3)	.19
TBV (mL), median (range)	1201 (475–3319)	1076 (630–3832)	1295 (635–2828)	.69
PBV/TBV, median (range)	2.91 (1.82–3.87)	2.86 (1.43–4.29)	2.86 (1.79–4.29)	.82
Time to interface formation (min), median (range)	NA	40 (15–65)	36 (12–82)	1.00
Time to interface formation/run time (%), median (range)	NA	17.0 (6.7–36.8)	25.0 (7.6–49.5)	.29
Inlet flow rate (mL/min), median (range)	23.0 (12.0–70.0)	16.0 (9.5–75.0)	32.0 (10.0–67.4)	**.04**
AC solution volume (mL), median (range)	350 (157–971)	258 (77–1118)	137 (64–360)	**.007**
Run time (min), median (range)	197 (120–302)	224 (117–274)	157 (103–227)	**.04**
$CD34^+$ CE2 (%), median (range)	37.3 (13.6–74.7)	54.6 (30.7–73.1)	35.2 (4.4–70.1)	**.04**
$CD34^+$ cell/patient BW ($\times 10^6$/kg), median (range)	4.06 (0.02–59.53)	7.73 (2.21–15.26)	7.14 (0.14–12.78)	.46
Number of leukocytapheresis (days), *n* (%)				
1 day	19 (67.9)	7 (100.0)	12 (92.3)	.07
≥2 days	9 (32.1)	0 (5.0)	1 (7.7)	

Note: Bold values indicate statistical significance ($p < .05$).

Abbreviations: AC, anticoagulant; BW, body weight; CE2, collection efficiency 2; CI, confidence interval; CIC, circulating immature cell; cMNC, continuous mononuclear cell collection; G-CSF, granulocyte-colony stimulating factor; Hct, hematocrit; HSC, highly concentrated sodium citrate; NSC, normal concentrated sodium citrate; MNC, mononuclear cell collection; PBV, processing blood volume; Plt, platelet; RBC, red blood cell; TBV, total blood volume; WBC, white blood cell.

pre-apheresis $CD34^+$ cell count also tended to correlate negatively (Figure 2B; $r = -.38$, $p = .09$). In contrast, pre-apheresis hematocrit (Hct) showed a weak positive correlation with CE2 (Figure 2C; $r = .24$, $p = .32$). None of these correlations reached statistical significance, indicating that baseline leukocyte or $CD34^+$ cell levels alone did not explain the observed variability in collection efficiency across procedures.

3.3 | Comparisons across the three protocol–anticoagulation groups

Because the use of HSC was predominantly limited to procedures performed under the cMNC protocol, we reclassified the cohort into three groups to address potential confounding: (1) NSC procedures performed with the MNC protocol ($n = 28$), (2) NSC procedures performed with the cMNC protocol ($n = 7$), and (3) HSC procedures performed with the cMNC protocol ($n = 13$). Descriptive comparisons of patient characteristics and apheresis-related variables across these three groups are summarized in Table 3. Baseline demographic parameters including age, sex, body weight, diagnosis category, and circulating immature cell count did not differ significantly among the three groups (all $p > .05$). Pre-procedure white blood cell counts differed markedly across groups, with the highest counts in the HSC + cMNC group, followed by NSC + cMNC, and NSC + MNC (median, $32.27 \times 10^3/\mu L$, $24.52 \times 10^3/\mu L$, and $4.67 \times 10^3/\mu L$; $p < .001$). Mobilization strategies also differed ($p = .002$). G-CSF combined with chemotherapy predominated in the NSC + MNC group (96.4%), whereas G-CSF alone was more frequently used in both cMNC groups (57.1% in NSC + cMNC and 53.8% in HSC + cMNC). Plerixafor use was also more frequent in the HSC + cMNC group (38.5%; $p = .04$).

Regarding procedural characteristics, the HSC + cMNC group most commonly underwent leukocytapheresis via central venous access (76.9%), whereas the NSC + MNC group predominantly used arterial access (80.0%) ($p < .001$). The HSC + cMNC group demonstrated a significantly higher initial inlet flow rate compared with the NSC + cMNC group and NSC + MNC group (median 32.0 mL/min, 16.0 mL/min, and 23.0 mL/min; $p = .04$). The total anticoagulant solution volume was lowest in the HSC + cMNC group compared with NSC + cMNC and NSC + MNC ($p = .007$). Run time was also shortest in the HSC + cMNC group (157 min vs. 224 min and 197 min, respectively; $p = .04$). In contrast, $CD34^+$ CE2 was significantly lower in the HSC + cMNC group than in the NSC + cMNC group (median 35.2% vs. 54.6%; $p = .04$) (Table 3).

3.4 | Regression analyses

To account for potential confounders, univariate and multivariable regression analyses were performed for three outcomes: run time, total anticoagulant solution volume, and CE2 (Table 4).

In univariate analysis, use of HSC was associated with shorter run time (coefficient, −37.5 min; 95% confidence interval [CI], −66.9 to −8.14; $p = .01$). In multivariable analysis, HSC remained an independent predictor of reduced run time (coefficient, −55.6 min; 95% CI, −106.2 to −5.04; $p = .03$), whereas vascular access type, protocol mode, mobilization method, plerixafor use, and baseline $CD34^+$ count were not significant predictors (all $p > .05$) (Table 4).

In univariate analysis, both cMNC protocol (coefficient, −157.1 mL; 95% CI, −288.5 to −25.7; $p = .02$) and HSC use (coefficient, −203.7 mL; 95% CI, −346.1 to −61.3; $p = .006$) were associated with lower anticoagulant volume. However, in multivariable analysis, no factor remained statistically significant, including HSC use (coefficient, −146.2 mL; 95% CI, −372.3 to 79.8; $p = .19$) (Table 4).

In univariate analysis, arterial/non-CV access was associated with reduced CE2 (coefficient, −19.1; 95% CI, −33.3 to −4.9; $p = .01$), as was HSC use (coefficient, −14.8; 95% CI, −29.5 to −0.01; $p = .049$). After multivariable adjustment, no evaluated factor—including HSC use—remained an independent predictor of CE2 (all $p > .05$) (Table 4).

3.5 | Sub-analysis of cMNC procedures: Effect of interface establishment delay on collection efficiency

Because both the MNC and cMNC protocols contain an interface formation process, the phase itself is not absent in MNC procedures. However, the intermittent collection–separation cycling in the MNC protocol prevents a consistent performance index for the interface establishment phase. In contrast, the cMNC protocol provides a continuous separation phase, allowing the time required for initial interface establishment to be evaluated relative to the overall run time. Therefore, a dedicated sub-analysis was performed restricted to procedures using the cMNC protocol ($n = 20$).

When using the cMNC protocol, after the valve is opened, harvesting into the collection bag begins even under the "establishing initial interface" phase. Moreover, after establishment of the initial interface is complete, the "collecting MNC" phase is performed. The phase of "collecting MNC" continues consistently until

TABLE 4 Univariate and multivariable analysis of factors affecting run time, anticoagulant solution volume, and collection efficacy.

	Univariate analysis			Multivariable analysis		
Factors	**Coefficient**	**95% CI**	***p*-value**	**Coefficient**	**95% CI**	***p*-value**
Run time						
Mode (MNC vs. cMNC)	16.2	−11.7 to 44.10	.25	–	–	–
Vascular access (Non-CV vs. CV)	−27.4	−58.6 to 3.7	.08	19.3	−70.1 to 31.5	.44
Sodium citrate (NSC vs. HSC)	−37.5	−66.9 to −8.14	**.01**	−55.6	−106.2 to −5.04	**.03**
$CD34^+$ cell (/μL)	−.14	−32.4 to .04	.12	−.09	−.27 to .08	.26
Mobilization (G-CSF vs. G-CSF + chemotherapy)	2.27	−32.1 to 36.6	.89	–	–	–
Use of plerixafor (No vs. Yes)	8.27	−27.4 to 43.9	.64	–	–	–
Anticoagulant solution volume						
Mode (MNC vs. cMNC)	−157.1	−288.5 to −25.7	**.02**	−22.7	−281.8 to 236.4	.86
Vascular access (Non-CV vs. CV)	−39.3	−197.7 to 119.0	.62	–	–	–
Sodium citrate (NSC vs. HSC)	−203.7	−346.1 to −61.3	**.006**	−146.2	−372.3 to 79.8	.19
$CD34^+$ cell (/μL)	−.02	−.004 to .04	.11	−.60	−1.5 to .31	.19
Mobilization (G-CSF vs. G-CSF + chemotherapy)	29.3	−139.7 to 198.4	.73	–	–	–
Use of plerixafor (No vs. Yes)	6.87	−169.3 to 183.0	.94	–	–	–
Collection efficacy						
Mode (MNC vs. cMNC)	.73	−17.5 to −18.9	.08	13.2	−6.5 to 32.9	.18
Vascular access (Non-CV vs. CV)	−19.1	−33.3 to 4.9	**.01**	14.9	−5.6 to 35.4	.15
Sodium citrate (NSC vs. HSC)	−14.8	−29.5 to −.01	**.049**	−11.0	−34.2 to 12.3	.34
$CD34^+$ cell (/μL)	−.03	−.10 to .04	.35	–	–	–
Mobilization (G-CSF vs. G-CSF + chemotherapy)	1.14	−15.4 to 17.6	.89	–	–	–
Use of plerixafor (No vs. Yes)	2.42	−14.5 to 19.3	.77	–	–	–

Note: Bold values indicate statistical significance ($p < .05$).

Abbreviations: CI, confidence interval; cMNC, continuous mononuclear cell collection; CV, central venous; G-CSF, granulocyte-colony stimulating factor; HSC, highly concentrated sodium citrate; NSC, normal concentrated sodium citrate; MNC, mononuclear cell collection.

the end of apheresis unless the interface is not stable. We empirically observed that delays in establishing the initial interface during pediatric PBSCH were even greater than those in adults. Therefore, we examined the impact of the long time required for establishing the initial interface ("delay") on collection efficiency. We investigated the correlation between the proportion of total operation time required to establish the initial interface and the collection efficiency. Among procedures performed under the cMNC protocol, the time required to establish the initial interface showed wide variability, ranging from 12 to 82 min and accounting for 6.7% to 49.5% of total run time (Table 3). As shown in Figure 3, longer interface establishment time was moderately associated with lower $CD34^+$ CE2 (Figure 3A; $r = -.55$, $p = .01$) and negatively correlated with pre-apheresis Hct levels (Figure 3B; $r = -.58$, $p = .007$). Furthermore, the proportion of run time spent in interface establishment demonstrated a strong inverse correlation with $CD34^+$ CE2 (Figure 3C; $r = -.73$, $p = .0003$).

4 | DISCUSSION

Optimizing leukocytapheresis in pediatric patients remains a clinical challenge because of their small blood volume (TBV) and unique physiological characteristics.[3,10,11] Efficient collection is particularly important for auto-PBSCHs, where minimizing extracorporeal circulation time and anticoagulant solution exposure directly affects both safety and tolerability. In the present study, the use of HSC was associated with a significant

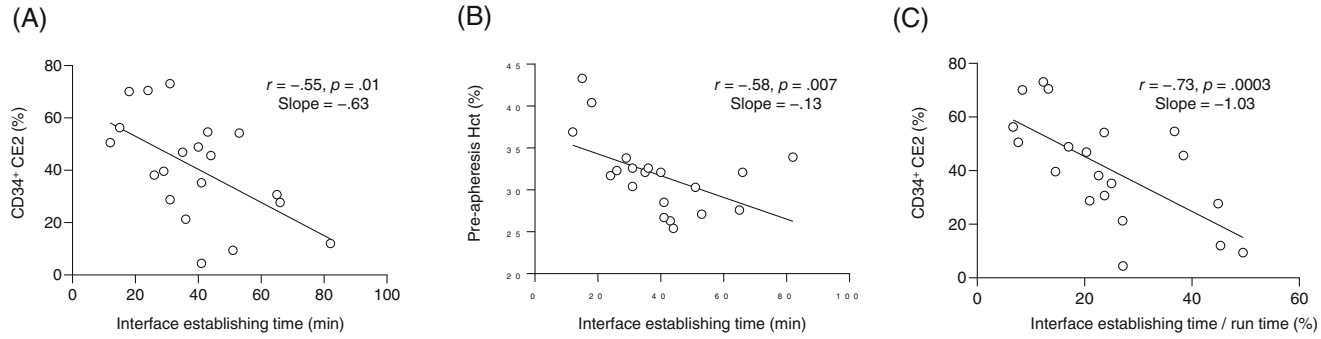

FIGURE 3 Correlations between CD34$^+$ cell collection efficiency 2 (CE2) or pre-apheresis hematocrit (Hct) and time up to the initial interface time establishment within the run time (i.e., interface establishing time) or the proportion of run time spent establishing the initial interface (i.e., interface establishing time/run time) in patients performed using a continuous mononuclear cell collection. Correlations between interface establishing time and (A) CD34$^+$ CE2 or (B) pre-apheresis Hct and (C) correlation between interface establishing time / run time and CD34$^+$ CE2.

reduction in run time, consistent with our previous observations in adult donors.[6,7] Importantly, our comparison focused on the total volume of anticoagulant solution administered. In pediatric leukocytapheresis, larger fluid volumes increase intravascular load and may result in dilutional toxicity irrespective of citrate concentration; therefore overall volume reduction remains clinically meaningful.

Although the unadjusted CD34$^+$ CE2 was lower in the HSC group, this difference was not attributable to HSC use itself. Multivariable regression analyses incorporating clinically relevant covariates—including pre-procedure PB CD34$^+$ counts, vascular access type, apheresis protocol, anticoagulation strategy, and mobilization modality—did not identify HSC as an independent predictor of reduced collection efficiency. These findings caution against attributing the lower unadjusted CE2 to citrate concentration alone; instead, they suggest that procedural dynamics are likely to play a substantial role.

The procedural context of the cMNC is particularly relevant. In cMNC, leukocyte collection begins immediately after the collection valve opens—even while the system is still in the "establishing initial interface" phase—resulting in the harvesting of cells under an unstable interface. This differs from the programmed "collecting MNC" phase, during which the interface has already stabilized. In our cohort, the proportion of run time allocated to interface establishment demonstrated a strong negative correlation with CD34$^+$ CE2, emphasizing the clinical importance of stabilizing the interface before substantial cell harvest occurs. This relationship was further illustrated in procedural tracings (Figure 4), in which some cases spent more

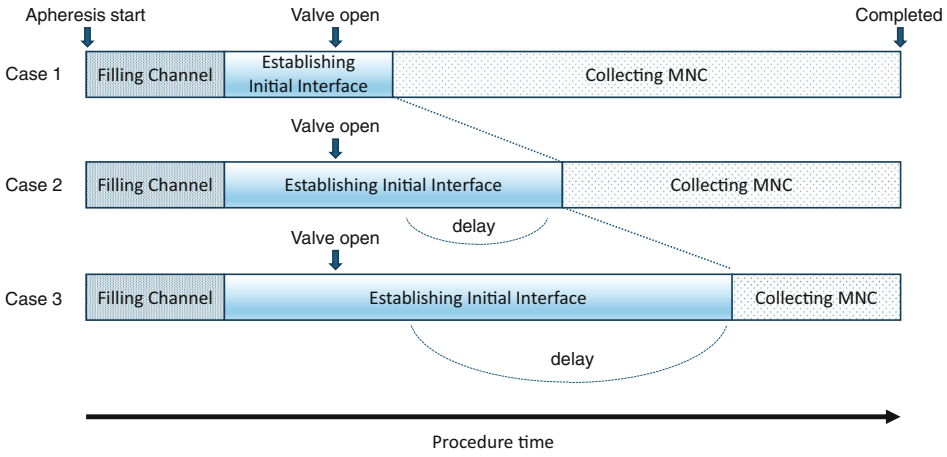

FIGURE 4 Representative cases illustrating the impact of prolonged establishing initial interface on collection efficiency. After the valve is opened, collection into the bag begins even under the establishing initial interface. After the establishing initial interface is complete, the "Collecting MNC" process is performed. "Collecting MNC" continues consistently until the end of apheresis. Compared with standard apheresis (case 1), if there is a delay in establishing initial interface, the proportion of the collecting MNC process per total run time becomes shorter (cases 2 and 3).

than half of their total run time in the interface-formation phase despite continuous processing.

Two mechanistic contributors to delayed interface establishment were evident. First, Hct input after RBC priming may be inaccurate because concentrate volume varies (260–280 mL in Japanese RBC units) and is sometimes entered without actual measurement. Second, insufficient initial inlet flow rate at the start of cMNC, especially in low-weight children, delays phase separation. These effects are amplified when the circuit-to-TBV ratio is high. Notably, in the HSC group the higher programmed anticoagulation ratio allowed higher inlet flow settings without increasing anticoagulant infusion, which may accelerate interface establishment but shorten stable collection time—potentially contributing to lower unadjusted CE2. This phenomenon reflects a protocol-dependent limitation of pediatric cMNC rather than a direct consequence of citrate concentration.

Consistent with our previous findings in adults,[8] this study demonstrated that lower Hct values were associated with the delayed establishment of the initial interface and diminished collection efficiency in pediatric patients. We also observed a strong negative correlation between the proportion of run time devoted to establishing the initial interface and $CD34^+$ CE2 levels, further emphasizing the importance of optimizing this step. The adoption of central venous catheters in our institution since 2023 has contributed to higher inlet flow rates and may represent an additional strategy to improve outcomes. This study focused solely on auto-PBSCH; however, because lymphocyte collection for tisagenlecleucel uses the same device and program, it warrants consideration for HSC use. Although heparin use in pediatric patients has been reported,[12,13] the manufacturer of the device does not recommend its concomitant use as an anticoagulant.

HSC reduced run time but was not an independent predictor of CE2 reduction after adjustment. Rather, prolonged interface establishment under cMNC disproportionately reduced the effective collection phase, explaining the lower unadjusted CE2. Optimization of inlet flow settings, priming accuracy, and vascular access strategy may therefore improve efficiency more than anticoagulant modification itself.

This study has some limitations, including its retrospective design, single-center setting, and limited sample size. Nonetheless, our findings suggest that optimization of the initial interface and flow conditions is crucial when applying HSC in pediatric leukocytapheresis. Prospective studies with larger pediatric cohorts are warranted to validate these observations and refine strategies to improve collection efficiency.

AUTHOR CONTRIBUTIONS

Keiko Fujii performed the conceptualization, formal analysis, investigation, methodology, supervision, validation, visualization, writing of the original draft, and editing. Wataru Kitamura performed the conceptualization, data curation, formal analysis, investigation, methodology, validation, and visualization. Kazuhiro Ikeuchi, Joji Shimono, Hiroyuki Murakami, Wataru Kitamura, and Fumio Otsuka reviewed and edited the manuscript. Yoshinobu Maeda and Nobuharu Fujii supervised, reviewed, and edited the manuscript. All the authors approved the final manuscript.

ACKNOWLEDGMENTS

We thank all the patients who participated in this study, the medical staff of the Division of Transfusion and Cell Therapy, and the Division of Clinical Laboratory of Okayama University Hospital. We are also grateful to Tomohiro Urata for valuable advice on statistical analyses. Naoe Takagi, Yachiyo Masuda, and Ayumi Okada for their assistance in the management of leukocytapheresis. The manuscript was edited and proofread by Editage (https://www.editage.jp/).

FUNDING INFORMATION

No funding was received for this study.

CONFLICT OF INTEREST STATEMENT

The authors report no declarations of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Keiko Fujii https://orcid.org/0009-0005-7614-2448
Wataru Kitamura https://orcid.org/0000-0002-8021-4732
Fumio Otsuka https://orcid.org/0000-0001-7014-9095
Nobuharu Fujii https://orcid.org/0000-0002-8043-7768

REFERENCES

1. Ward DM. Conventional apheresis therapies: a review. J Clin Apher. 2011;26:230–8.
2. Lee G, Arepally GM. Anticoagulation techniques in apheresis: from heparin to citrate and beyond. J Clin Apher. 2012;27:117–25.
3. Michon B, Moghrabi A, Winikoff R, Barrette S, Bernstein ML, Champagne J, et al. Complications of apheresis in children. Transfusion. 2007;47:1837–42.
4. DeSimone RA, Myers GD, Guest EM, Shi PA. Combined heparin/acid citrate dextrose solution a anticoagulation in the

Optia continuous mononuclear cell protocol for pediatric lymphocyte apheresis. J Clin Apher. 2019;34:487–9.
5. Grewal S, Hassanein R, Mendoza S, Sajdak E, Sullivan HC, Jacob R. Feasibility and safety of peripheral blood stem cell collection in children with extremely low body weight: a single center study. Transfusion. 2025;65:1640–9.
6. Abe M, Fujii K, Mitsuhashi T, Kitamura W, Ikeuchi K, Fukumi T, et al. Novel method of leukocytapheresis using a highly concentrated sodium citrate solution alternative to acid citrate dextrose solution a. Transfusion. 2025;65:1223–30.
7. Kitamura W, Fujii K, Abe M, Ikeuchi K, Shimono J, Washio K, et al. Novel method for autologous peripheral blood stem cell harvest using highly concentrated sodium citrate solution replacing acid citrate dextrose solution a. Transfusion. 2025;65:1662–72.
8. Kondo T, Fujii N, Fujii K, Sumii Y, Urata T, Kimura M, et al. Low hematocrit reduces the efficiency of CD34(+) cell collection when using the spectra Optia continuous mononuclear cell collection procedure. Transfusion. 2022;62:1065–72.
9. Cancelas JA, Scott EP, Bill JR. Continuous CD34+ cell collection by a new device is safe and more efficient than by a standard collection procedure: results of a two-center, crossover, randomized trial. Transfusion. 2016;56:2824–32.
10. Cooling L, Hoffmann S, Webb D, Meade M, Yamada C, Davenport R, et al. Procedure-related complications and adverse events associated with pediatric autologous peripheral blood stem cell collection. J Clin Apher. 2017;32:35–48.
11. Aksoy BA. Apheresis in pediatric patients: current differences and difficulties. Transfus Apher Sci. 2023;62:103679.
12. Dettke M, Buchta C, Wiesinger H, Maas JH, Strate A, Chen Y. Anticoagulation in large-volume leukapheresis: comparison between citrate- versus heparin-based anticoagulation on safety and CD34 (+) cell collection efficiency. Cytotherapy. 2012;14:350–8.
13. Qayed M, McGuirk JP, Myers GD, Parameswaran V, Waller EK, Holman P, et al. Leukapheresis guidance and best practices for optimal chimeric antigen receptor T-cell manufacturing. Cytotherapy. 2022;24:869–78.

How to cite this article: Fujii K, Kitamura W, Washio K, Ikeuchi K, Shimono J, Murakami H, et al. Pediatric autologous peripheral blood stem cell collection without heparin using a highly concentrated sodium citrate anticoagulant: A retrospective comparison with standard ACD-A. Transfusion. 2026. https://doi.org/10.1111/trf.70102