Procedure-related complications and adverse events associated with pediatric autologous peripheral blood stem cell collection

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ABSTRACT

Introduction: Autologous peripheral blood hematopoietic progenitor cell collection (A-HPCC) in pediatric patients is considered relatively safe although technically challenging. Very little is known regarding the incidence, risk factors and impact of procedure-related adverse events (AE) on pediatric A-HPCC outcomes.

Methods: Prospective 4.5 year review of AE associated with pediatric A-HPCC. AE were graded by severity and type. Potential demographic and procedural risk factors, and the impact on product quality, were compared by t-test, chi-square, and linear regression.

Results: Sixty-two children underwent 110 A-HPCC, including 36 (58%) under 20 kg. Fifty-five AE were documented in 25.4% A-HPCCs and 39% of children (citrate 25%, access 19%, technical 11%, cardiovascular 0%, allergic 1.8%). No AE were noted in children < 10 kg anticoagulated with heparin. Access and technical AE accounted for 73% of severe AE, with line-related problems underlying most technical AE (87.5%, p=0.006). AE were more likely in older (p=0.012), heavier patients (p=0.02), who frequently required more than one A-HPCC (p=0.012). In contrast, young children were more likely to experience citrate AE with gastrointestinal symptoms (median age, 6 years; p=0.076). AE had no impact on CD34 collection rates; however, mean CD34 yields (4.2 vs 20.4 million/kg; p=0.0035) were decreased in patients with technical AE due to lower peripheral CD34 counts and a high number of aborted procedures (37.%).

Conclusion: Venous access and flow-related issues are a major factor associated with moderate and severe AE, effecting approximately 10% of patients. AE are more frequent with increasing patient age, weight and number of procedures.

Introduction

Peripheral blood hematopoietic progenitor cell collection (HPCC) is considered a relatively safe procedure both in adults and pediatric patients. Young pediatric patients, however, can present unique challenges due to their size and small blood volume, which may increase their risk of adverse events (AE). The latter includes hypotension and hemodynamic instability due to fluid shifts, citrate anticoagulation, dilutional and iatrogenic anemia [1-5]. Adequate venous access is another challenge with all the attendant risks associated with central venous catheters (CVC) [1-3]. Finally, the need for a blood prime and slow inlet rates required for A-HPCC in very small children can lead to delays in establishing and maintaining a stable interface [2], increasing both total volume processed and procedure times [6].

Large studies on procedural AE in pediatric apheresis are relatively scarce [3,7]. Pediatric bone marrow registry studies have predominantly focused on pediatric allogeneic HPC donations, with reported AE commonly restricted to procedural cell losses, G-CSF toxicity, and catheter-related complications [8-11]. AE data associated with pediatric autologous collection (A-HPCC) is more limited, with many studies more than 20 years old. Nearly all published reports are small retrospective, single-institution studies with less than 40 patients, varying anticoagulation and priming protocols, and may include both auto- and allo-donors [2-4,12-20] In addition, reported data seldom includes AE due to technical issues during the procedure.

In April 2009, our institution began prospectively documenting AE associated with A-HPCC in both adult and pediatric patients. We now report our results and analysis

of procedure-related AE in 62 pediatric patients and 110 A-HPCC procedures over a 4.5 year period. This is the largest, and only the second, prospective study of procedure-related AE associated with pediatric A-HPCC [17]. It is also one of the largest reported studies in children < 20 kg undergoing large volume leukapheresis (LVL).

MATERIALS AND METHODS:

Documentation and Prospective Collection of Adverse Events:

In April 2009, the University of Michigan redesigned the HPC collection procedure flowsheet that included a dedicated mandatory field for documentation of observed AE. AE were defined as: 1) growth factor (granulocyte colony-stimulating factor; G-CSF), 2) anticoagulation, 3) venous access, 4) technical and 5) "other". AE were also graded as mild, moderate or severe (Table 1). Any event graded as moderate or severe required a written descriptor in a free text field. Flowsheets were reviewed daily by peer staff members for completion and accuracy. An audit was performed 9 months after implementation of the new form for compliance and consistency in grading [21]. Procedure-related data for each collection, including AE, were recorded by the Cell Therapy Laboratory staff as part of the department's internal quality assurance program. All AE, treatment and clinical outcomes were also included in the daily medical procedure note by the apheresis attending physician.

Patients and Study Design:

The study was a 4.5 year retrospective review of prospectively-collected, procedure-related AE during HPCC at the University of Michigan between 4/2009 and 12/2013. Inclusion criteria included an age < 18 years of age at time of A-HPCC. Only AE attributed to citrate anticoagulation, venous access, technical issues and "other" were studied. Only 3 children (ages 9-16) had documented AE due to G-CSF, defined as symptoms and complaints present prior to A-HPCC procedure. AE due to G-CSF were excluded from further analysis due to the small number of documented AE available and potential underreporting in very young children, which depended on second-hand observations by parents.

Primary data elements included AE type, AE description, and any medical intervention required. Procedure-related information included type of venous access, anticoagulation regimen, blood prime, volume processed, inlet flow rates, procedure time and product characteristics. Patient demographic and laboratory information included age, sex, weight, medications, total blood volume (TBV), primary diagnosis, mobilization regimen, and pre-procedure blood counts (CBC, CD34, WBC differential).

As a comparison group, AE were also examined in 82 adult multiple myeloma patients who underwent 210 A-HPCC during the 2013 calendar year. Adult myeloma patients were collected for a target of 6 million CD34/kg. In adults, data was limited to AE type and venous access.

Venous Access

Most patients underwent short-term, double lumen CVC placement the morning of their first A-HPCC. Line care was per institutional guidelines [22]. Following each apheresis session, the catheter ports were flushed initially with 5 mL saline, followed by heparin (1:1000 units/mL, 0.9-1.3 mL fill volume) and then capped. Femoral and other non-tunneled catheters were removed within 24 hours of the last A-HPCC.

HPC Collection

All patients underwent LVL by continuous-flow centrifugation (COBE Spectra, Gambro BCT, Lakewood, CO) using the WBC collection set [23]. A total of 3 TBV were processed per procedure [23-25]. MNCs were collected at a blood plasma interface of 1% to 2% hematocrit, a mean inlet volume of 1 mL/kg/min, and a collection volume of 1.0 mL/min. For patients weighing less than 10 kg body weight, A-HPCC was performed in the pediatric intensive care unit, using a reconstituted whole blood prime and systemic heparin anticoagulation (30 units/kg) [24]. Heparin was monitored by the activated clotting time (therapeutic range, 180-220 sec). To prevent clotting of the product, ACD-A was manually added to the final product (10% final product volume).

Patients weighing more than 10 kg were anticoagulated with ACD-A at an anticoagulant: whole blood (AC:WB) ratio of 1:12. To mitigate against dilutional anemia

and hypotension, a RBC prime was used if the extracorporeal volume was greater than 10% of the patient's total blood volume. Patients received prophylactic calcium gluconate (3%, 22.6 mg/mL in 100 mL normal saline) on a weight-based scale range to prevent citrate toxicity [24], where flow rate range = [(patient weight in kg] x (30 to 90 mg/mL)] ÷ 22.6 mg/mL calcium gluconate solution ÷ 3 hours (approximate total infusion time). RBC for machine priming were ABO/Rh compatible, pre-storage leukoreduced, and irradiated. Patients who required a blood prime were premedicated with antihistamine and acetaminophen [24]. To avoid volume overload, no rinse-back was performed at the end of the procedure.

Product Analysis

Product volume, WBC count, WBC differential and CD34 count were determined on all collected units. Cell counts and WBC differential were performed on the Sysmex XE 5000 (Sysmex, Kobe, Japan). CD34 yields were determined by flow cytometry (Gallios™; Beckman-Coulter, Brea, CA) as recommended by the International Society of Hematology and Graft Engineering (ISHAGE) [25,26]. Sterility testing of each product was performed before and after processing using the USP culture method per 21 CFR 610.12 [23,24,27]. All cell processing was performed in biosafety hoods, located within a certified clean room. Cells were volume adjusted and frozen in 10% dimethyl sulfoxide as described previously [23-25].

Data Analysis

Quantitative data were reported as the mean ± standard deviation (SD) unless noted otherwise. Variables with wide inter-patient values were reported as mean, median and range. CD34 and MNC collection efficiencies (CE) were calculated as described by previously [23,28]. Categorical data were analyzed by chi-square and odds ratio (OR) using EpiInfo™ (Centers for Disease Control and Prevention, Atlanta, GA). Linear regression, graphics and t-test were performed with commercial software (Kaleidograph, Synergy Software, Reading, PA). A p value < 0.05 was considered significant.

RESULTS

Patient Demographics

Sixty-two pediatric patients underwent 110 procedures over a 4.5 year period (Table 2). Nearly all patients were collected during recovery from treatment-related chemotherapy (60/62, 97%). Patients ranged in age from 1 to 18 years of age, with a median age of 4.5 years. Forty-three percent of children were less than 3 years of age and 56% weighed less than 20 kg. Sixty-one percent of patients required a blood prime. No patient was on an ACE (angiotensin converting enzyme) inhibitor at the time of A-HPCC.

The majority (72%) of subjects had a diagnosis of neuroblastoma or central nervous system tumors, and accounted for the predominance of very young children in

our study cohort. Per treatment-specific protocols, these patients were collected for a final target yield of 10-15 million CD34/kg to support three consecutive stem cell rescues following myelo-ablative chemotherapy (Table 2) [24]. The remaining children carried a diagnosis of lymphoma (Hodgkin's lymphoma, n=8; B cell lymphoma, n=2), hepatoblastoma (n=1), germ cell tumor (n=1), Ewing's sarcoma (n=2) and carcinoma (n=3). In general, these children were older (median 14 years, $p<0.0001$) and were collected following chemotherapy for a single transplant of 3-5 million CD34/kg. Overall, 61% of patients collected in a single procedure.

AE in pediatric A-HPCC

A total of 55 individual AE were documented in 24 (38.7%) children and 27 procedures (24.5%) (Table 3). The majority of AE were graded as moderate (54.5%) or severe (27.3%). Most severe AE (73%) were technical and venous access.

Patients requiring more than one procedure were more likely to experience at least one AE over the course of treatment (58% vs 27%, $p=0.012$). In general, AE tended to be more common in older and larger children, who were also more likely to require several procedures (Fig. 1, $p=0.07$). When examined by diagnosis, AE were also higher in children with "other" diagnoses (71% vs 29%, $p=0.008$), most of whom were older (9.5 ± 4.4 yrs). In contrast, no AE were observed in 6 children $\leq$ 10 kg, who were systemically anticoagulated with heparin and required only a single procedure.

AE due to citrate anticoagulant

Side effects due to citrate anticoagulant were noted in 25% of patients (14/56) and 14% (15/106) of procedures (Table 4). Severe AE (5.4% patients) were limited to gastrointestinal symptoms: no hypotension or tetany were noted. Gastrointestinal symptoms composed the majority of AE (75%, 9/14) and included abdominal pain, nausea, vomiting and diarrhea. Neurologic symptoms were recorded in 7 (12%) patients and included parasthesias, lightheadedness and agitation. Agitation occurred in one child in three successive collections within 30 minutes of starting cell collection. Neurologic symptoms, including agitation, were mild and responsive to increasing the calcium replacement infusion rate.

The incidence of citrate AE increased over time (Fig. 2C), with citrate AE accounting for 100% of all AE documented after the first 2 days. Likewise, there was an increase in citrate AE in heavier children, who were more likely to require several procedures (Fig. 2B). Like adults [29,30], citrate AE tended to be more common in females although the difference did not reach clinical significance (30% vs 15.6% males, p=0.18). There was no correlation between citrate AE by patient diagnosis (p=0.19-0.47). Young and small children were more likely to experience gastrointestinal symptoms (median age 6 years; median weight 24 kg) whereas neurologic symptoms were more common in older children (median age 11.8 years, p=0.076) (Fig. 2A). Ten procedures in 8 patients were associated with a specific nurse operator.

Because all patients responded to medical management, no intra-procedure electrolytes were drawn. A review of pre- and 24 hour post-procedure electrolytes showed a mildly decreased total calcium in one patient (8.0 mg/dL).

AE due to Venous Access

Ninety-two percent of patients had a CVC for venous access on day 1 (Table 3), including all patients under 11 years of age. A short-term femoral dialysis catheter was the predominant catheter in 76% of patients. Five older patients (12-18 years) were originally scheduled for A-HPCC using peripheral IV access (PIV).

A total of 18 venous AE were documented in 13 procedures (12%) and 19% (12/62) of patients (Table 4). Six patients had more than one documented AE per procedure. Six AE were mild (33%), 7 were moderate (39%) and 5 severe (28%). Venous AE included bleeding, pain; occluded CVC requiring multiple flushes throughout the procedure, reversal of arterial and venous lines, PIV for draw, or positional maneuvers; multiple venipunctures, and procedure cancelled or terminated early due to access issues. In 47 patients with femoral lines, approximately 15% had at least one line-associated AE. Patients with internal jugular and subclavian CVC had a line-associated AE rate of 50% ($p= 0.013$).There were no instances of infection, thrombosis or arteriovenous fistula associated with femoral CVC. One patient developed a small hematoma following CVC removal.

Five procedures in four patients were associated with PIV and multiple venipunctures. All four patients were considerably older (16.8 ± 1.2 years) and larger (70 ± 20 kg, range 57-103). In one patient, the procedure was cancelled pending emergent CVC placement. One patient lost a PIV midway through the procedure and required several attempts to re-establish venous access. Two patients with poorly functioning CVCs required placement of a PIV for draw (n=1) or return (n=1).

Like pediatric allogenic donors [10], bleeding and pain were usually associated with newly placed CVC and tended to occur in younger patients (median 5.5 years). Bleeding was minimal and limited to oozing around the insertion site and responded to pressure (n=1) or a topical clotting agent (n=1). No patient required a blood transfusion for catheter-related blood loss. Mild pain was treated with acetominophen in two patients: one patient required morphine for pain relief.

Technical AE

Twenty-two moderate to severe technical AE were documented in 7 patients (11%) and 9 procedures (8%). AE included slow inlet rates, multiple alarms, an unstable interface, prolonged procedure times, clotted circuit with blood loss and premature termination of the procedure (Table 4, Supplemental Data, Table S1). Most AE (8/9) were due to poorly functioning CVCs. In patients with newly placed femoral lines, catheter-related issues were encountered after day 1. In one older patient, poor CVC function was attributed to placement of a left subclavian CVC that was too small for her age and size.

Prolonged procedure times occurred in 66% (6/9) of affected procedures. In four procedures, inlet rates were less than 1 ml/kg/min (range, 0.31-0.72; Table S1). In one patient, prolonged procedure times were due to a long delay in establishing the interface following a RBC prime [6]. In general, technical AE increased procedure time by 35 minutes (range 8-105 minutes) relative to case-matched controls (TBV ± 5%). Due to slow inlet rates, three procedures did not achieve a full LVL collection. Prolonged procedure times may have contributed to citrate AE in two patients.

Other AE

One patient had a mild allergic reaction to the RBC prime. Symptoms were limited to pruritis and rash and responded to additional antihistamines. No patient had hypotension or hemodynamic instability.

Comparison of AE in pediatrics and adults

Pediatric allogeneic donors are reported to have less AE than adults due, in large part, to significantly less G-CSF toxicity [10]. To determine whether the same was true for A-HPCC, we compared procedure-related AE in pediatric patients to 82 adult myeloma patients who underwent a total of 210 A-HPCC during the 2013 calendar year. Overall, AE were slightly more frequent in adult patients (48% vs 39%) although the AE rate per procedure was identical (~25%, Table 4). There was no significant difference in citrate-

related AE between children and adults. Children, however, were three times more likely to experience gastrointestinal symptoms (16% vs 9%, OR=3.38) whereas parasthesias were common in adults (31% vs 9%, p=0.002). Children were also more likely to have technical problems due to CVC-related issues (85.7%, p=0.006; OR=27). Unlike pediatric patients, most adult patients were collected using PIV (75.6%, p<0.00001). Most alarms in adults were attributed to slow inlet flow, high return pressures or machine obstructions/malfunctions associated with a new blood separator.

Impact of AE on cell collection

We also examined the impact of AE on CD34 collection and HPC product quality. Technical AE due to flow problems have the potential to interfere with a stable interface. Furthermore, reversal of arterial and venous lines using dual lumen catheters is reported to increase recirculation by 7% to 20%, with decreases in CD34-CE [17,31,32]. Finally, multiple venipunctures, repeated line flushing and manipulation could increase the risk for bacterial contamination.

AE had no impact on the CD34 collection rate per peripheral CD34 count: the rate of collection was similar, regardless of the presence or type of AE (Fig 3A-D). There was also no significant difference in MNC-CE (range, 47-56%) or CD34-CE (54-57%) by AE (Supplemental Data, Table S2). There was also no difference in the mean CD34 and MNC yields. When examined by specific type of AE, products associated with technical AE tended to have lower CD34 yields (4.2 vs 21.6 million/kg, p=0.00035) due to lower

peripheral CD34 counts and a high number of aborted procedures (3/8, 37%). There were no positive cultures with any product collected.

DISCUSSION

Children are often perceived to tolerate HPCC better than adults, with significantly less G-CSF toxicity [10]. In adults, procedure-associated AE average 9-13% per procedure and 12 to 42% per patient, with higher rates observed in women and low body weights [7,29,30]. In healthy pediatric donors, procedural AE rates range from 20-40% per patient [10,11], which is compatible with our results (39%).

Young age, small size and an increasing number of procedures are all reported AE risk factors in pediatric apheresis [3,4,11]. In pediatric HPCC, Sevilla et al have reported an inverse relationship between patient size and AE rates, with the highest rates in children < 10 kg (>90%), falling to 51% in children < 20 kg and 20% in children > 20 kg [4,11]. In contrast, we found that older and heavier children had the highest AE rates, reaching 56% in children > 40 kg (Fig 1). Older children were also more likely to have more procedures, increasing the likelihood for procedure-associated AE (Table 3). Michon also noted a correlation between AE rates and number of apheresis procedures, with 82% of children eventually experiencing at least one AE during the course of treatment [3].

Citrate was the most common AE encountered, with at least one citrate AE observed in 25% of children. This is consistent with prior pediatric (7-25%, Table 5) and

adult studies [2,3,8,10,13,14,16, 29, 30, 33]. In our study, the risk of citrate AE was higher in older and heavier patients, who often required several procedures (Fig 2). We did not observe citrate or other AE in children ≤ 10 kg, who were systemically anticoagulated with heparin. A marked reduction in citrate AE is also reported using low dose-citrate and heparin anticoagulant regimens (10 units/mL heparin in ACD-A), although citrate-associated hypotension and hypocalcemia can still occur in ~ 5% of patients [2,17,20,33,34]. Other cited advantages of heparin-based anticoagulation are decreases in net fluid balance, hypokalemia and base excess and an increase in blood volume processed per unit time [17,33,34]. Disadvantages of heparin are greater procedural platelet losses, prolonged coagulation abnormalities, bleeding and potential heparin sensitization. [17,33-35]

We did not observe any episodes of hypotension, which have been reported in 0.7 to 90% of pediatric patients (Table 5) [2-4,10-12,14-16]. Very young children are at particular risk for procedure-associated hypotension due to small blood volumes, fluid shifts, dilutional anemia and iatrogenic blood losses associated with infectious disease testing, cell counts and other laboratory tests [5]. Hypotension can occur early in the procedure, particularly when using older blood separators with large extracorporeal priming volumes [1,2,5,12,14,18]. Hypotension and hemodynamic instability occurring late in the procedure is typically attributed to citrate [2] or can follow blood losses arising from catastrophic instrument malfunctions [4,5].

To avoid hypotension, most centers, including our own, perform a blood prime whenever the extracorporeal volume exceeds 10-15% of the patient's blood volume [1-

5,8-10, 12-15,17-20]. In addition, most centers have policies specifying a minimum pre-procedure hemoglobin, which can range from 8 to 12 gm/dL [3,16]. Although we did not observe hypotension, other studies have still documented episodes of hypotension despite a RBC prime (Table 5)-- especially in children anticoagulated with citrate [3,12-15]. It is possible this could reflect a combination of endothelial dysfunction and lower endogenous ACE levels by G-CSF [36], coupled with a bradykinin release syndrome analogous to that described in small pediatric dialysis patients [37,38]. Infants are particularly dependent on the renal angiotensin system for blood pressure control and are highly sensitive to decreases in ACE activity [39]. Disadvantages of a RBC prime are delays in establishing an interface, the potential for transfusion reactions and higher procedural platelet losses [6,12,17]

Some institutions use albumin to prime the circuit, sometimes coupled with higher pre-procedure hemoglobin levels [Table 5]. A review of the literature, however, suggests that albumin may not be appropriate in all patients. As shown in Table 5, some of highest rates of hypotension in children < 20 kg were associated with albumin priming [11,16]. Sevilla et al reported cardiovascular symptoms in 48% of healthy pediatric donors < 20 kg undergoing allogeneic HPCC [11]. A similar cardiovascular AE rate was noted by Orbach et al associated with A-HPCC in small children [16].

Few studies have documented technical AE associated with pediatric A-HPCC. We recorded technical AE in 7 patients (11.3%) and 9 procedures (8%). Unlike adult patients, technical AE in pediatric patients were overwhelming CVC-related (85.7%, p=0.002), leading to interface delays, prolonged procedures and/or short collections. We

observed one severe technical AE due to clotting of the circuit. Technical AE due to instrumentation and circuit loss tend to be severe, often leading to aborted procedures, blood loss and potentially product loss [2-5,16,30]. Fortunately, instrumentation problems are relatively uncommon during HPCC, with reported rates ranging from 0.4-7% in children and adults [3,14,30]. This is sharp contrast to pediatric dialysis where alarms, instrument shutdowns and circuit loss eventually occur in the majority of patients [37].

The rate of technical AE in our study is equivalent to that reported by others. Michon et al reported technical AE in 19.7% of all pediatric apheresis procedures, including A-HPCC [3]. In pediatric HPCC, technical AE have been reported in up to 24% of patients and 12% to 20% of procedures [14-17]. Like our study, most technical AE were CVC-related, requiring reversal of lines or "extreme positional maneuvers" to complete the procedure [14,17]. In contrast, CVC-related technical AE were significantly less common in our adult patients, who are routinely collected by PIV whenever possible. In a recent study, alarms due to a slow inlet flow rate occurred in only 0.69% of adult A-HPCC [30].

The high frequency of CVC-related technical AE in our pediatric patients reflects the difficulty and importance of establishing adequate venous access in this population [1,2]. In one of our patients, the catheter (9 French) was clearly too small for the patient's age and weight (68 kg) [40]. Four of our patients had a left-sided subclavian CVC, with line-related AE documented in 2 patients and 3 procedures, and decreased CD34-CE (40% vs 58%, p=0.03). Left-sided subclavian CVC have a documented higher rate of

malfunction [21,41-43], including HPCC. In a study of Neostar® CVC, 42% of patients with left-sided CVCs had at least one AE ($p<0.001$), including significant procedural delays (24%) and aborted procedures (8%) [43]. Likewise, we have reported a 19% severe AE rate with left-sided CVC in adult A-HPCC [6]. At our institution, left subclavian placement is avoided whenever possible.

Our practice has favored the placement of a temporary, dual-lumen femoral dialysis catheter in young children undergoing A-HPCC. Patients generally undergo line placement the morning of their first scheduled collection, followed by LVL in the afternoon. Nearly half of our patients collected in a single session and 36% within two sessions: Only 6 patients (13%) required a femoral CVC more than 3 days (range, 1-6 days). Flow-related AE were observed in 15% of patients and 5% of procedures on day 2 or later. Access and flow-related AE were significantly less common with femoral CVC than all other types of venous access (43% AE), especially subclavian and internal jugular CVC (50% AE). Femoral CVC also have a relatively low rate of flow-related in adult A-HPCC patients, with alarms and occlusion occurring after 2-3 days [44]. Patients undergoing A-HPCC may be at higher risk for CVC occlusion, in general, due to G-CSF's prothrombotic effects on platelet reactivity and coagulation factors [36,45-49]. Prophylactic calcium gluconate infusion through the return line may also increase the risk for catheter malfunction [50].

Heparin and heparin-citrate anticoagulation might reduce catheter malfunction rates since it would avoid or minimize the need for calcium replacement, while increasing the blood volume processed per unit time. Studies using heparin-based anticoagulation in

small children rarely encounter issues with short-term catheters [4,18]. Likewise, we did not observe any catheter-related technical or other AE in 6 patients receiving systemic heparin anticoagulation — although all of these patients collected in one day. A randomized adult trial comparing ACD-A versus heparin-based anticoagulation found no difference in catheter malfunction rates, however, the study limited its analysis to the first A-HPCC only [33]. An earlier study by Reik et al reported CVC-related flow problems in 13% of adult A-HPCC using a 6 U/mL heparin/ACD-A regimen [34]. In pediatric dialysis, citrate appears superior to heparin anticoagulation, with significantly fewer episodes of clotting, circuit loss and a longer circuit life [37,50].

In summary, our study shows an overall AE rate of 39% of patients and 14% of procedures. As a single institutional study, inherent weaknesses are the number of patients, which are heterogenous relative to age, underlying diagnosis and target CD34 yields. Despite the latter, this is the largest study limited to autologous pediatric patients, and is more homogenous and detailed than most other published studies. Contrary to earlier studies, we found that older and heavier children were at greater risk for procedure-related AE. Unlike younger patients, older children were more likely to use alternate venous access, with an increased incidence of venous and flow-related issues. In addition, older and heavier children often required more procedures and more total citrate exposure. Possible methods to decrease AE in this population include optimizing CD34 mobilization and use of citrate-heparin anticoagulation. Active monitoring of venous access issues and have led to discontinuation of some brands, early venous access assessment in older children by apheresis nursing staff, and avoidance of outside CVC placement for HPCC.

Abbreviations: ACE, angiotensin converting enzyme; AE, adverse event, CE, collection efficiency; CVC, central venous catheter, (A-)HPCC, (autologous) peripheral blood human progenitor cell collection; LVL, large volume leukapheresis; PIV, peripheral IV; TBV, total blood volume.

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Table 1: Grading of Procedure-Associated AE

AE Category	Mild	Moderate	Severe
Anticoagulant (ACD-A)	Mild parasthesia resolved by increasing calcium infusion rate	Parasthesia at maximum calcium infusion rate	Any of the following: Nausea, vomiting, abdominal pain, hypotension, tetany
		Calcium boluses	
		Pause in the procedure	
Venous Access	One restart of line	Restart of lines $\geq$ 2 times	Chronic access issues requiring multiple saline flushes
	Mild bleeding at line site, controlled by dressing	Positional changes	Urokinase required
	Mild pain at catheter site	Line reversal	Procedure aborted/terminated due to access issues
		Recirculation	Hematoma/venous thrombosis
		Peripheral IV for draw and catheter/port for return	Blood loss requiring transfusion
		Bleeding requiring pressure	
		Pain requiring narcotics	
Technical	Occasional alarm	Interface instability	Machine malfunction
		Multiple alarms	Circuit clotting
		Slow inlet rate	Tubing breach or kinking
		Prolonged procedure	Circuit change required
			Blood loss in circuit
			Procedure terminated early

Table 2: Patient Demographics

Variable	Number Patients
No. Patients	62
Sex (M/F)	30/32
Median Age, years (%)	4.5 (range, 1-18)
≤ 3 years	27 (44%)
> 4 years	35 (56%)
Median weight (%)	18 (range, 8-103)
<10 kg	6 (10%)
10-20 kg	30 (48%)
20-40 kg	10 (16%)
40-60 kg	8 (13%)
> 60 kg	8 (13%)
Blood Prime	38 (61%)
Diagnosis (%)	
Neuroblastoma	25 (40%)
Brain Tumor	20 (32%)
Lymphoma	10 (16%)
Hepatoblastoma	1 (1.6%)
Germ Cell Tumor	1 (1.6%)
Ewing's Sarcoma	2 (3.2%)
Ovarian Cancer	2 (3.2%)
Testicular Cancer	1 (1.6%)
Mobilization (%)	
Chemotherapy	60 (97%)
Growth-factor only	2 (3%)
Remobilization	0
Blood Counts Day 1	
WBC (10^9/L)	24.5 ± 17 (range, 4.6-64.4)
% MNC	18.3 ± 12.6 (range, 3-56%)
MNC (10^9/L)	3.9 ± 4.6 (range, 1-54.2)
% CD34[a]	1.34 ± 0.23 (range 0.01-7.43%)
CD34 per uL[a]	211.2 ± 42 (range, 1.1-1957)
CD34 Target Yields	
3-5 x 10^6/kg	16
10-15 x 10^6/kg	46

a. mean ± SEM.

Table 3: Comparison of Patients with and without AE

		Adverse Event (AE)		
Variable	**All**	**Yes**	**No**	**P**
No. Patients (%)	62 (100%)	24 (39%)	38 (61%)	-
Age, years (median)	6.6 ± 5.5 (4.5)	8.8 ± 6.0 (8.5)	5.2 ± 2.3 (3)	0.012
M/F	30/32	10/15	20/17	0.28
Weight, kg (median)	29.1 ± 24 (18)	37.7 ± 28 (27)	23.7 ± 20 (16)	0.02
TBV, mL (median)	1988 ± 1388 (1357)	2600 ± 1581 (2606)	1574 ± 1080 (1150)	0.005
Diagnosis (%)				
Neuroblastoma	25 (40%)	7 (28%)	18 (72%)	0.11
Brain Tumor	20 (32%)	8 (40%)	12 (60%)	0.88
Lymphoma	10 (16%)	4 (40%)	6 (60%)	0.98
Other	7 (11%)	5 (71%)	2 (29%)	0.008
Total No. Procedures	110	52	58	-
Volume processed (L)	6.86 ± 6.10	7.06 ± 4.8	6.80 ± 6.5	0.85
Avg. Procedures/Patient	1.8 ± 1.2	2.0 ± 1.1	1.5 ± 1.1	0.14
Patients > 1 Procedure (%)	24 (39%)	14 (58%)	10 (27%)	0.012
No. Procedures ≥ 1 AE	110	27	83	-
No. Proc/AE per day (%AE)				
Day 1	62	13 (21%)	49	ref
Day 2	24	8 (33%)	16	0.23
Day 3	14	4 (29%)	10	0.54
Day 4-6	10	2 (20%)	8	0.95
Anticoagulation				
Heparin	6	0	6	0.04
ACD-A	56	24	32	0.04
RBC prime	38	12	26	0.15
Venous access				
PIV	5	3	2	0.32
CVC	57	21	36	0.32
CVC Brand[a]				
medCOMP®	5	1	4	0.41
Arrow International®	25	6	19	0.08
Mahurkur™	20	10	10	0.13
NeoStar™	4	2	2	0.57

Powerline®	3	2	1	0.27
Anatomic Placement				
Femoral	47	16	31	0.35
Left subclavian	4	2	2	0.57
Right internal jugular	6	3	3	0.48

a. Double lumen, dialysis CVC included Mahurkar™ (8-12 F, Covidein, Mansfield, MA), medCOMP (7 F, Haleysville, PA) and Arrow International (12 Fr, Reading, PA). Tunneled CVC included Powerline® (Bard, Tempe, AZ) and NeoStar™ (Angiodynamics, Latham, NY).

Table 4: Comparison of Procedure-related AE in Pediatric and Adult Patients

AE Type	Pediatric Patients (n=62)		Adult Patients (n=82)		AE in Peds vs Adults	
	No. AE[a]	% AE[b]	No. AE	% AE	P	OR (95% CI)[c]
Heparin Anticoagulant[d]	0/6	0	0/1[e]	0	-	
Citrate Anticoagulant[d]	14/56[a]	(25%)	27/81	(33.3%)	0.29	
Gastrointestinal	9	(16.1%)	4	(4.9%)	0.09	OR=3.68 (1.1-12.6)
Nausea/vomiting	3	(5.3%)	4	(4.9%)	0.91	
Abdominal pain	5	(8.9%)	0	0	0.006	
Diarrhea	1	(1.8%)	0	0	0.23	
Neurologic	7	(12.5%)	26	(32.1%)	0.008	OR=0.30 (0.12-0.76)
Parasthesia	5	(8.9%)	25	(30.9%)	0.002	OR=0.22 (0.07-0.60)
Lightheaded	1	(1.8%)	0	0	0.23	
Agitation	1	(1.8%)	0	0	0.23	
Muscle cramping	0	0	2[f]	(2.5%)	0.24	
Cold	0	0	1	(1.2%)	0.40	
Venous Access	12/62[a]	(19%)	14/82	(17.1%)	0.72	
Restart PIV	5	(6.4%)	8	(9.7%)	0.48	
CVC line reversal	2	(3.2%)	1	(1.2%)	0.40	
CVC positional	1	(1.6%)	1	(1.2%)	0.84	
CVC recirculation	0	0	1	(1.2%)	0.85	
Occluded CVC[g]	2	(3.2%)	2	(2.4%)	0.78	
PIV draw, CVC return	1	(1.6%)	2	(2.4%)	0.73	
Pain	3	(4.8%)	2	(2.4%)	0.44	
Bleeding	2	(3.2%)	1	(1.2%)	0.10	
Tape allergy	0	0	2	(2.4%)	0.22	
Infection	0	0	1	(1.2%)	0.38	
Cancelled/terminated	2	(3.2%)	0	0	0.10	
Technical	7/62[a]	(11.2%)	11/82	(13.4%)	0.70	
Multiple alarms	4	(6.4%)	7	(8.5%)	0.64	
Slow inlet	5	(8.1%)	6	(7.3%)	0.87	
Unstable interface	3	(4.8%)	2	(2.4%)	0.42	
Machine malfunction	0	0	2	(2.4%)	0.22	
Clotted circuit	1	(1.6%)	1	(1.4%)	0.73	
Blood loss[h]	1	(1.6%)	0	0	0.25	
Prolonged procedure	5	(8.1%)	6	(7.3%)	0.88	
Short procedure	3	(4.8%)	3	(3.6%)	0.63	
CVC-related[i]	6/7	(85.7%)	2/11	(16.7%)	0.006	OR=27 (2 - 468)
Total Patients[j]	24/62	(38.7%)	39/82	(47.6%)	0.29	
Total Procedures[k]	28/110	(25.4%)	54/210	(25.7%)	0.96	

a. Number of AE/No. patients. Note that some patients had more than one AE per procedure and the entire course of A-HPCC. As a result, the total number of AE exceeds the number of total number of patients in each category.
b. % AE
c. Odds ratio and 95% confidence interval
d. AE in patients anticoagulated with heparin versus ACD-A.
e. One adult patient with end stage renal disease, anticoagulated with 10% heparin in ACD-A at AC:WB ratio 1:22.
f. Tetany, chest pain in two adult patients.
g. CVC requiring multiple flushes and/or urokinase to access.
h. Unable to return blood to patient
i. No. and percent technical AE due to CVC malfunction.
j. Total number of patients with at least one procedure-related AE
k. Total number of procedures with at least one AE

Table 5. Review of Published Procedural AE during Pediatric HPC collection[a]

Study, year	No. Pts	No. Proc	Donor Type	Weight (in kg)	RBC Prime	Anti-coagulant[b]	AE[a] (% Patients / (% Procedures))				
							Citrate	Venous Access	Technical	CV[c]	Other[d,e]
Takaue [12], 1995	38	81	Auto	< 20	Y	ACD-A	-	-	-	5.3 (2.5)	2.6[d] (1.2)
Diaz [13], 1996	31	48	Auto	12 < 25 26 - 60	Y N	ACD-A	16.1[f] (10.4)	-	-	6.4 (4.2)	3.2[e] (2.1)
Gorlin [2], 1996	14	85	Auto	< 25	Y	Heparin[g]	7.1[g] (1.2)	64	-	7.1[g] (1.2)	-
Madero [14], 1997	56	71	Auto	9 < 25 25 - 62	Y N	ACD-A	23.2[h] (18.3[f])	17.8[i]	12.5[i] (10[f])	3.5 (2.8)	-
Pulsipher [8],2005	201	218	Allo	< 20 > 20	Y N	Heparin or ACD-A[j]	10.1[j]	41[j]	-	-	-
Sevilla [4], 2007	12	13	Auto Allo	7.5-10.9 9	Y N[l]	Heparin[k]	16.7 (15.3)	-	8.3 (7.7)	91.7 (85)	-
Michon [3], 2007	-	305	Auto, Allo	< 15-20 > 20	Y[m] N	ACD-A	24.9 (11.8)	-	-	20.5 (9.5)	-
Sevilla [11], 2008	66	152	Allo	< 20	N[l]	Heparin[k]	8 (6.4[f])	7 (6.4[f])	-	48 (38.7)	-
				> 20	N	ACD-A	19.5 (15[f])	-	-	2.4 (1.8)	-

DeSilvestro [7], 2009	210	291	Auto	-	-	ACD-A (Heparin?)[n]	4.3[f] (3.1)	2[f] (1.4)	5[f] (3.8)	0	-
Fishmeister [15], 2000	51	165	Auto	9 < 20 20 - 30 30 - 92	Y N[l] N	ACD-A	6.5[f] (21.2)	39.2[f,o] (12.1)	23.5[f,o] (18.8)	5.9[f] (1.8)	-
Orbach [16], 2003	24	48	Auto	< 20	N[l]	ACD-A	20.8 (10.4[f])	-	16.7 (8.3[f])	20.8 (10.4[f])	-
Bolan [17], 2004	38	74	Auto, Allo	11 - 29	Y	Heparin, ACD-A[p]	7.1[p] (3.8)	26.3[q] (13.5[f])	23.9[q] (12[f])	0	-
Syczynsnski [10], 2012	140	220	Allo	12 - 114	-	ACD-A (Heparin?)[n]	21	15 (9.5[f])	-	0.7 (0.4)	-
Salazar-Riojas [187], 2014	22	24	Auto, Allo	< 20	Y	Heparin[k]	0	0	-	4.5 (4.2)	-
This study	6	6	Auto	< 10	Y	Heparin	0	0	0	0	0
	30	40	Auto	11-20	Y	ACD-A	17	13	10	0	3.8[d]
	26	64	Auto	> 20	Y/N	ACD-A	35	27	15	0	0
Total	62	110		8-103			25 (14)	19 (12)	11.3 (7.3)	0	1.8[d] (0.9)

Abbreviations: -, not reported or available; ACD-A, acid citrate dextrose; Auto, autologous donor; Allo, allogeneic donor; Pt, patient; Proc, procedure; RBC prime, Y=RBC used in priming circuit, N=no RBC used for circuit priming.

a. AE were classified as described in Table 1. The adjusted AE rate (% patients, % procedures) was determined for citrate, venous access, technical, cardiovascular, and "other" AE.

b. Anticoagulant used during HPCC.

c. Cardiovascular symptoms, defined as hypotension, tachycardia and/or hemodynamic instability.
d. Allergic transfusion reactions to RBC prime.
e. Hypothermia.
f. Estimated incidence based on number of patients and procedures.
g. Low dose ACD-A (AC:WB ratio=1:25-1:30) and systemic heparinization. Hypotension, emesis and diaphoresis in one infant attributed to citrate. No problems with subsequent procedures using only systemic heparin and no ACD-A.
h. Citrate AE includes documented hypocalcemia, parasthesias, nausea and vomiting.
i. Venous AE includes nonfunctioning catheter requiring replacement, urokinase treatment and malfunctioning CVC requiring extreme positional maneuvers. Seven venous AE were associated with slow inlet rates.
j. Multi-institutional survey of 22 pediatric collection centers. ACD-A discussed as primary anticoagulant for most donors (83% > 7 years of age). Hypocalcemia or symptoms documented in 19/188 donors. Venous AE limited to catheter pain and bleeding in 44/106 patients with CVC.
k. Heparin (10 Units/mL) in ACD-A, administered at AC:WB ratios 1:20-1:30.
l. Circuit primed with albumin.
m. RBC prime if < 15 kg or if severely anemic.
n. Multi-institutional registry study. No details regarding anticoagulation in younger patients.
o. 31 technical AE, 19 due to flow-related issues and 12 due to instrumentation or software problems. Data reported as AE per procedure.
p. Three anticoagulation protocols over 9 year period including systemic heparinization, systemic heparinization with low dose citrate (AC:WB ratio 1:20-1:30) and standard ACD-A (AC:WB ratio=1:10-1:13). Citrate AE rates calculated for patients anticoagulated with ACD-A (n=53)
q. One patient had persistent bleeding and hematoma with a femoral catheter. Documented flow problems in 9/38 patients with CVC.

Figure Legends

Figure 1. AE rate in pediatric A-HPC patients. A) The percentage of patients with at least one AE by patient weight. B) The number of HPC procedures by patient weight (mean ± SD). Patients greater than 20 kg required significantly more HPC procedures than children less than 20 kg (p=0.003).

Figure 2. Citrate AE. A) Distribution of gastrointestinal (black) and neurologic (white) citrate AE by patient age. B) Frequency of citrate AE by patient weight. Note that patients < 10 kg were anticoagulated with heparin. C) Frequency of citrate AE by procedure day.

Figure 3. Impact of AE on CD34 collection rate: A) All AE, B) Citrate AE, C) Venous Access, and D) Technical AE. Legend, —•— procedures with AE, - -o- - procedures with no AE.

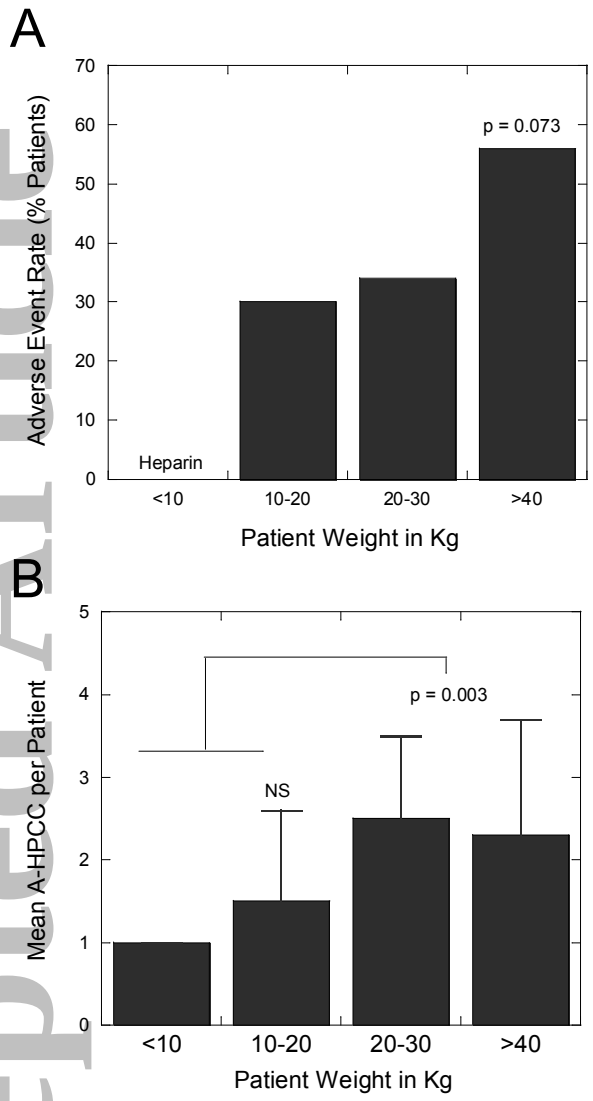

Figure 1

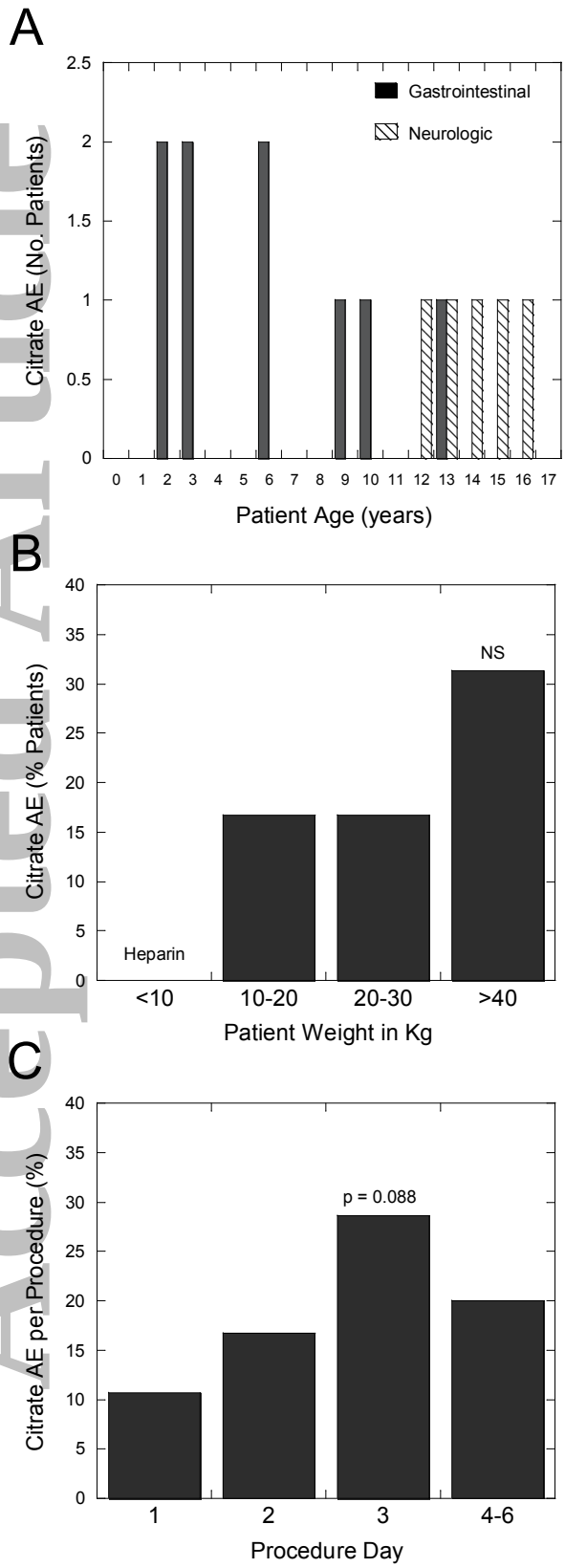

Figure 2

Figure 3