Large volume leukapheresis is efficient and safe even in small children up to 15 kg body weight

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Background. The collection of peripheral blood stem cells, although now a routine procedure, is still a challenge in low body weight children because of specific technical and clinical issues. For paediatric patients it is crucial to obtain an adequate number of CD34+ cells with the minimum number of procedures: this can be done using large volume leukapheresis (LVL).

Materials and methods. We analysed the efficacy and safety of 54 autologous LVL performed in 50 children (33 [66%] males and 17 [34%] females), median age 2 years (range, 1-5) and median body weight 12 kg (range, 6-15). The procedures were performed with a COBE Spectra previously primed with red blood cells; ACD-A solution and heparin were used as anticoagulants.

Results. The target CD34+ cell dose (≥5×10/kg body weight) were collected with one LVL in 46 (92%) patients, while four (8%) patients needed another procedure. All our LVL were well tolerated. Side effects were observed in five (9.2%) patients and one procedure had to be discontinued because of catheter-related haemorrhage. The platelet count decreased significantly ($p<0.001$) after each procedure but without bleeding or need for transfusion support.

Discussion. Our experience confirms that LVL is efficient and safe even in small children, if the procedure is adjusted considering the weight and age of child. The most important factors are good venous access, adequate preparation of the child's electrolyte status, and surroundings in which the small child as well as parents feel comfortable, and can tolerate the procedure better. Although a median platelet loss of 50% can be expected, LVL is safe and reduces the overall number of procedures required. It can be recommended for peripheral blood stem cell collection even in small body weight children with malignant diseases, particularly those who mobilise low numbers of CD34+ cells.

Keywords: apheresis, peripheral blood stem cell collection, paediatric patients.

Introduction

Although the collection of autologous peripheral blood stem cells (PBSC) is now a routine procedure, apheresis remains challenging in low body weight children because of technical and clinical issues related to vascular access, low total blood volume, anticoagulation and side effects. Since rapid and sustained engraftment following high-dose therapy depends on the numbers of stem cells infused, efforts are directed towards harvesting sufficient numbers of CD34+ cells. In paediatric patients it is very important to collect as many PBSC as possible during leukapheresis and to obtain an adequate number of CD34+ cells with a minimum number of procedures. One way to increase the number of PBSC collected is to process a larger volume of the patient's blood during a so-called large-volume leukapheresis (LVL) procedure which involves processing the patient's total blood volume at least three times. LVL differs from standard leukapheresis by processing a larger volume of blood, an increased blood flow rate, additional heparin anticoagulant and longer duration of the procedure[1-6]. Our previous experience with LVL in adult patients, as well as the results of other authors who performed LVL in paediatric patients encouraged us to process larger blood volumes in small body weight children[7-9].

The purpose of this study was to investigate the efficiency and safety of LVL in small children up to 15 kg body weight (BW). We analysed the characteristics of the PBCS collection with LVL, the leukapheresis products, and adverse reactions which occurred during procedures. Finally, we evaluated whether LVL is efficient in obtaining the target cell number in poor and in good mobilisers.

Materials and methods

This study was performed in the Department of Transfusion Medicine and Transplantation Biology,

University Hospital Centre (UHC) Zagreb, between 2007 and 2013, on a group of 50 children weighing up to 15 kg who had been previously diagnosed and treated at UHC Zagreb and the Children's Hospital Zagreb (Table I). All children were candidates for high-dose chemotherapy followed by autologous PBSC transplantation. PBSC were mobilised in all children by a combination of disease-specific chemotherapy cycles and 10 μg/kg/day s.c. granulocyte colony-stimulating factor (Neupogen, Roche, Switzerland). The study was approved by the local ethics committee, and written informed parental consent for the PBSC collection by LVL was obtained from all parents.

PBSC were collected using a COBE Spectra cell separator (MNC programme, software version 6.0; Terumo BCT, Lakewood, CO, USA). The cell separator was primed with leucocyte-depleted and irradiated packed red blood cells before the procedure in order to prevent haemodynamic complications and dilutional anaemia. A venous access was established using a dual lumen central venous catheter: in 43 (86%) patients the catheter was placed in the femoral vein, in five (10%) patients in the subclavian vein and in two (4%) patients in the jugular vein.

Leukapheresis started when the peripheral blood CD34+ cell count reached 10×10^6/L. A minimum of 30×10^9/L platelets was required before leukapheresis.

Table I - Patients' characteristics.

Number of patients	50
Gender (male/female)	33 (66%)/17 (34%)
*Age (years)**	2 (1-5)
*Body weight (kg)**	12 (6-15)
*Total blood volume (mL)**	901 (440-1,272)
Diagnosis (n)	
Neuroblastoma	31
Rhabdomyosarcoma	7
Acute myeloid leukaemia	2
Primitive neuroectodermal tumour	2
Wilms' tumour	2
Hodgkin's disease	1
Non-Hodgkin's lymphoma	1
Nephroblastoma	1
Meduloblastoma	1
Hepatoblastoma	1
Retinoblastoma	1
Prior therapy	
Cycles of prior chemotherapy (n)*	6 (3-21)
Previous radiotherapy (n, %)	2 (4%)

* Median (range).

Patients with pre-apheresis counts of $\geq20\times10^6$/L CD34+ cells were considered good mobilisers, while those with a CD34+ count $<20\times10^6$/L were considered poor mobilisers. Twelve patients were poor mobilisers, and 38 were good mobilisers.

The target yield of CD34+ cells was 5×10^6/kg of body weight, although a yield of 3.5×10^6/kg BW was considered minimally acceptable. If possible we tried to collect more than the target number of CD34+ cells in case the children gained weight at the time of transplantation. If the target dose of CD34+ cells was not collected, another apheresis procedure was performed the following day.

LVL was performed in all patients and the children's total blood volume was processed 4.0 to 7.4 (median 5.4) times. The total blood volume was calculated as 80 mL × total body weight (kg). The inlet flow rate was set according to the instrument's calculations. The collection rate was 1.0 mL/min while the collected fraction was maintained under manual control at a haematocrit of approximately 1%.

A combination of citrate dextrose formula A solution (ACD-A, Baxter, Deerfield, IL, USA) and heparin (Heparin, Belupo, Croatia) was used for anticoagulation. The addition of 6 IU of heparin per 1 mL of ACD-A allowed an increase in the ACD-A to whole blood ratio to 1:24. The inlet flow rate was, therefore, doubled and twice the blood volume was processed in the same time. Platelet clumping was prevented by adding ACD to the collection bag.

The collected product was replaced with the same volume of 5% human albumin, given as a continuous infusion. Blood warmers ((Spectratherm, Gambro BCT, Lakewood, CO, USA) were used during all procedures. In order to prevent volume overload during rinse-back at the end of apheresis, blood was collected into a bag and centrifuged, the plasma was removed and an autologous red blood cell unit was prepared for autologous transfusion. In order to prevent symptoms of hypocalcaemia, all patients received prophylactic calcium gluconate (1 g×10 kg BW) in 100 mL of normal saline in a slow infusion controlled by an automatic infusion pump throughout the LVL procedure. Our patients were monitored regularly throughout the procedure with a cardiac monitor and pulse oximetry. Vital signs were monitored closely at the beginning of the procedure when complications such as hypovolaemia are most prevalent. The observation interval was gradually prolonged to every 30 minutes once the procedure was ongoing, and all adverse reactions were recorded.

Peripheral blood samples taken before and after leukapheresis, and samples from leukapheresis products were analysed for white blood cell, mononuclear cell, platelet and CD34+ cell counts.

Complete blood counts of the peripheral blood samples and leukapheresis products were obtained using an automated cell counter, ADVIA 120 (Bayer, Leverkusen, Germany). White blood cell differential counts were determined manually using Wright-Giemsa-stained specimens. Mononuclear cells were defined as the sum of monocytes and lymphocytes.

CD34+ cells were analysed by flow cytometry using a FACSCalibur (BD Biosciences, Heidelberg, Germany) following the standard ISHAGE procedure for cell staining with anti-CD34-PE (clone 8G12) and anti-CD45-FITC (clone 2D1) monoclonal antibodies (BD Biosciences)[10].

Data were tested for normality with the Kolmogorov-Smirnov test. All distributions were normal, thus parametric procedures were used for all analyses. Differences between pre-apheresis and post-apheresis peripheral blood cell counts were tested with a paired samples *t*-test, while differences between poor and good mobilisers were tested with the *t*-test for independent samples. We used Pearson's correlation to test the association between CD34+ cell total yield and pre-apheresis CD34+ cell count. The level of statistical significance was set at 0.05 for all analyses and statistical computations were performed using IBM SPSS 21.0 for Windows (Chicago, IL, USA).

Results

Fifty-four apheresis procedures, performed in 50 small children up to 15 kg BW, were analysed. Leukapheresis procedures and products characteristics are shown in Table II. Pre-apheresis and post-apheresis peripheral blood cell counts are presented in Table III. All analysed blood cell counts were significantly lower after leukapheresis (p<0.0001).

There was a strong correlation between the pre-apheresis peripheral blood CD34+ cell count and the total CD34+ cell yield (r=0.836, p<0.001). In both poor and good mobilisers, the total CD34+ yield correlated significantly with the pre-apheresis CD34 count (r=0.653, p<0.05 and r=0.786, p<0.0001, respectively). The target number of CD34+ cells (≥5×10/kg BW) for PBSC transplantation was collected with only one LVL procedure in 46 (92%) patients, whereas an additional procedure was required in the other four (8%) patients.

The pre-apheresis CD34+ cell count and LVL characteristics in poor and good mobilisers are shown in Table IV. Total CD34+ cell yield was 9.7×10⁶/kg BW (2.1-46.4×10⁶/kg BW) and was significantly higher in good mobilisers than in poor mobilisers (p<0.0001).

Table II - Leukapheresis procedures and product characteristics.

Leukapheresis procedures	**Median (range)**
Procedure time (min)	255 (174-303)
Processed total blood volume (×)	5.4 (4.0-7.4)
Processed blood volume (mL)	4,957 (2,101-8,672)
Inlet blood flow rate (mL/min)	22 (10-47)
AC volume (mL)	240 (113-416)
Product volume (mL)	242 (191-304)
Leukapheresis products	**Median (range)**
Total nucleated cells ×10⁸/kg BW	15.5 (2.33-42.17)
Mononuclear cells ×10⁸/kg BW	9.8 (0.82-20.36)
Haematocrit (%)	1.6 (0.1-5.2)
CD34+ cells ×10⁶/kg BW	9.76 (2.1-46.4)
Mononuclear cell collection efficiency (%)	65.1 (25.5-97.8)

AC: anticoagulant; BW: body weight.

Table III - Pre-apheresis and post-apheresis peripheral blood cell counts.

	Pre-apheresis*	**Post-apheresis***	**p****
Leucocytes ×10⁹/L	16.9 (3.95-42.5)	10.6 (3.2-31.0)	<0.0001
Mononuclear cells ×10⁹/L	4.3 (0.97-21.92)	2.8 (0.79-20.01)	<0.0001
Haemoglobin (g/L)	95 (67-126)	84 (63-126)	<0.0001
Haematocrit (%)	28 (21-36)	24 (20-36)	<0.0001
Platelets ×10⁹/L	99 (35-387)	50 (20-127)	<0.0001
CD34+cell ×10⁶/L	56 (5.6-351.2)	-	-

* Median (range); ** paired samples *t*-test.

Table IV - Pre-apheresis CD34+ cell count and LVL characteristics in poor and good mobilisers.

	Poor mobilisers CD34+ cell count <20×10⁶/L	**Good mobilisers CD34+ cell count ≥20×10⁶/L**	**Total**	**p****
Patients (n)	12 (24%)	38 (76%)	50	-
CD34+ cells ×10⁶/L (peripheral blood)*	17.5 (5.6-19.5)	82 (20.1-351.2)	56 (5.6-351.2)	<0.0001
Total number of aphereses (n)	16	38	54	-
Total blood volume processed (×)	6.0 (4.3-7.4)	5.1 (4.0-6.4)	5.4 (4.0-7.4)	0.279
Mononuclear cell collection efficiency (%)	67.9 (46.5-90.7)	61.0 (25.5-97.8)	65.1 (25.5-97.8)	0.338
CD34+ cells ×10⁶/kg BW (yield)*	4.1 (2.1-8.9)	14.8 (4.3-46.4)	9.7 (2.1-46.4)	<0.0001

* Median (range); ** independent samples *t*-test.

All LVL were well tolerated, and only one procedure had to be discontinued. Apheresis-related side effects were experienced during only five (9.2%) procedures. Mild symptoms of citrate-induced hypocalcaemia were observed in two children. Problems with the central venous catheter were observed in three children. Two children had problems with access blood flow caused by catheter occlusion, and required adjustment and reduction of inlet flow rate. One child had a catheter-related haemorrhage because the femoral vein was perforated during insertion of the catheter, which had to be replaced, and consequently required administration of blood products to manage bleeding. The platelet count decreased significantly ($p<0.001$) after each procedure: 99×10^9/L (35-387) *vs* 5 (20-127), but no bleeding was observed due to low platelet count and there was no need for transfusion support.

Discussion

Even though PBSC collection in paediatric patients is technically similar to that performed in adult patients, there are some issues that require special attention: adequate venous access, the relatively large blood volume in extracorporeal circulation, choice of anticoagulant, compliance of the patient which can be demanding as well as the availability of support services. A paediatric patient in an adult apheresis setting creates an additional challenge[11]. Our previous experience with LVL in adult patients, as well as results of other authors who performed LVL in paediatric patients encouraged us to process larger blood volumes in children[6-9].

The amount of transplanted CD34+ cells is the most important predictor for safe engraftment[12], and in children it is particularly important to optimise PBPC harvesting and to reduce the number of leukapheresis per patient. Processing larger volumes of blood in a single LVL may increase CD34+ cell yield, consequently reducing the number of procedures required and diminishing the total cost of collections[1,5]. Another rationale for using LVL is the narrow peak of CD34+ cells in the peripheral blood, present only for a short period after mobilisation, and therefore the optimal time for successful collection could be missed[13]. Several studies have confirmed that CD34+ cells are collected at a steady rate throughout LVL and that the relative composition of the harvested CD34+ cells does not change significantly[14-18]. Dubrovsky *et al*.[7] analysed the relationship between CD34+ cell collection efficiency and processed blood volumes, and concluded that the efficiency of CD34+ cell collection for paediatric autologous PBSC transplantation on the first day of harvest did not decrease with larger processed blood volumes. Their results indirectly indicated that bone marrow CD34+ cell mobilisation occurred with longer apheresis procedures in paediatric patients. In adult patients, higher CD34+ cell counts harvested by LVL was explained by steady recruitment of PBSC during leukapheresis[15,16,19,20]. In our previous study, three times more CD34+ cells were collected than were present in the blood before leukapheresis, and the recruitment factor for CD34+ cells was significantly higher in poor mobilisers than in good mobilisers, which points to the importance of LVL in patients who mobilise low numbers of CD34+ cells[20-24]. Few authors have dealt with recruitment of CD34+ cells in children[8,21,25]. Delgado *et al*.[25] showed that the recruitment of CD34+ cells in low weight children was significantly greater in the LVL group and that, apart from the well-known influence of the pre-apheresis CD34+ cell count, two other factors had a major impact on the CD34+ cell yield: patient's diagnosis and processed blood volume. Gorlin *et al*.[8] documented the usefulness of LVL in children of various ages and sizes, but pointed out that intra-apheresis recruitment of progenitor cells during LVL in paediatric patients was not as great as that observed in adults. It may be that the higher number of total blood volumes processed in small patients may both mask recruitment and lead to modest depletion of progenitors by the fourth hour of collection. Sevilla *et al*.[26] reported significantly higher recruitment of CD34+ cells in children anticoagulated with additional heparin than in children in whom only citrate was used.

The peripheral veins in small children cannot accommodate needles large enough to maintain the blood flow rates needed for apheresis, and central venous access is often required. Although the majority of children referred for PBSC collection already have long-term central venous catheters used for the administration of chemotherapy or other medications, these catheters are not suitable for apheresis because they are too soft and their lumen may collapse under negative pressure. For the access line in small children, some centres use an arterial catheter inserted in the radial artery, in combination with a central venous catheter, and provisional catheters in larger blood vessels or peripheral veins for the return line[27-29]. However, most centres use a double lumen dialysis catheter inserted into a femoral[9], subclavian or jugular vein[30,31], as we did in our patients. The central venous catheters in our institution were inserted by experienced paediatric anaesthesiologists or paediatric intensivists, usually the day before apheresis, and removed as soon as enough hematopoietic stem cells had been collected and frozen. Apheresis catheters carry a risk of causing vein occlusion, erosion or perforation of the vein and bleeding, while femoral catheters also carry a higher risk of infection[31]. Catheter-related haemorrhage occurred in one child, and although the catheters were inserted in the femoral vein in the majority of our children, no catheter-related infections were observed.

One of the main issues in low body weight children is their small total blood volume which requires special attention considering the blood volume in extracorporeal circulation, the volume of collected product, as well as the blood that remains in a separator set after collection. Since the extracorporeal volume of the apheresis set along with the blood warmer exceeds 10-15% of the total blood volume of a small child, most centres prime the separator with irradiated leucocyte-depleted packed red blood cells[31]. Blood priming diminishes an initial fluid volume deficit, but also carries all the risks involved in allogeneic blood transfusion. The extracorporeal blood returned to the patient at the end of run would be the equivalent of a 280 mL transfusion over 15 minutes into a small child[31]. If extracorporeal blood is rinsed into a transfer blood bag its volume can be reduced and it can be return in a slower reinfusion later[31]. Our practice is to prime the set with irradiated leucocyte-depleted compatible red blood cells and at the end of collection to rinse blood into a transfer blood bag which, after centrifugation, serves as an autologous red blood cell transfusion. In smaller patients, the volume of product collected may represent more than 20% of the patient's blood volume, and normovolaemia during apheresis is maintained by replacing the collected product with a continuous infusion of the same volume of 5% albumin via the return line at the same rate as the collection[31]. We did not observe any reaction due to blood volume imbalance in our patients.

Citrate in combination with heparin is the preferred anticoagulant for paediatric apheresis, although some authors performed LVL in low body weight children without heparin, using only citrate[32-34]. A standard ACD-A/whole blood flow rate (ACD/WBFR) ratio of 1:12 is not enough for low body weight children to achieve an adequate inlet flow rate[30]. Addition of heparin to ACD-A (6 IU/1 mL) and an increase of the ACD/whole blood flow rate ratio to 1:24 or 1:30 enable a reduction of the volume of citrate infused while maintaining relatively high inlet flow rates[30,31]. Heparin might be an additional risk factor for bleeding complications in thrombocytopenic patients with central venous catheters[35], development of heparin-induced thrombocytopenia (HIT) and associated thrombotic complications[36], but none of these complications was observed in our patients.

In our study the majority (76%) of patients were good mobilisers and successfully mobilised $\geq 20\times10^6$/L CD34+ cells, which is comparable with yields in other studies[27-29,37]. According to our results, as well as to those of Diaz *et al*.[38] and Kanold *et al*.[39], even in small body weight children, the pre-apheresis CD34+ cell count is still the best predictor of the outcome of PBSC collection in the LVL setting, although the volume of blood processed during LVL also affects the total yield[2,14,27,40-43]. The optimal target number of CD34+ cells ($\geq 5\times10$/kg BW) was collected with only one LVL procedures in 46 (92%) patients, while four (8%) patients needed one additional procedure. In our experience, LVL can be repeated daily providing children are haemodynamically stable[31].

The CD34+ cell yields in our patients were similar to those obtained in other studies, and were significantly higher in good mobilisers than in poor mobilisers ($p<0.001$)[27,29,37,44,45]. Using LVL in good mobilisers, there is a possibility of collecting more CD34+ cells than needed for transplantation, which can lead to long-term storage of unnecessary transplants.

LVL is an efficient procedure, but there still remains the question of safety[4,5]. The main objection to LVL is that the larger volume of infused anticoagulants can cause electrolyte imbalances such as hypocalcaemia, metabolic alkalosis, hypokalaemia and hypomagnesaemia along with a more pronounced decrease of platelets[9,46]. Thrombocytopenia can be avoided by using separation techniques in which the platelets are elutriated from collected mononuclear cells[47,48]. In agreement with previous reports, we did not observe an increase in the number of adverse events during LVL collection[46,49]. All our LVL procedures were well tolerated, and only one procedure had to be discontinued because of catheter-related haemorrhage caused by femoral vein perforation. Mild symptoms of citrate-induced hypocalcaemia were observed in two children, and two other children had problems with access blood flow caused by catheter occlusion, and required adjustment and reduction of inlet flow rate. The platelet count decreased significantly after each procedure, but no bleeding due to a low platelet count was observed and there was no need for transfusion support. Although LVL takes longer, children probably tolerate an extra hour of collection better than another procedure on consecutive days which would increase the total cost of treatment and expose them to risks of central venous line complications and additional leukapheresis procedures[23].

Symptoms of citrate toxicity are non-specific and can be difficult to detect in young children. Such toxicity can manifest with abdominal pain, pallor, sweating and nausea followed by tachycardia and hypotension[31]. Some authors reported that citrate toxicity is rare in children and that prophylactic calcium administration is seldom necessary[27,31]. Buchta *et al*.[50] showed that prophylactic calcium infusion during LVL reduced the incidence of citrate-related symptoms without affecting the technical performance or the number of CD34+ cells collected, as was confirmed by our results. In our institution, all patients received prophylactic infusion of calcium gluconate in order to prevent

symptoms of hypocalcaemia. We also encourage the intake of calcium-rich fluids and food during the procedure. Children were closely monitored, and if symptoms suggestive of hypocalcaemia were observed, ionised calcium levels were determined and calcium replacement adjusted. Mild symptoms of citrate-induced hypocalcaemia were observed in two patients. Therefore, in our experience LVL can be performed safely with appropriate monitoring, as has already been shown by other authors[27,51]. Warming the blood and solutions administered during apheresis reduces the risk of hypothermia and, theoretically, citrate toxicity[31]. In our centre a blood warmer is routinely used for paediatric apheresis, which may contribute to the observed low incidence of symptoms of hypocalcaemia.

Processing of the large blood volumes was accomplished by doubling the inlet flow rate and additional use of heparin, along with prolongation of the procedure to 5 hours. The LVL could possibly have been prolonged even more, but was limited to 5 hours for the children's comfort and tolerance, and consistent with the working hours of the apheresis unit, quality control and cell-processing laboratories. Time-consuming LVL raises an issue of patient's compliance, but previous studies showed that even children could cooperate with prolonged apheresis procedures[8,17,21,30]. Gorlin *et al*.[8] stated that longer periods of collection might be tolerated physiologically, but would challenge behavioural limits. In uncompliant small children, Ravagnani *et al*.[29] placed peripheral vascular accesses and performed leukapheresis at the same time under general anaesthesia. Although apheresis lasted for 5 hours, according to our experience LVL was well tolerated by both children and staff, and there was no need for sedation. We prefer that parents are present in the apheresis unit and encourage them to take care of children who then feel safer and calmer. Children are allowed normal oral intake during the procedure and they can be given milk and dairy products during apheresis. At the beginning of paediatric apheresis programme in our institution several patients were sedated before the procedure. Children received phenobarbitone 2 hours before the start of the procedure and they would usually fall asleep immediately after the start of the apheresis. This did not make the staff more relaxed; it only obstructed communications and put extra pressure on monitoring the child who was not able to show any discomfort. Our experience confirms that mild sedation has a role only in the management of selected paediatric patients with seizure activity or neuromuscular instability[31].

The results of our study are in favour of LVL but some drawbacks should also be mentioned. LVL is definitely time-consuming because processing five total blood volumes requires up to 5 hours. The use of LVL has implications for the working hours of the apheresis department as well as quality control and cell-processing laboratory. LVL may result in an excess of collected CD34+ cells and should not, therefore, be used in patients who mobilised a high number of CD34+ cells.

Conclusions

Our experience confirms that LVL is efficient and safe even in small children, if the procedure is adjusted considering the weight and age of the child. The most important factors are good venous access, adequate preparation of the child regarding electrolyte status, and surroundings in which the small child as well as parents feel comfortable, and can tolerate the procedure better. Although a median platelet loss of 50% can be expected, LVL is safe and reduces the overall number of apheresis procedures required. It can be recommended for PBSC collection even in children with a low body weight with malignant diseases, particularly those who mobilised low numbers of CD34+ cells.

Authorship contributions

IB and BGC designed the study. SM, LR, GJ, JS collected clinical and laboratory data. IB analysed data and prepared the manuscript. All Authors revised manuscript and approved the final version.

The Authors declare no conflicts of interest.

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