Large volume leukapheresis: efficacy and safety of processing patient's total blood volume six times

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Abstract

Large volume leukapheresis: Efficacy and safety of processing patient's total blood volume six times

Large-volume leukapheresis (LVL) differs from standard leukapheresis by increased blood flow and altered anticoagulation regimen. An open issue is to what degree a further increase in processed blood volume is reasonable in terms of higher yields and safety. In 30 LVL performed in patients with haematologic malignancies, 6 total blood volumes were processed. LVL resulted in a higher CD34+ cell yield, without change in graft quality. Although a marked platelet decrease can be expected, LVL is safe and can be recommended as the standard procedure for patients who mobilize low numbers of CD34+ cells and when high number of CD34+ cells are required.

1. Introduction

Peripheral blood has become widely accepted source of hematopoietic stem cells because of easier accessibility and faster engraftment [1]. Since rapid and sustained engraftment following high-dose therapy depends on the numbers of stem cells reinfused, efforts are directed towards harvesting sufficient numbers of CD34+ cells. Quality of the peripheral blood stem cell (PBSC) graft is affected by the effectiveness of mobilization of stem cells, proper timing of collection, and the efficiency of collection technique [2]. Although sufficient PBSCs may be obtained in a single leukapheresis, the majority of patients require repeated procedures. An alternative to repeated aphereses could be processing larger volumes of blood during one procedure. Large-volume leukapheresis (LVL) involves processing patient's total blood volume (TBV) at least three times during single procedure, and differs from standard leukapheresis by processing larger blood volume, increasing blood flow rate, use of additional anticoagulant heparin and longer duration of procedure [1-5]. Unfortunately, there is no standardized protocol for LVL and between centers there are differences regarding blood volume processed and duration of procedure. An open issue is to what degree a further increase in processed blood volume is reasonable in terms of higher cell yields and safety.

The purpose of this study was to investigate efficiency and safety of processing 6 TBVs. We prospectively analyzed the kinetics of peripheral blood cells and CD34+ cell yield during LVL, and intraapheresis recruitment of CD34+ cells. Finally, we evaluated whether the prolonged procedure and processing 6 instead of 4 TBVs altered the quality of leukapheresis product.

2. Patients and methods

2.1. Patients

Study was performed on group of 30 patients treated at University Hospital Centre Zagreb (table 1.). All patients were candidates for high-dose chemotherapy followed by autologous PBSC transplantation. PBSCs were mobilized by combination of disease specific chemotherapy protocols and 10 μg/kg/day s.c. granulocyte colony-stimulating factor (G-CSF) filgrastim (Neupogen, Roche, Switzerland). Study was approved by the local ethics committee, and written informed consent for the PBSC collection by LVL and additional testing was obtained from all patients.

2.2. Methods

2.2.1. PBSC collection

PBSCs were collected using a Cobe-Spectra cell separator (MNC program, software version 6.0) (Gambro BCT, Lakewood, CO, USA). Venous access was established using a dual lumen central venous catheter (Dualise-Cath, Vygon, France), placed in subclavian (27 patients), jugular (2 patients) or femoral vein (1 patient).

Leukapheresis started when the peripheral blood CD34+ cell count reached $10x10^6$/L. A minimum of $30x10^9$/L platelets was required before leukapheresis. Patients with preapheresis counts of $\geq 20x10^6$/L CD34+ cells were considered good mobilizers, while those with a CD34+ count $<20x10^6$/L were considered poor mobilizers. Twelve patients were poor mobilizers, and 18 were good mobilizers.

The target yield of CD34+ cells was $3.5x10^6$/kg of body weight (BW), except in patients with multiple myeloma who were scheduled for double transplantation with target yield of $7x10^6$/kg BW.

During LVL the total volume of processed blood equaled 6 patients' TBVs, calculated by the instrument, based on weight, height and gender. Inlet flow rate was set according to instrument calculations. Collection rate was 1.0 ml/min while the collected fraction was maintained under manual control at a hematocrit of approximately 1%.

A combination of solution citrate dextrose formula A (ACD-A, Baxter, Deerfield, IL, USA) and heparin (Heparin, Belupo, Croatia) were used as anticoagulants. The addition of 6 IU of heparin per 1 ml of ACD-A allowed us to enhance the ACD-A to whole blood ratio to 1:24. Therefore the inlet flow rate was doubled and in the same time twice the blood volume was processed.

In order to investigate the volume-dependent kinetics of CD34+ cell yield after each TBV processed, WBC collection set was modified. Collection bag was replaced with a six collection bag set which were connected to the WBC set collection line by sterile connection device. Modified set allowed removal of the collected cells every time one TBV had been processed and therefore product collected during processing each TBV could be analyzed.

When the optimal interface for MNC collection was established, the first collection bag was opened; bags were changed after processing 1x, 2x, 3x, 4x, 5x and 6x the patients' TBV. All aphereses were performed by the same operator and parameters were held constant throughout the procedure.

Patients were monitored for adverse reactions and recorded reactions were classified according to National Cancer Institute Common Toxicity Criteria (CTC-NCI) Classification, Version 2 [4]. In order to prevent symptoms of hypocalcemia all patients during LVL received 0.5 g calcium chloride/10 kg BW in 100 ml of saline solution.

2.2.2. Laboratory evaluation of peripheral blood and leukapheresis products

Peripheral blood samples taken preapheresis and after processing each TBV, and samples from each leukapheresis bag were analyzed for WBC, MNC, platelet and CD34+ cell counts. Afterwards, products collected in the first four bags were pooled into one bag, while products collected in the fifth and sixth bag were pooled into another bag. Samples were taken from each pooled bag and quality of products was compared with respect to CD34+ cell count. An intraindividual comparison of the collected CD34+ cells was done with respect to their differentiation- (CD38, CD90, HLA-DR) and lineage-associated markers (CD117, CD33, CD41). Numbers of colony-forming units: granulocyte macrophage (CFU-GM), burst forming units-erythroid (BFU-E) and mixed units (CFU-MIX) were determined in two pooled bags as well. Finally, products from both bags were pooled into one final bag and analysed for WBC, MNC, platelet and CD34+ cell count.

Cell yields were expressed as both the content of cells in a product and the cell yield per kg of patient's BW. Total yield was defined as the sum of the yields of 6 bags. Cumulative yield was defined as the sum of the yields collected during processing each TBV. CD34+ cell collection efficiency [6, 7] and recruitment factor [8-10] for the various cell populations were calculated as previously described.

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

CD34+ cells were analysed by flow cytometry using FACSCalibur (BD Biosciences, Heidelberg, Germany) following standard ISHAGE procedure for cell staining with anti-CD34-PE (clone 8G12) and anti-CD45-FITC (clone 2D1) monoclonal antibodies (BD Biosciences) [11]. CD34+ cell subsets were analyzed using monoclonal antibodies to CD38, HLA-DR, CD90, CD117, CD41 and CD33 (BD Biosciences).

Short-term culture assay for CFU-GM, BFU-E, CFU-MIX) was performed using MethoCult H4433 medium (StemCell Technologies, Vancouver, Canada) [12]. The number of colonies was evaluated after 14 days of incubation at 37°C in a humidified atmosphere with 5% CO_2.

Electrolytes calcium, potassium, phosphorus, and magnesium were tested in peripheral blood samples obtained pre- and postapheresis using Olympus AU 400 analyzer (Olympus Diagnostica, Tokyo, Japan).

2.2.3. Statistical analysis

Data were tested for normality using Kolmogorov Smirnov test. All distributions were normal, thus means and standard deviations as descriptors and parametric procedures were used for all analyses.

Repeated measures ANOVA was used to test changes in peripheral blood cells during LVL. If repeated measures ANOVA resulted in statistically significant F-ratio, we

used paired samples t-tests to test the differences between neighbouring points of measurement. In these cases we used Bonferonni's correction for multiple comparisons: to preserve the overall $p<0.05$ level of statistical significance, $p<0.008$ was considered statistically significant when there were 6 comparisons, and $p<0.007$ when there were 7 comparisons. Furthermore we used within-between subjects ANOVA to test the possible effects of diagnosis (multiple myeloma vs. lymphoma) or level of CD34+ cell mobilization (good vs. poor mobilizers) on changes in peripheral blood cells during LVL. A 2x2 between subjects ANOVA was used to test the effect of diagnosis and level of CD34+ cell mobilization on recruitment factor. Independent samples t-test was used to test the differences in CD34+ cell yield between patients with multiple myeloma and those with lymphoma, as well as between good and poor mobilizers. We used Spearman's rho coefficient to test the association between CD34+ cell total yield with preapheresis CD34+ cell count. The influence of processing 6 TBVs as opposed to standard processing of 4 TBVs on success of collecting necessary amount of CD34+ cells in patients with lymphoma was tested using McNemar's test. Differences in CD34+ cell subpopulations in product collected during processing the first 4 TBVs and product collected during processing the fifth and sixth TBVs were tested using paired samples t-test. Level of statistical significance was set at 0.05 for all analyses and they were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL).

3. Results

Thirty aphereses performed in 30 patients were analyzed. A mean volume of 26.97±5.48 l (range 17.83-37.29 l) was processed during 305.8±7.6 min (range 290-324 min) with a mean flow rate of 91.7±18.8 ml/min (60.2-131.9 ml/min). The patients received a mean volume of 1120.8±230.1 ml ACD-A (range 740-1510 ml) and 6725.2±1380.6 U of heparin (range 4440-9060 U). The mean volume of total apheresis products was 291.9±13.2 ml (range 255-335ml). After processing 6 TBVs the mean total CD34+ cell yield was 4.71±3.79 (range 0.91-16.90) $x10^8$/kg BW, and was significantly higher in good mobilizers than in poor mobilizers, (6.54±3.93 $x10^6$/kg vs. 1.98±0.79 $x10^6$/kg, *$p<0.001$).* There were no significant differences in CD34+ yield between the myeloma and lymphoma patients.

There was a strong correlation between the preapheresis peripheral blood CD34+ cell count and the total CD34+ cells yield *($\rho=0.860$, $p<0.001$)*. In both good and poor mobilizers, the total CD34+ yield correlated significantly with the preapheresis CD34 count *($\rho=0.83$ and $\rho=0.89$, respectively; $p<0.0001$).*

3.1. Kinetics of peripheral blood cells during LVL

The baseline hematological parameters and their changes during apheresis after processing each of the 6 TBVs are summarized in table 2. and figure 1. LVL significantly decreased the circulating WBC, MNC, and CD34+ cell counts *($p<0.001$).* Post-hoc analyses revealed that this effect was due to the significant difference in number of WBC, MNC and CD34+ cells between the baseline values and values after processing the first TBV *($p<0.001$).* We found no significant interaction between the change in peripheral

blood CD34+ cell count and diagnosis *(p=0.212)* or the level of mobilization of CD34+ cells *(p=0.62).* Platelet count decreased significantly during LVL through each of the seven points of measurement (*p<0,001).*

3.2. Kinetics of PBSC collection

PBSCs harvested during processing each TBV volume were collected in a separate bag, with mean volume of 48.6±5.1 ml. Yields of cells and CD34+ cell collection efficiency during LVL are summarized in table 3. and figure 2. Statistically significant change in yield of WBC, MNC, and CD34+ cells during LVL were found (*p<0.001).* Post-hoc analyses revealed that this effect was due to the significantly higher yield of all cells while processing the second TBV compared to the first TBV*(p<0.001).* Afterwards the mean yield of WBC, MNC and CD34+ cells remained stable while processing the remaining 5 TBVs. No significant interaction between the kinetic of CD34+ cell yield and diagnosis *(p=0.380)* or level of CD34+ cells mobilization were found *(p=0.886).* Although the total amount of harvested CD34+ cells was higher for good mobilizers, the kinetics of CD34+ cell collection during different stages of LVL were the same for all patients.

Cummulative yield of CD34+ cells increased continuously during LVL with each processed TBV *(p<0.001),* which confirmed that the number of CD34+ cells collected during apheresis is related to the volume of blood processed.

The average total CD34+ cell collection efficiency during LVL was 70.9±33.2%. CD34+ cell collection efficiency was lowest during the first TBV processing, but afterward remained stable. No significant interaction between the change in CD34+ cell

collection efficiency and diagnosis *(p=0.309)* or the level of CD34+ cell mobilization were found *(p=0.173)*.

3.3. Comparison of the total CD34+ cell yield collected during processing 4 TBVs vs. 6 TBVs

Processing 6 TBVs versus 4 TBVs resulted in significantly higher CD34+ cell yields; 4.71±3.79x10^6/kg vs. 3.28±2.89x10^6/kg, respectively *(p<0.001)*. If leukapheresis was stopped after processing 4 TBVs, the target CD34+ cell yield would have been achieved after only one procedure in 9 (30%) patients. However, when the procedure was prolonged to processing 6 TBVs, the target dose was successfully achieved in 20 (66.7%) patients *(p=0.001)*.

The results for the group of 20 myeloma patients were analyzed separately because their target dose was doubled. With processing 6 TBVs, satisfactory CD34+ counts for tandem transplantation was achieved in 6 (30%) patients, for single transplantation in 9 (45%) patients, while counts <3.5x10^6 CD34+/kg were collected in 5 (25%) patients. If leukapheresis was stopped after processing 4 TBVs, only one patient (5%) would have collected enough CD34+ cells for tandem transplantation and 6 (30%) for single transplantation

3.4. Quality of products collected during different stages of LVL

The intraindividual comparison of quality of cells collected while processing 4 TBVs and cells collected while processing the fifth and sixth TBVs are presented in table 4. The CD34+ cell subset analysis revealed no significant difference in the composition

of products collected during different stages of LVL. Likewise, the number of CFU-GM, BFU-E and CFU-MIX collected from processing 4 TBVs was not significantly different from the number of CFUs collected from processing the fifth and sixth TBVs.

3.5. Recruitment of cells during LVL

Total number of cells in peripheral blood preapheresis and postapheresis, total number of cells in the leukapheresis products, and recruitment factor for WBC, MNC, granulocytes, CD34+ cells and platelets are presented in table 5. The recruitment factor was calculated for each of these cells in order to find out whether cell-specific recruitment occurred during LVL. The highest recruitment factor (3.2±1.6) was found for CD34+ cells. Statistically significant difference in CD34+ cell recruitment factor was found between good (2.8±0.4) and poor mobilizers (4.1±0.5), respectively *(p=0.044).* There was no difference in recruitment factors concerning patients' diagnoses *(p=0.112).*

3.6. Side effects and electrolyte changes during LVL

All LVLs were well tolerated, and none of procedures had to be discontinued. Apheresis related adverse reactions were restricted to mild symptoms of citrate toxicity. Only one patient experienced mild perioral paresthesias, classified as grade 1. Although significant decreases in platelet count were observed after LVL, no bleeding episodes occurred, and there was no need for transfusion support.

All analysed electrolytes decreased significantly with respect to the basal values: calcium 5.18±4.10%, *(p=0.005),* potassium 11.75±7.46% *(p<0.001),* magnesium 11.98±5.44% *(p<0.001),* and phosphorus 17.22±16.06% *(p=0.024)*. Although calcium

concentration decreased significantly with respect to basal values, it still remained within referent values (2.38±0.40 mmol/L vs. 2.24±0.22mmol/L) [13]. Other analysed electrolytes decreased markedly below referent interval: potassium 3.92±0.35 mmol/L vs. 3.47±0.38 mmol/L, magnesium 0.71±0.08 vs. 0.65±0.09, and phosphorus 0.87±0.28 mmol/L vs. 0.73±0.2 mmol/L [13]. Nevertheless, none of our patients experienced clinically relevant side effects related to changes in these electrolytes.

4. Discussion

Leukapheresis has been successfully used in PBSC collection for over 20 years, however several topics still remain unresolved regarding the optimal method of collection and the volume of processed blood. Processing of larger blood volumes in single LVL may improve CD34+ cell yield, consequently reducing the number of required procedures and diminishing the total cost of collections [2, 4]. Another rationale for use of LVL is the narrow peak of the CD34+ cells in the peripheral blood, present only for a short period after mobilization, and therefore the optimal time for successful collection would not likely be missed [14].

Results of previous studies which compared standard vs. LVL processing varied considerably [2, 4, 10, 14-18]. It is difficult to establish a control group with comparable patients due to high interindividual variability regarding patients' characteristics, such as age, gender, TBV, stage of disease, previous treatment, and baseline blood count values. Even if the same patient is analyzed the following day, peripheral blood CD34+ cell count could halve or double overnight. The optimal study design would be to compare products collected during the same procedure at different time, which was done in this study, and differences caused by interindividual variability were excluded. Studies of kinetics of PBSC enrichment during LVL showed variable results [3, 8-10, 19-31], which could be consequence of difference in volumes of processed blood. Therefore, processed blood volume in our study was standardized and strictly related to the patient's TBV. Processing 6 TBVs was accomplished by doubling inlet flow rate and additional use of heparin, along with prolongation of procedure to 5 hours. Duration of LVL could have been prolonged even more, but was limited to 5 hours because of patients’ comfort and

tolerance, and consistence with working hours of apheresis unit, quality control and cell processing laboratories. Time-consuming LVL raises an issue of patient's compliance, but previous studies showed that cooperation for prolonged apheresis procedure could be achieved even in pediatric patients [17, 25, 26].

Strong correlation between the preapheresis CD34+ cell count and the total CD34+ cell yield was found, both in good and poor mobilizers. Accordingly to our results as well as other reports [5, 22, 27, 29], even in LVL setting, the preapheresis CD34+ cell count is still the best predictor of the outcome of PBSC collection, although the volume of blood processed during LVL also affects the total yield.

LVL is feasible only if the level of CD34+ cells in the blood is maintained throughout the procedure. Although a decline in CD34+ cell level was documented in all patients, our study showed that even after processing 6 TBVs there was no exhaustion of the peripheral blood CD34+ cells. Kinetic study showed that decrease in CD34+ count is related to the number of the TBVs processed. The drop in CD34+ cell count was most evident at the beginning of leukapheresis, as cells were packed into the apheresis device set [3, 26]. At the same time, separator draws patient's blood while returns saline which filled set lines, which can cause hemodilution and contribute to CD34+ cell decrease.

Our results confirm that CD34+ cells were collected at a steady rate throughout the LVL [9, 19, 26, 30]. The lowest CD34+ cell yield and collection efficiency were observed at the very beginning of LVL, which can be explained by the time required to establish interface, and by a dilution effect in the first collection bag, caused by the automated filling with saline [19, 29, 31]. In the remaining collection period, the CD34+ cell yield and collection efficiency were stable, without any time-dependent changes, as

observed by others [9, 10]. Furthermore, the MNC purity remained constant during LVL, confirming that enhanced blood volume processing doesn't affect the product quality in terms of higher granulocyte contamination during the final stages of apheresis [19]. It is important to emphasize that in spite of decrease of peripheral blood CD34+ cell count during LVL, the total cumulative CD34+ cell yield increased steadily after each blood volume processed. Kinetics of CD34+ cell collection were similar and stable regardless of diagnosis or level of CD34+ cell mobilization, therefore all patients could have benefit from LVL [32].

Use of modified sets enabled us to compare how many cells would be collected after processing standard 4 TBVs vs. 6 TBVs. CD34+ cell yield was higher when leukapheresis was extended, which could be particularly beneficial in poor mobilizers. Our results confirmed that likelihood for collecting target number of CD34+ cells was greater with use of LVL than with standard leukapheresis [4, 28].

Current protocols for myeloma patients require tandem transplantation [33, 34], and it is common practice to collect sufficient cells for two grafts. That is sometimes challenging in heavily pretreated patients, particularly those who previously received lenalidomide [4, 35]. Moreover, some data demonstrated different mobilization of PBSCs and myeloma cells, with peak levels of myeloma cells occurring few days after CD34+ cells [36-38]. Since LVL allows collection of more CD34+ cells, fewer days are required to obtain target cell dose, and use of LVL could result in lower tumor contamination of graft [36-38]. Zubair et al. [33] estimated that using LVL in myeloma patients, the cost savings could be greater than $7,597 per patient.

Leukapheresis product consists of hematopoietic stem and progenitor cells in different stages of differentiation [39-41]. Previous studies, [39, 41, 42] as well as ours, reported that the majority of collected CD34+ cell coexpressed CD38, HLA-DR or CD33 associated with commitment to different hematopoietic lineages. Our study first analysed CD34+ cell subpopulations in product collected while processing as many as 6 TBVs, and showed no difference in the composition of products collected during different stage of LVL. This finding argues against a preferential release of particular CD34+ cell subsets during the LVL [27]. Our data confirm that LVL results in a higher CD34+ cell yield without change in graft quality.

Higher CD34+ cell yield harvested by processing a larger volume of blood was explained with a steady recruitment of PBSCs during leukapheresis [8, 9, 18, 19]. In our study, threefold more CD34+ cells were collected than were present in blood before leukapheresis. The recruitment was limited to MNC and CD34+ cells, which were predominantly removed during the procedure, which was also observed by other authors [8, 23, 24, 43]. Interestingly, recruitment factor for CD34+ cells in our study was significantly higher in poor mobilizers than in good mobilizers, which points to the importance of LVL in patients who mobilize low number of CD34+ cells [17]. The underlying mechanisms of PBSCs recruitment during LVL are not clear, and few hypotheses have been proposed. Some authors explained recruitment of PBSCs merely by the stimulating effect of G-CSF administration [44], while others suggested that it was mediated by a negative feedback mechanism caused by the decline of the peripheral blood CD34+ cell count during LVL [8, 10]. Another possible mechanism is modification

of PBSCs homing and transmigration caused by changes in their microenvironment, induced by use of heparin [15, 45, 46] and citrate-induced hypocalcemia [27, 47].

Results of our study are in favour of LVL but some drawbacks should also be mentioned. LVL is definitely time-consuming because processing 6 TBVs requires 5 hours. Implementation of LVL has implications on working hours of apheresis department as well as quality control and cell processing laboratory. Timely completion of LVL requires high blood flow rates. In some studies it wasn't possible to establish the required inlet flow rate, and the procedures were shortened because of catheter related problems [10, 18]. We didn't observe any problem related to high inlet flow rate, but all our patients had apheresis catheter which we recommend for LVL. LVL may result in an excess of collected CD34+ cells, therefore it shouldn’t be used in patients who mobilized high number of CD34+ cells.

A limiting factor for widespread use of LVL may be patients’ tolerance of the procedure [2, 3]. Larger volumes of infused anticoagulants causes electrolyte imbalance such as hypocalcemia, metabolic alkalosis, hypokalemia and hypomagnesemia [48]. In agreement with previous reports [33, 48], we did not observe increase in number of adverse events during LVL collection. Buchta et al. [49] 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. Our study also confirmed marked platelet decrease after processing each TBV with halved platelet count after LVL [3, 48]. The additional heparin administration enables the reduction of infused volume of citrate anticoagulant, but might represent an additional risk factor for bleeding complications in thrombocytopenic

patients with central venous catheters [50]. Heparin-induced thrombocytopenia (HIT) and associated thrombotic complications could develop in a newly exposed patient, or rapid-onset complications might occur in a patient with a recent prior history o HIT [51]. Processing up to 6 TBVs according to our results could be performed safely because no severe adverse reactions occurred, including bleeding complications. Although it lasts longer, patients will probably tolerate an extra hour of collection easier 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 leukaphereses [24].

LVL could be strongly recommend for patients who mobilized low number of CD34+ cells, and patients who need high dose of CD34+ cells, including double transplantation or *in vitro* processing of leukapheresis product where a significant loss of cells is expected. If role of LVL is considered in setting of new mobilizing agents, such as plerixafor used in case of previously unsuccessful mobilization [52, 53], there is no doubt that LVL should be performed to collect maximum PBSCs in one procedure. From the data presented, we conclude that processing of 6 TBVs during LVL is efficient and safe technique, which significantly reduces the number of aphereses needed to obtain target number of CD34+ cells. Whether or not further increase of the processed blood volume could improve the quality of graft must be evaluated in forthcoming studies.

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Tables

Table 1. Patients' characteristics

Gender (male / female)	16 / 14
Age (years)*	50.1±10.9 (19-61)
Body weight (kg)*	78.3±15.4 (57–108)
Total blood volume (ml)*	4618.6±140.5 (3463–6761)
Diagnosis (N)	
Multiple myeloma	20
Non-Hodgkin's lymphoma	8
Hodgkin's disease	2
Mobilization (N)	
cyclophosphamide (4 g/m^2)	20
HDIM	7
ICE	1
mini BEAM	1
DHAP	1

**mean±SD (range); HDIM=high-dose ifosfamide, mitoxantrone; ICE=ifosfamide, carboplatin, etoposide; mini BEAM=carmustine, etoposide, cytarabine, melphalan; DHAP=dexamethasone, cytarabine, cisplatin*

Table 2. Kinetics of WBC, MNC, CD34+ cells and platelets in the peripheral blood during LVL (mean ± SD). Levels of statistical significance were calculated comparing the results after each processed total blood volume (TBV) with the following

	Peripheral blood cells							
Processed TBV	WBC x 10^9/L	WBC decrease from baseline %	MNC x10^9/L	MNC decrease from baseline %	CD34+ x10^6/L	CD34+ cell decrease from baseline %	Platelet x 10^9/L	Platelets decrease from baseline %
Baseline	14.39 ±7.68	100	2.02± 0.81	100	48.35 ±44.80	100	99.75 ±40.17	100
*p**	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
1x	12.10 ±6.91	83.20 ± 10.74	1.38 ±0.57	69.27 ± 12.69	27.86 ±23.19	63.46 ±20.38	79.06 ±33.55	79.26 ±9.06
*p**	*NS*	*NS*	*p=0.004*	*NS*	*NS*	*NS*	*<0.001*	*<0.001*
2x	11.83 ±6.69	82.48 ± 12.64	1.26 ±0.51	64.23 ± 14.71	26.71 ±25.29	58.17 ±18.01	71.75 ±32.69	72.24 ±12.73
*p**	*NS*	*NS*	*0.005*	*0.007*	*NS*	*NS*	*<0.001*	*<0.001*
3x	11.64 ±6.57	80.07 ± 10.20	1.15 ±0.41	58.58 ± 10.37	23.42 ±23.00	51.20 ±17.02	63.41 ±25.46	64.55 ±11.23
*p**	*NS*	*NS*	*0.006*	*0.003*	*NS*	*NS*	*<0.001*	*<0.001*
4x	11.41 ±6.59	78.01 ± 12.39	1.05 ±0.38	53.98 ± 11.91	21.02 ±19.73	47.72 ±21.27	58.75 ±25.41	59.88 ±13.61
*p**	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*<0.001*	*<0.001*
5x	11.16 ±6.47	76.67 ± 14.50	1.01 ±0.35	52.54 ± 13.01	19.63 ±16.13	46.47 ±19.08	55.20 ±25.14	56.65 ±16.21
*p**	NS	NS	NS	NS	NS	NS	*<0.001*	*<0.001*
6x	11.09 ±6.53	76.01 ± 14.56	12.14 ±8.46	50.35 ± 16.21	17.61 ±14.05	45.95 ±18.64	49.44 ±21.46	51.65 ±15.89
$F_{6,156}$	***19.168***	***46.828***	***42.029***	***138.430***	***15.368***	***61.054***	***62.312***	***130.902***
p**	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***

p* *paired samples t-tests;* ***p***** *repeated measures ANOVA*

Table 3. Yields of WBC, MNC and CD34+ cells collected in separate bags and CD34+ cell collection efficiencies (CE) during each total blood volume processed (mean±SD). Levels of statistical significance were calculated comparing the results after each processed total blood volume (TBV) with the following

	Yield						
Processed TBV	**WBC $x10^9$/L**	**WBC $x10^8$/kg**	**MNC $x10^9$/L**	**MNC $x10^8$/kg**	**CD34+ $x10^6$/L**	**CD34+ $x10^6$/kg**	**CD34+ cell CE (%)**
1x	149.7 ±61.4	0.87 ±0.43	80.3 ±39.5	0.45 ±0.23	12.6 ±10.7	0.71 ±0.61	40.6 ±18.7
p*	*NS*	*p<0.001*	*p<0.001*	*p<0.001*	*NS*	*p=0.004*	*p<0.001*
2x	162.1 ±57.8	1.02 ±0.41	88.4 ±36.6	0.56 ±0.22	14.3 ±14.2	0.87 ±0.73	68.6 ±25.3
p*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
3x	150.9 ±68.3	0.97 ±0.36	82.2 ±33.5	0.53 ±0.19	14.5 ±16.2	0.87 ±0.84	74.4 ±32.6
p*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
4x	152.6 ±48.6	0.96 ±0.32	80.5 ±29.8	0.50 ±0.19	13.4 ±14.1	0.82 ±0.82	73.8 ±29.2
p*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
5x	156.7 ±46.3	0.98 ±0.28	81.6 ±32.1	0.48 ±0.18	13.6 ±10.1	0.80 ±0.60	74.4 ±24.2
p*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
6	159.1 ±46.0	1.01 ±0.34	79.6 ±27.5	0.49 ±0.19	12.9 ±9.1	0.82 ±0.51	79.5 ±28.3
$F_{5,125}$	***1.489***	***3.187***	***3.979***	***4.808***	***0.242***	***3.082***	***9.927***
p*	***0.198***	***0.041***	***0.002***	***<0.001***	***0.943***	***0.012***	***<0.001***
Total product	**149.7 ±61.4**	**5.71 ±1.97**	**80.3 ±39.5**	**3.32 ±3.86**	**126.2 ±107.2**	**4.71 ±3.79**	**70.9 ±33.2**

p* *paired samples t-test;* ***p**** *repeated measures ANOVA*

Table 4. Comparison of quality of leukapheresis products collected during different stage of LVL: subset analysis of CD34+ cells and numbers of CFU-GM, BFU-E and CFU-MIX colonies /10^5 cells after short-term culture

Subsets of CD34+ cells	**TBV processed 1.-4. TBV**	**TBV processed 5th and 6th times**	***p****
CD38+ (% CD34+ cells)	98.90±2.33	99.07±1.67	*0.669*
CD34+CD38+ (x10^8/L)	12.74±10.47	12.13±11.09	*0.238*
HLA-DR+ (% CD34+cells)	98.67±1.84	99.02±1.30	*0.100*
CD34+HLA-DR+ (x10^8/L)	11.90±10.29	11.30±10.45	*0.147*
CD90+ (% CD34+ cells)	46.69±19.77	47.72±20.82	*0.320*
CD34+CD90+ (x10^8/L)	6.80±6.7	6.76±6.3	*0.884*
CD117+ (% CD34+ cells)	94.15±4.75	94.48±4.64	*0.529*
CD34+CD117+ (x10^8/L)	11.39±9.12	11.12±9.19	*0.349*
CD41+ (% CD34+ cells)	21.97±19.28	19.74±20.09	*0.080*
CD34+CD41+ (x10^8/L)	2.34±2.20	2.21±2.16	*0.508*
CD33+ (% CD34+ cells)	89.10±13.49	89.52±12.33	*0.416*
CD34+CD33+ (x10^8/L)	10.91±8.44	10.68±8.55	*0.483*
Number of colonies /10^5 cells			
CFU-GM	77.63±31.88	73.65±24.27	*0.344*
BFU-E	47.19±27.72	48.94±25.23	*0.558*
CFU-MIX	16.28±7.57	15±5.31	*0.301*

p* *paired samples t-tests*

Table 5. Preapheresis and postapheresis total number of cells in peripheral blood, total number of collected cells in the leukapheresis products and recruitment factor

Parameters	**Preapheresis**	**Postapheresis**	**Total number of harvested cells**	**Recruitment factor***
WBC ($x10^8$)	676.70±416.73	527.36±368.78	439.69±156.81	**1.64**±0.54
MNC ($x10^8$)	96.32±45.07	46.56±20.49	205.47±103.26	**2.72**±0.69
Granulocyte ($x10^8$)	580.37±385.53	480.79±355.94	234.21±137.95	**1.42**±0.51
CD34+ cells ($x10^6$)	226.80±204.88	83.37±68.77	540.12±562.95	**3.23**±1.61
Platelets ($x10^9$)	469.47±210.93	227.70±87.77	313.72±183.12	**1.17**±0.20

***Recruitment factor** = absolute number of cells in blood postapheresis + absolute number of cells collected/ absolute number of cells in blood preapheresis*

Figures

Figure 1. Decrease of WBC, MNC, CD34+ cells and platelets during LVL expressed as a mean percentage from baseline values

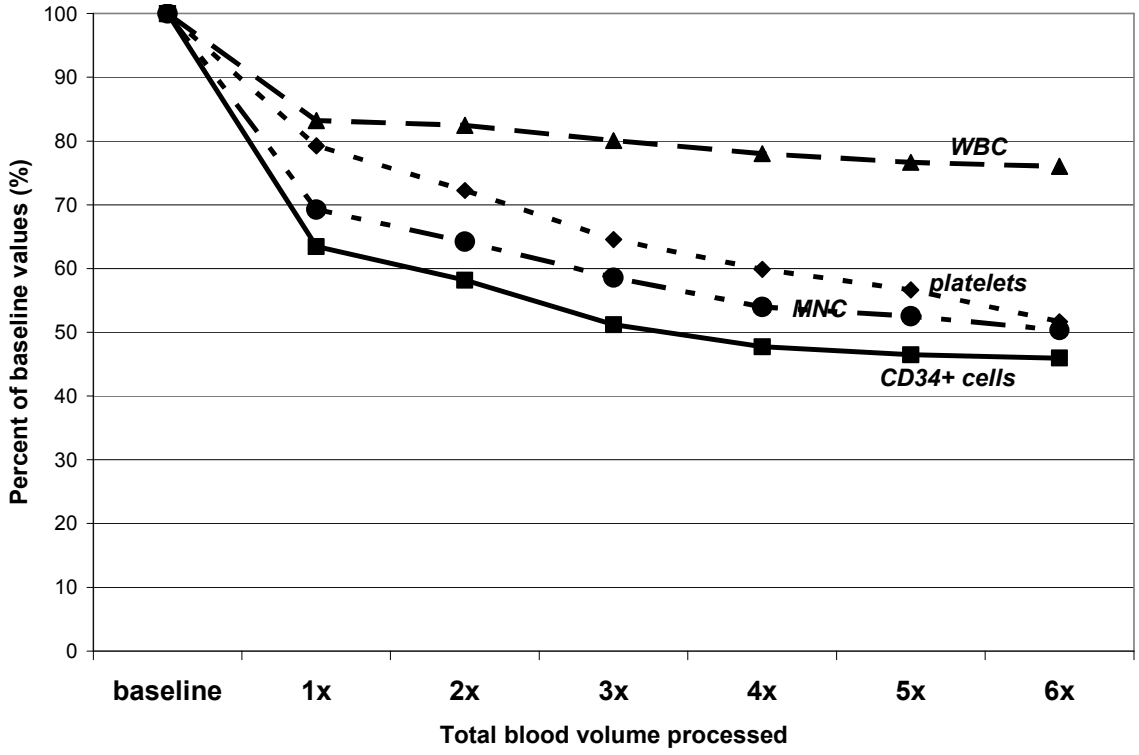

Figure 2. Yields of WBC, MNC and CD34+ cells / kg BW collected after each blood volume processed during large volume leukapheresis expressed as a mean