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# Post-thaw Viability of Cryopreserved Hematopoietic Progenitor Cell Grafts: Does It Matter?

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## ABSTRACT

Cell viability in peripheral blood progenitor cell (PBPC) grafts and its influence on the clinical course following transplantation was evaluated in 81 consecutive transplantations (72 autologous, 9 allogeneic) performed in patients with hematological diseases. Viability of cells in PBPC grafts immediately upon collection was 98.6±3.5%, after addition of dimethyl sulfoxide (DMSO) 73.3±21.8%, and post-thaw 65.2±16.1%. It did not differ significantly between patients with different diagnoses, gender, age, type of priming used, dose of G-CSF administered or number of CD34+ cells collected. However, grafts stored for more than 60 days showed lower post-thaw viability compared to the ones thawed in the 60 days following cryopreservation (56.6±15.2% vs. 67.6±15.5%,  $p=0.04$ ). Post-thaw graft viability did not influence engraftment time, but there was a predisposition towards infectious complications in the post-transplant period in patients receiving grafts with lower percentage of viable cells. They developed febrile neutropenia more often (72.2% vs. 50% of patients,  $p=0.05$ ) and had more febrile days (2.4±2.6 vs. 1.5±2.3,  $p=0.05$ ) following transplantation. We have demonstrated that PBPC grafts are capable of long term engraftment regardless of the graft storage time or percentage of viable cells post-thaw, which confirms the robustness of CD34+ cells during the freeze/thaw procedures carried out in daily clinical practice. Granulocyte concentration in PBPC grafts could have an influence on infectious complications following transplantation and needs to be further investigated on a larger number of patients.

**Key words:** hematopoietic progenitor cells, cryopreservation, viability

## Introduction

Over the last two decades, the clinical applications of hematopoietic stem cell transplantation have considerably increased<sup>1</sup>. Peripheral blood has become a preferred source of hematopoietic progenitor cells (HPCs) and has replaced bone marrow for virtually all autologous transplants and for most allogeneic transplants. Advantages include more rapid engraftment, reduced morbidity and duration of hospital stay<sup>2,3</sup>. Both fresh and cryopreserved peripheral blood progenitor cells (PBPCs) are used for transplantation. Cryopreserved cells, a standard in autologous setting, are being used more often in the allogeneic setting as well<sup>4,5</sup>. However, the freezing procedure can be harmful to HPCs and can cause their injury by direct effects of low temperature and by the formation of ice crystals<sup>6</sup>. Therefore, a cryoprotectant is needed to protect the integrity, viability and function of the hema-

topoietic cells during the procedure. Dimethyl sulfoxide (DMSO) is the most widely used cryoprotectant; it can penetrate cells, reducing the proportion of water incorporated into ice and the extent of cellular dehydration<sup>7,8</sup>. In spite of all precautions, it is well known that addition of DMSO, together with cryopreservation and thawing change the biological characteristics of cells, resulting in variable loss of cell viability and a reduction in the absolute number of viable CD34+ cells available for transplantation<sup>9–13</sup>.

The absolute mononuclear cell count was initially used to predict the engraftment potential of bone marrow<sup>14</sup> until the CD34+ surface antigen, expressed on early progenitor cells, present on about 1–4% of the mononuclear cells in the marrow and less than 0.1% in

peripheral blood, was found to correlate with colony-forming capability and engrafting potential of the peripheral blood HPCs<sup>15–17</sup>. Recommendation for HPCs transplant today relies on the CD34+ cell dose, and it is generally accepted that the minimal acceptable dose for successful transplantation is  $2 \times 10^6$  CD34+ cells *per* kg of recipient weight<sup>3,18</sup>, and  $3 \times 10^6$  CD34+ cells *per* kg of recipient weight for allogeneic transplantation<sup>19</sup>. Several studies have demonstrated a correlation between the number of CD34+ cells infused and predicted engraftment<sup>13,20–21</sup>. However, the vast majority of studies rely on pre-cryopreserved CD34+ cell dose, while still little is known about the quality of the post-thaw product and its impact on the course of transplantation, since not nearly as many studies have addressed these questions. A few studies have shown that the number of viable post-thaw CD34+ cells infused predicts engraftment in autologous hematopoietic stem cell recipients<sup>13,23</sup>. One study has demonstrated that viable CD34+ stem cell content of a cord blood graft assessed before transplantation is most representative of hematologic recovery<sup>24</sup>.

Prior to transplantation, cell viability of post-thaw HPCs grafts is often less than 70% which raises concerns regarding the accuracy of the cell dose and the quality of the graft that is administered<sup>25</sup>. However, viability of the total nucleated cell in post-thaw HPCs grafts does not accurately represent the survival of the CD34+ cells and no apparent loss of progenitor activity was demonstrated in most studies<sup>26–28</sup>. Although it has been reported that stem cells retain viability and engraftment capacity even after extended storage in liquid nitrogen<sup>29,30</sup>, some studies have demonstrated that prolonged graft storage can lead to decreased total cell viability in post-thaw HPCs products<sup>31</sup>.

The primary aim of this study was to investigate cell viability in grafts used for autologous and allogeneic peripheral blood progenitor cell transplantation in patients with hematological diseases before cryopreservation and after thawing, and to evaluate the impact of cell viability on patients' clinical course following transplantation.

Secondary aims of the study were to evaluate PBPC mobilization efficacy in this group of patients and to correlate the CD34+ cell dose infused with hematopoietic recovery following transplantation.

## Material and Methods

### *Mobilization and collection*

Peripheral blood progenitor cell grafts used for 81 consecutive transplantations from January 2007 to June 2009 in Department of Medicine, University Hospital »Merkur« were included in this study. Nine PBPCs used for allogeneic transplantation were obtained from related donors, and the remaining 72 were collected for autologous transplantations from patients with different hematological diseases. Characteristics of patients included in this study are summarized in Table 1. Autologous PBPCs were mobilized with chemotherapy and

granulocyte colony stimulating factor (G-CSF). All myeloma patients received cyclophosphamide 3 g/m<sup>2</sup> as priming. Out of 45 lymphoma patients, 37 received mini-BEAM (carmustin 60 mg/m<sup>2</sup> on day 1, ara-c 2×100 mg/m<sup>2</sup> from day 2 to 5, etoposide 75 mg/m<sup>2</sup> from day 2 to 5, melphalan 30 mg/m<sup>2</sup> on day 6), while 8 patients received other chemotherapeutic regimen. The majority of patients with acute leukemia (8/13) received high dose ara-c 2×1g/m<sup>2</sup> day 1 to 6 and mitoxantron 10 mg/m<sup>2</sup> from day 5 to 7. Lymphoma and myeloma patients were discharged after chemotherapy, only to be readmitted 9 days later. G-CSF was administered at 10 mcg *per* kg of body weight *per* day, starting either on the day when WBC counts decreased to levels  $< 1 \times 10^9/L$ , or, if this did not occur, one day after WBC count nadir. G-CSF was continued until leukapheresis, which was initiated when the blood CD34+ count was at least  $10 \times 10^6/L$ . PBPCs from related donors were in all cases mobilized with G-CSF (10 mcg *per* kg of body weight × 5 days). Cell collection procedures were performed with a continuous flow blood cell separator (COBE Spectra, Lakewood, USA). All cell collection procedures were performed with program version 6.1.

**TABLE 1**  
PATIENTS' CHARACTERISTICS

Total number		81
Gender		
Male		40
Female		41
Age	Median	47.5
	Range	19–71
Diagnosis		
Non Hodgkin's Lymphoma		35
Multiple Myeloma		20
Hodgkin's Disease		10
Acute Myeloid Leukemia		7
Acute Lymphoblastic Leukemia		6
Aplastic Anemia		2
Prolymphocytic Leukemia		1
Previous Treatment		
Lines of chemotherapy	Median	2
	Range	1–7
Cycles of chemotherapy	Median	7
	Range	1–17
Type of Transplantation		
Autologous		72
Allogeneic		9
CD34+ cells ( $\times 10^6/kg$ ) infused	Mean	12.7
	Range	1.6–84.2
Mononuclear cells ( $\times 10^8/kg$ ) infused	Mean	6.9
	Range	1.1–20.7

### *CD34+ enumeration*

Absolute numbers of CD34+ cells were enumerated immediately following harvest. Analysis of CD34+ cells was performed on flow cytometer (Coulter Epics XL, Fullerton, USA) according to the manufacturer's instructions with the commercially available CD34 antibody (BD Bioscience, San Jose, USA).

### *Cryopreservation and thawing*

Hematopoietic progenitor cells were cryopreserved at a concentration of  $2\text{--}3 \times 10^8$  total nucleated cell *per* milliliter to  $-160^\circ\text{C}$  by a controlled rate freezer (Planer Kryo 560–16, Sunbery on Thames, UK) in a final concentration of 10% dimethyl sulfoxide (DMSO) (WAK Chemie Medical GmbH, Steinbach, Germany) and 4.5% human serum albumin. The controlled-rate freezing started at  $12^\circ\text{C}$ . In the first step of the run, the chamber was cooled to  $+4^\circ\text{C}$  at a rate of  $4^\circ\text{C}$  *per* minute; This temperature was held for 5 minutes. This was followed by a cooling-rate of  $2^\circ\text{C}$  *per* minute down to  $-5^\circ\text{C}$ . Further cooling was performed with  $1^\circ\text{C}$  *per* min down to  $-40^\circ\text{C}$ , and the procedure was completed by a final cooling rate of  $5^\circ\text{C}$  *per* min down to a temperature of  $-160^\circ\text{C}$ . After the cryopreservation procedure, grafts were stored in liquid nitrogen at  $-196^\circ\text{C}$ . On the day of transplantation they were thawed in a water bath at  $37^\circ\text{C}$  for 1–3 min. A sample of 1 ml was taken for viability analysis just before transplantation. No additional washing was performed and all testing was performed on samples from the complete products.

### *Viability measurement*

The viability assay was performed using trypan blue dye exclusion test. Non viable cells, due to damage to the integrity of their membrane, are stained blue by the dye and can be identified under a light microscope. Trypan blue 0.1% solution (Sigma, St. Louis, USA) was diluted with an equal volume of cells. At least 200 cells were microscopically analyzed in duplicate for viability in a hemocytometer chamber. Analysis was performed immediately with a single observer for all samples. Viability was calculated as the mean percentage of viable cells of the two analyses. Cell viability in PBPC grafts was assessed at 3 timepoints: 1. immediately after cell collection, 2. before cryopreservation, after addition of DMSO, and 3. immediately after thawing.

### *Conditioning, engraftment and supportive care*

Patients received high-dose chemotherapy appropriate for the disease prior to PBPC infusion. Conditioning regimen included BEAM (carmustin  $300\text{ mg/m}^2$  on day  $-6$ , ara-c  $2 \times 200\text{ mg/m}^2$  from day  $-5$  to  $-2$ , etoposide  $200\text{ mg/m}^2$  from day  $-5$  to  $-2$ , melphalan  $140\text{ mg/m}^2$  on day  $-1$ ), high dose melphalan ( $100\text{ mg/m}^2$  from day  $-4$  to  $-3$ ), BuCy (busulfan  $4\text{ mg/kg}$  from day  $-8$  to  $-5$  and cyclophosphamide  $60\text{ mg/kg}$  from day  $-4$  to  $-3$ ), Flu-BU-ATG (fludarabine  $30\text{ mg/m}^2$  day  $-10$  to  $-5$ , busulfan  $4\text{ mg/kg}$  day  $-6$  to  $-5$ , ATG day  $-4$  to  $-1$ ). Peripheral blood progenitor cell grafts were infused into patients through central

venous catheters in 30–45 min. Routine peripheral blood cell counts were performed daily using an automated hematology analyzer (Sysmex XE-2100, Kobe, Japan). Two independent parameters were used to determine the time to engraftment post-transplant. These included the first day out of 2 sequential days post-transplant in which an absolute neutrophil count (ANC) of  $1 \times 10^9/\text{L}$  was achieved, and the first day out of 2 in which  $20 \times 10^9/\text{L}$  platelets were achieved without transfusion. All patients, except patients with acute myeloid leukemia in the autologous setting, received G-CSF  $10\text{ mcg/kg/day}$  during neutropenia following transplantation. Ciprofloxacin  $2 \times 250\text{ mg}$  and fluconazole  $200\text{ mg}$  were given orally as anti-infective prophylaxis. Body temperature was monitored every 2h; in case of fever  $\geq 38.5^\circ\text{C}$  or  $\geq 38^\circ\text{C}$  in two consecutive measurements microbiological work-up was done and empirical antimicrobial treatment was started. In the absence of other clinical reasons for patients' transfusion support, two units of packed red cells were transfused in all cases when the hemoglobin was less than  $75\text{ g/L}$  and 8 units of random donor platelets if platelets were less than  $5 \times 10^9/\text{L}$ .

### *Statistical analysis*

Analysis included descriptive statistics, with data expressed as mean  $\pm$  standard deviation, unless otherwise indicated. Standard regression analysis was used to determine possible correlations between variables. Chi square, t-test and the nonparametric Mann-Whitney and Kruskal-Wallis tests were used to assess for statistical differences between two or more groups. A p value less than 0.05 was considered statistically significant. All statistical analyses were performed with StatView™ statistical program, version 5.0.1. (SAS Institute, Cary, NC, USA).

## **Results**

Sufficient number of PBPCs for one transplantation ( $>2 \times 10^6$  and  $>4 \times 10^6$  CD34+ cells *per* kg of patient's BW for autologous and allogeneic transplantation, respectively) was collected during a single apheresis in 56/72 patients and in 2/9 related donors. In 12 patients and 6 donors, 2 aphereses were needed and in 4 patients and 1 donor, 3 aphereses were necessary to achieve this goal. HPCs mobilization was suboptimal in 2 patients with multiple myeloma:  $1.64$  and  $1.95 \times 10^6$  CD34+ cells *per* kg of BW were collected. Although target CD34+ cell dose was not collected in these patients, clinical decision was to use these grafts for transplantation.

As part of the PBPC collection procedure for autologous transplantation, G-CSF was administered for a median of 7.5 days (mean  $7.6 \pm 1.4$ , range 4–11) at a median dose of  $4350\text{ mcg}$  (mean  $4575 \pm 990$ , range 2400–9000). PBPCs collection was most successful in patients with lymphoma, which is why they received a higher CD34+ cell dose following high dose chemotherapy conditioning than patients with multiple myeloma or acute leukemia ( $15.9 \pm 14.8$  vs.  $8.6 \pm 5.9$  vs.  $8.8 \pm 9.1$ , respectively. Kruskal-

-Wallis,  $p=0.03$ ). Mean numbers of CD34+ and mononuclear cells infused for all patients were  $12.7 \pm 12.6 \times 10^6$  and  $6.9 \pm 4.6 \times 10^8$  per kg of body weight, respectively (Table 1). There was no correlation between the number of CD34+ cells collected and age, gender or number of lines/cycles of previous therapy received.

A strong, statistically significant correlation was found by simple regression between pre-cryopreserved CD34+ cell dose and hematopoietic recovery in patients, expressed as time to reach  $ANC > 1 \times 10^9/L$  and platelets  $> 20 \times 10^9/L$  ( $R^2=0.14$   $p=0.001$ ,  $R^2=0.12$   $p=0.005$ , respectively). This was confirmed when patients were divided in two groups according to pre-cryopreserved CD34+ cell dose, the cutoff point being the median value of  $9.4 \times 10^6$  per kg of body weight (Mann Whitney, ANC recovery  $p < 0.0001$ , platelet recovery  $p=0.0005$ ). Occurrence of febrile neutropenia and total number of febrile days did not differ significantly between patients depending on the dose of CD34+ cells infused.

Viability of the cells in PBPC grafts was found to be different at the three predetermined timepoints (Figure 1). Immediately upon collection, mean cell viability was  $98.6 \pm 3.5\%$ , after addition of DMSO  $73.3 \pm 21.8$ , and post-thaw  $65.2 \pm 16.1\%$ . Mean graft storage time was  $105.9 \pm 246$  days with a median of 40.5 days.

Post-thaw viability of the grafts did not differ significantly between patients with different diagnoses, gender, type of priming used or dose of G-CSF administered. Also, no correlation was found between post-thaw viability and patients age or number of collected CD34+ cells. However, graft storage time was found to influence post-thaw viability of HPCs grafts (Figure 2); grafts stored

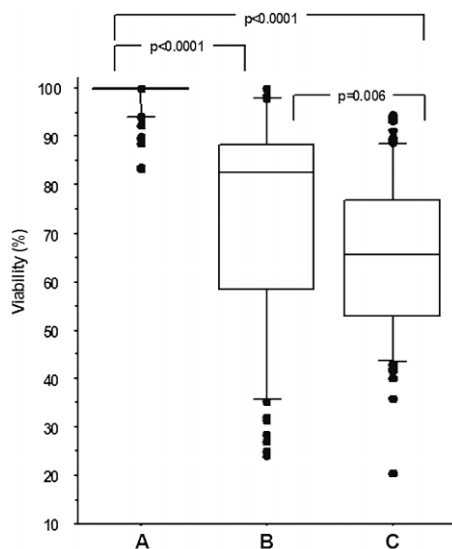


Fig. 1. Viability of cells in PBPC grafts assessed by trypan blue exclusion test: a) after collection b) after addition of DMSO, prior to cryopreservation c) post-thaw. Statistical differences between A, B and C were assessed with paired t-test. The lower and the upper lines of the box show the 25<sup>th</sup> and 75<sup>th</sup> percentiles. The line within the box is the median value, the whiskers show the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Black dots represent the outliers.

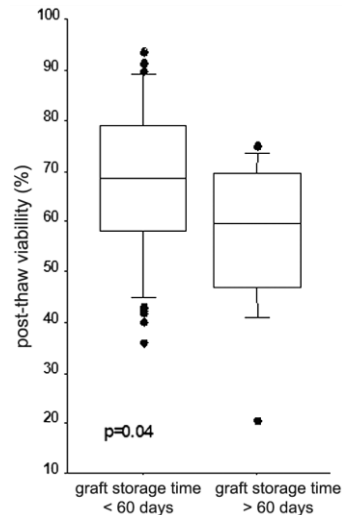


Fig. 2. Post-thaw viability of cells in PBPC grafts according to duration of graft storage time. The lower and the upper lines of the box show the 25<sup>th</sup> and 75<sup>th</sup> percentiles. The line within the box is the median value, the whiskers show the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Black dots represent the outliers.

for more than 60 days showed lower post-thaw viability compared to the ones thawed in the 60 days following cryopreservation ( $56.6 \pm 15.2$  vs.  $67.6 \pm 15.5$ , Mann Whitney  $p=0.04$ ). Mean graft storage time for myeloma patients was somewhat longer compared to graft storage time for patients with other diagnoses, but these differences did not reach statistical significance.

Median time post-transplantation to reach absolute neutrophil counts  $> 1 \times 10^9/L$  and platelets  $> 20 \times 10^9/L$  in two consecutive days was 10 and 11 days, respectively. Patients on average received  $401 \pm 657.3$  ml of packed RBC and  $11.5 \pm 13.4$  doses of platelet transfusions. In order to evaluate not only the effect of CD34+ dose (see above) but also the influence of post-thaw graft viability on engraftment time we have correlated post-thaw viability with time to reach target ANC and platelet counts and have not found significant correlations by simple regression (Figure 3a and b). Additionally, two equal groups ( $\gg$ low $\ll$  and  $\gg$ high $\ll$  post-thaw viability grafts) were formed, using the median viability observed in the whole group (65%) as the cutoff point. Hematopoietic recovery (time to  $ANC > 1 \times 10^9$  and platelets  $20 \times 10^9/L$ ) as well as need for transfusions was not found to be significantly different between the two groups.

Data on infectious complications in the post-transplant period were available for 72 patients. We have discovered a clear predisposition towards infectious complications in patients receiving grafts with lower percentage of viable cells. These patients developed febrile neutropenia more often than patients receiving grafts with higher post-thaw viability [72.2% of patients vs. 50% of patients,  $\chi^2$  ( $p=0.05$ ), Figure 4] and also differed in number of febrile days observed ( $2.4 \pm 2.6$  vs.  $1.5 \pm 2.3$ , Mann Whitney  $p=0.05$ ). Furthermore, post-thaw viability values were found to correlate by simple regression ( $R^2=0.06$ ,

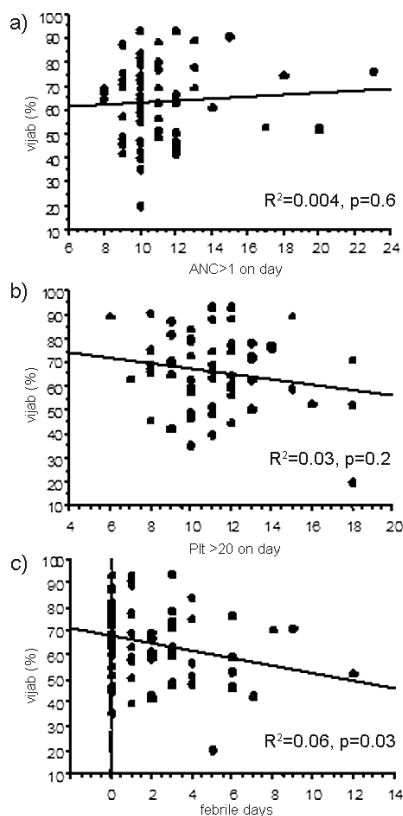


Fig. 3. Post-thaw viability of cells in PBPC grafts in relation to post-transplant clinical course: A – number of days needed to reach absolute neutrophil counts (ANC)  $>1 \times 10^9/L$ , B – number of days needed to reach platelet counts  $>20 \times 10^9/L$ , C – number of febrile days.

$p=0.003$ , Figure 3b) with number of febrile days observed in the post-transplant period. The influence of other relevant factors for occurrence of febrile neutropenia (CD34+ cell dose, patients' age, diagnosis, autologous or allogeneic transplantation, number of lines/cycles of therapy received prior to transplantation) has also been investigated. Compared to patients with lymphoma or myeloma, patients suffering from acute leukemia had a significantly higher incidence of febrile neutropenia ( $\chi^2$ - $p=0.001$ ) following transplantation. Influence of other previously mentioned parameters on development of febrile neutropenia was not statistically significant.

## Discussion

The mobilization scheme, developed and applied in our hospital with G-CSF administered according to a modulating schedule, resulted in mobilizing sufficient numbers of CD34+ cells in the vast majority of our patients. It can be considered both economical and successful, with fewer days of hospitalization needed and total dose of filgrastim used lower than usually reported<sup>32</sup>. It is well known that prior use of stem cell toxic agents, such as nitrogen mustard, procarbazine, melphalan, car-

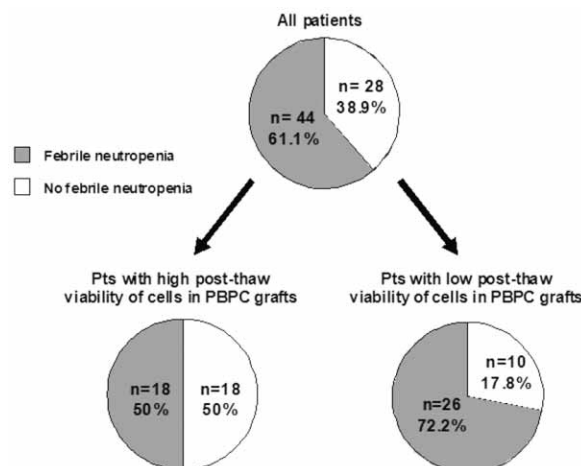


Fig. 4. Incidence of febrile neutropenia in patients following hematopoietic stem cell transplantation according to post-thaw viability of cells in PBPC grafts.

mustine or high-dose cytarabine can result in poor and unpredictable mobilization<sup>33</sup>. The use of miniBEAM as priming regimen in our lymphoma patients, although controversial for previously mentioned reasons, did not affect stem cell mobilization potential, and as we have reported previously, was more successful in yielding sufficient CD34+ numbers compared to the scheme with cyclophosphamide used for priming<sup>34,35</sup>. The lack of significant stem cell toxicity of miniBEAM observed in our patients can probably be explained by the fact that it was administered to these patients for the first time. The choice of miniBEAM for priming brought an additional benefit, providing instant and »in vivo« information about chemosensitivity of the lymphoma to this combination of drugs, used later in higher doses for myeloablative conditioning.

In our patients, as in a number of other studies<sup>13, 20–22</sup>, faster hematopoietic recovery was observed in patients that received a higher CD34+ cell dose. No correlation was found with the CD34+ dose and the occurrence of febrile neutropenia or number of febrile days in the post-transplant period, which is also in concordance with other published results<sup>36</sup>.

We assessed PBPC viability using trypan blue dye exclusion test, which remains the most common membrane integrity stain used to distinguish between viable and nonviable nucleated cells. This test was used, sometimes in combination with other tests, in most of the other reported studies<sup>26, 37–39</sup>. The fact that we have evaluated viability of not only CD34+ cells, but all cells in the PBPC graft has to be underlined and taken into account when comparing our results with the results of other studies. Namely, due to their different membrane content of lipids and proteins, HPCs appear to be more resistant to cryopreservation injury than other mononuclear cells<sup>25, 27, 28</sup> which is reflected, post-thaw, in their higher viability compared to the viability of all nucleated cells. Determination of PBPC graft viability after thawing takes into

account the effects of freezing, thawing and DMSO, and therefore represents the actual composition of the graft prior to (re)infusion. Therefore, for the purpose of correlating PBPC viability with clinical data, in our study we have chosen post-thaw viability results (Figure 1c) as the most relevant ones.

Cell viability in PBPCs grafts registered at the predetermined timepoints in our study showed the expected decreasing trend from the time of collection to post-thaw. Similar results with loss of viability of both total nucleated and CD34+ cells in post thaw samples compared with fresh samples were observed by other investigators as well<sup>23,26,30,37,40</sup>. We, as others, have also found important inter-patient differences of graft viability, especially at the second and third timepoint (Figures 1b and c). PBPC graft viability after addition of DMSO prior to cryopreservation ranged in our study from 24% to 100%, and post-thaw from 20.4% to 94.4%. No correlation was found between viability and the number of CD34+ cells present in grafts, patients' age, gender, diagnosis or extent of previous treatment, but contrary to observations from some other studies<sup>30,41</sup>, we have found that grafts stored for more than 60 days had lower post-thaw viability. Generally, storage time of most stem cell grafts is less than 3 months (in our study: mean 105±246 days, median 40.5 days). The question of graft durability, defined as the time that stem cells can safely remain cryopreserved still remains somewhat controversial<sup>42,43</sup>. However, most of the studies have demonstrated that even PBPC grafts that have been stored for several years are capable of long term engraftment<sup>44,45</sup>. Our results confirm these findings, since all our patients engrafted, regardless of their graft storage time or percentage of viable cells post-thaw.

The design of our study did not envision quantification of CD34+ cells after cryopreservation, nor their viability assessment pre- and post-thaw. These procedures are still not routinely performed in the vast majority of transplant centers. Some authors have shown that quantification of post-thaw viable CD34+ cells seems to be the most valuable predictor of platelet engraftment<sup>13,23</sup>. One could therefore argue that assessing CD34+ viability at different stages of processing could, besides providing accurate quality control, maybe help in identifying patients at risk of delays in hematopoietic reconstitution and alter some aspects of their care following transplantation. However, our results question the necessity of performing these controls, confirming the relative ro-

bustness of CD34+ cells during the freeze/thaw procedures which are carried out in daily clinical practice.

An interesting and, to our knowledge previously unreported phenomenon was found regarding PBPC graft post-thaw viability and infectious complications in patients following transplantation. Namely, patients with higher PBPC graft viability were less prone to develop febrile neutropenia and had less febrile days in the post-transplant period (Figures 3c and 4).

It has been demonstrated that granulocytes collected after the administration of G-CSF seem to retain functional and biochemical characteristics for at least 24 hours of storage, which suggests additional effects of G-CSF mobilization beyond enhancing yields and the possibility of storage of these components after collection<sup>46</sup>. However, the tolerance of granulocytes to DMSO is lower than that of lymphocytes or immature HPCs<sup>47</sup> and a number of studies have shown that the majority of cells lost during cryopreservation are granulocytes, while most lymphocyte-like cells, including CD34+ cells survive the process of freezing and thawing<sup>12,26,48</sup>. There have been publications that claim successful cryopreservation of granulocytes but huge variations are found between results depending on the viability assay used<sup>49</sup>. Viability tests usually reflect an intact cell membrane, while they do not provide information on typical granulocytic functions such as chemotaxis or bactericidal activity and are therefore limited in their ability to predict what is going to happen in vivo. Fois and colleagues have shown that post-thaw cell viability is inversely correlated with the granulocytic cell concentration before cryopreservation<sup>30</sup>. Not knowing the granulocyte concentration before and after thawing in our samples, we can only speculate that those grafts showing low post-thaw viability could have had high pre-thaw concentrations of fragile granulocytes, vulnerable to cryopreservation and thawing. On the contrary, those grafts showing high post-thaw viability could have had either lower pre-thaw granulocyte concentrations, or more robust granulocytes, that might have survived cryopreservation and thawing and that might have, to some extent, contributed to protection of those patients from infections in the post-transplant period. Knowing the short half-life of granulocytes, and their relatively low total dose in the PBPC graft, this explanation remains highly speculative. However, it is an intriguing finding that deserves further investigation with additional tests, on a larger number of patients, less heterogeneous in terms of underlying disease, type of transplantation or conditioning regimen used.

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## VIJABILNOST PRIPRAVKA KRVOTVORNIH MATIČNIH STANICA NAKON ODMRZAVANJA: IMA LI KLINIČKO ZNAČENJE ILI NE?

### SAŽETAK

Vijabilnost pripravka krvotvornih matičnih stanica iz periferne krvi (KMSPK) i njen utjecaj na klinički tijek nakon transplantacije istraživani su kod 81 uzastopne transplantacije (72 autologne i 9 alogeničnih) provedene u bolesnika oboljelih od hematoloških bolesti. Vijabilnost stanica KMSPK odmah nakon sakupljanja bila je  $98,6 \pm 3,5\%$ , nakon dodatka dimetil sulfoksida  $73,3 \pm 21,8\%$ , a nakon odmrzavanja  $65,2 \pm 16,1\%$ . Nije se bitno razlikovala kod bolesnika različitih dijagnoza, dobi, spola, načina na koji su KMSPK mobilizirane, dozi G-CSFa ili broju prikupljenih CD34+ stanica. Pripravci zamrznuti dulje od 60 dana imali su slabiju vijabilnost nakon odmrzavanja u usporedbi s onima koji su bili zamrznuti manje od 60 dana ( $56,6 \pm 15,2\%$  prema  $67,6 \pm 15,5\%$ ,  $p=0,04$ ). Vijabilnost nakon odmrzavanja nije utjecala na dinamiku hematopoetskog oporavka, no kod bolesnika kojima je vijabilnost pripravka KMSPK bila slabija uočena je sklonost infektivnim komplikacijama. Češće su razvili febrilnu neutropeniju ( $72,2\%$  prema  $50\%$  bolesnika,  $p=0,05$ ) te su imali više febrilnih dana ( $2,4 \pm 2,6$  prema  $1,5 \pm 2,3$ ,  $p=0,05$ ) nakon transplantacije. Pokazali smo da transplantacija KMSPK dovodi do hematopoetskog oporavka neovisno o tome koliko je dugo pripravak KMSPK bio zamrznut i neovisno o vijabilnosti stanica nakon odmrzavanja. Time se potvrdila otpornost CD34+ stanica na postupke zamrzavanja i odmrzavanja koji se rutinski provode u svakodnevnoj praksi. Moguće je da koncentracija granulocita u pripravku KMSPK ima utjecaj na razvoj infektivnih komplikacija nakon transplantacije, no taj je fenomen potrebno detaljnije istražiti na većem broju bolesnika.