Poor Mobilization of Hematopoietic Stem Cells—Definitions, Incidence, Risk Factors, and Impact on Outcome of Autologous Transplantation

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As more efficient agents for stem cell mobilization are being developed, there is an urgent need to define which patient population might benefit from these novel drugs. For a precise and prospective definition of "poor mobilization" (PM), we have analyzed the efficiency of mobilization in patients intended to receive autologous transplantation at our center in the past 6 years. Between January 2003, and December 2008, 840 patients with the following diagnoses were scheduled to undergo leukapheresis: multiple myeloma (MM, n = 602) and non-Hodgkin lymphoma (NHL, n = 238). Most patients mobilized readily: close to 85% of the patients had a level of 20/μL to >500/μL of CD34$^+$ cells at the peak of stimulation. Of the 840 patients, 129 (15.3%) were considered to be PMs, defined as patients who had a peak concentration of <20/μL of CD34$^+$ cells upon stimulation with granulocyte-colony stimulating factor (G-CSF) subsequent to induction chemotherapy appropriate for the respective disease. Among them, 38 (4.5%) patients had CD34$^+$ levels between 11 and 19/μL at maximum stimulation, defined as "borderline" PM, 49 (5.8%) patients had CD34$^+$ levels between 6 and 10/μL, defined as "relative" PM, and 42 patients (5%) with levels of <5/μL, defined as "absolute" PM. There was no difference in the incidence of PM between patients with MM versus those with NHL. Sex, age, body weight (b.w.) and previous irradiation therapy did not make any significant difference. Only the total number of cycles of previous chemotherapy (P = .0034), and previous treatment with melphalan (Mel; P = .0078) had a significant impact on the ability to mobilize. For the good mobilizers, the median time to recovery of the white blood cells (WBCs) to 1.0/nL or more was 13 days with a range of 7 to 22 days, whereas for the PM group it was 14 days with a range of 8 to 37 days. This difference was statistically not significant. The median time to recovery of the platelets counts to an unmaintained level of >20/nL was 11 days with a range of 6 to 17 days for the good mobilizers, whereas for the PM it was 11 days with a range of 7 to 32 days. Again, this difference was not significant. The majority of the patients today intended for autologous transplantations were able to mobilize readily. As long as ≥2.0 × 10^6 of CD34$^+$ cells/kg b.w. have been collected, PM was not associated with inferior engraftment.

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INTRODUCTION

Peripheral blood stem cells (PBSCs) have largely replaced bone marrow (BM) cells in autologous trans-

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plantations, and have become the source of stem cells in the majority of allogeneic transplantations. In the mid-1980s several institutions demonstrated that PBSCs could represent viable alternatives to BM cells as a source of hematopoietic stem and progenitor cells for autologous transplantation [1-5]. PBSCs offer several advantages such as harvest of cells without general anesthesia, elimination of pain after multiple aspirations from the BM, and above all, they are associated with more rapid engraftment [6-8].

In the meantime, autologous PBSC transplantation is the treatment of choice within the primary treatment strategy for multiple myeloma (MM) [9-12], and is also the preferred treatment option for relapsed/refractory B cell non-Hodgkin lymphoma (NHL) [13-17]. The main disadvantage of PBSCs is that they exist in the circulation in very small numbers. Less than 0.06% of white blood cells (WBCs) are

CD34$^+$, which is a cell surface protein that is expressed on hematopoietic stem (HSCs) and progenitor cells and represents a reliable surrogate marker for hematopoietic progenitor cells [18-20].

HSCs and progenitor cells reside in the BM and they have to be mobilized into the circulation prior to being collected by apheresis. The number of apheresis procedures needed and the success of transplantation are determined by the efficiency of stem cell mobilization ([21,22], review in [23]). Stem cells adhere to their BM niche by interactions between SDF1α, which is produced by BM stromal cells, and CXCR4, which is expressed on CD34$^+$ cells [24,25]. Granulocyte colony-stimulating factor (G-CSF), which has been in clinical use for more than 2 decades, mobilizes stem cells from the BM niche by secretion of neutrophil-associated extracellular proteases, such as MMP-9, which subsequently releases HSC from their niche [26]. In contrast, Plerixafor (formerly known as AMD3100) is a novel mobilization agent that directly inhibits the CXCR4-SDF1α cell-cell interaction [27,28].

Several stem cell mobilization strategies have been employed since development of PBSC transplantations. In the early days of transplantation, stem cell mobilization was achieved with chemotherapeutic drugs because the use of chemotherapy causes a significant increase in the number of PBSCs at the time of recovery [1,3,5]. However, many patients failed to mobilize sufficient PBSCs for transplantation in response to chemotherapy. In the late 1980s, hematopoietic growth factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and G-CSF have been made available [29-33]. Their administration subsequent to chemotherapy has been shown to mobilize PBSCs efficiently [34]. G-CSF and GM-CSF were approved for use as HSC mobilizing agents, but G-CSF (in combination with chemotherapy or alone) has become standard (review in [23]). Unfortunately, some patients fail to mobilize sufficient numbers of PBSCs for transplantation in response to G-CSF with or without chemotherapy [22,30,35-39]. There is thus far no consensus on the definition of poor mobilizers (PM).

Based on this retrospective analysis of 840 patients who were mobilized with chemotherapy and growth factors with the intent of autologous transplantation at a single center, we have provided a more precise definition of "poor" mobilization, and have evaluated the incidence, risk factors, and impact on transplantation outcome in a modern setting.

PATIENTS AND METHODS

Patients

We analyzed data from 602 patients with MM and 238 patients with NHL, who were scheduled to receive autologous PBSC transplantation (PBSCT) between 2003 and 2008 at the Department of Internal Medicine V in Heidelberg. Retrospective data analysis was approved by the Ethics Committee of the Medical Faculty of Heidelberg. The median age was 59 years, with a range from 12 to 75 years (MM: 60 years, range: 27-75 years; NHL: 54 years, range: 12-74 years). PBSCs were mobilized with chemotherapy (CT) followed by G-CSF. The appropriate regimen as CT was used to reduce the tumor burden and to facilitate PBSC harvesting.

Mobilization Regimens

For patients with MM, the following chemotherapy regimens were used for remission induction and for mobilization. CAD (cyclophosphamide [Cy] 1,000 mg/m^2/d day 1, doxorubicin 15 mg/m^2/d days 1-4, dexamethasone 40 mg/day orally days 1-4), TCED (thalidomide 400 mg/day orally, etoposide 40 mg/m^2/d days 1-4, Cy 400 mg/m^2/d days 1-4, dexamethasone 40 mg/day orally days 1-4), HD-Cy (Cy 2000 mg/m^2/day days 1-2) or lenalidomide/dexamethasone (lenalidomide 25 mg/day orally days 1-21, dexamethasone 20 mg/day orally days 1-4, 9-12 and 17-20).

For patients with NHL, the following regimens were used for remission induction and, in case chemosensitivity is demonstrated, the same regimen will be used for mobilization: Dexa-BEAM (dexamethasone 3 × 8 mg/day days 1-10, carmustine 60 mg/m^2/day day 2, melphalan [Mel] 30 mg/m^2/day day 2, cytarabine 2 × 100 mg/m^2/day days 3-6, etoposide 75 mg/m^2/day days 3-6), R-Dexa-BEAM (rituximab 375 mg/m^2/day day 0, Dexa-BEAM), CHOP (Cy 750 mg/m^2/day day 1, doxorubicin 50 mg/m^2/day day 1, vincristine 1.4 mg/m^2/day [max. 2.0 mg] day 1, prednisone 100 mg/day days 1 -5), CHOEP (CHOP plus etoposide 100 mg/m^2/day days 1-3), R-CHOP (rituximab 375 mg/m^2/day day 0, CHOP), R-CHOEP (rituximab 375 mg/m^2/day day 0, CHOP plus etoposide 100 mg/m^2/day days 1-3), DHAP (dexamethasone 40 mg/day days 1-4, cisplatin 100 mg/m^2/day day 1, cytarabine 2 × 2000 mg/m^2/day day 2), R-DHAP (rituximab 375 mg/m^2/day day 0, DHAP) or HD-Cy (Cy 2000 mg/m^2/day days 1-2).

All patients received G-CSF starting 4 to 5 days after completion of chemotherapy in dosages of 5-10 μg/kg/day subcutaneously (s.c.) until the end of the collection period.

If the patients failed to reach target collections, they could have a second or third attempt to mobilize an adequate amount of stem cells for transplantation. The following options were adopted: (1) another attempt to mobilize with chemotherapy and G-CSF; (2) G-CSF alone after a "rest" period of at least 21 days without chemotherapy; (3) BM harvest as an

alternative; (4) Plerixafor within a compassionate use program.

Immunofluorescence Staining and Flow Cytometry

PB CD34$^+$ cell measurements were started after a WBC count of $\geq 5.0 \times 10^9$/L was reached. CD34$^+$ cell measurements during mobilization were performed daily on weekdays. The absolute number of CD34$^+$ cells was evaluated by flow cytometry using a FACScan analyzer (Becton Dickinson; Heidelberg, Germany) and the appropriate isotype-matched negative control, as has been described previously [19,20]. We used a forward scatter versus CD45 fluorescence dot plot to discriminate between the smallest hematopoietic cell population and erythrocytes or debris. The percentage of CD34$^+$ cells relative to the percentage of CD45$^-$ cells and the absolute number of CD34$^+$ cells were calculated as previously described.

To increase the sensitivity of progenitor cell detection in steady-state PB, a gated acquisition on CD34$^+$ cells was performed [18,19]. CD34$^+$ PBSCs were monitored daily as soon as the WBC recovered ($\geq 5.0 \times 10^9$/L PB). Apheresis was scheduled to start when there were >20 CD34$^+$ cells/μL of PB. If this target was not reached, other parameters, like increasing WBC or platelet reconstitution, were used to decide on leukapheresis attempts. The minimal collection target was 2.0×10^6 CD34$^+$ cells/kg body weight (b.w). Our aim was to process 3 times the patient's blood volume daily through an indwelling central or peripheral venous catheter using a cell separator (Spectra; COBE Laboratories, Heimstetten, Germany). Each leukapheresis product was cryopreserved in nitrogen until the day of transplantation.

Collection of PBSC

Harvesting was performed with a COBE Spectra Blood Cell (Spectra; COBE Laboratories). We started the leukapheresis procedure if the CD34$^+$ counts in PB were >20/μL. In cases when the leukocyte count under daily G-CSF-stimulation after chemotherapy has reached a plateau for more than 2 days, the patients underwent a leukapheresis procedure attempt even if the CD34$^+$ count was <20 cells/μL. All patients described in this study underwent leukapheresis.

Definition of PM

Patients with CD34$^+$ levels of <20/μL in peripheral blood at maximum stimulation were considered to be PM. This group was further subdivided: patients with CD34$^+$ levels between 11 and 19/μL were defined as "borderline" PM; patients with CD34$^+$ levels between 6 and 10/μL were defined as "relative" PM, and patients with levels of <5/μL were regarded as "absolute" PM.

Reconstitution

The definitions of engraftment and supportive care after autologous transplantation has been described in detail previously [17,19,20,34]. Hematopoietic reconstitution was defined from the day of PBSC reinfusion (day 0) until leukocyte counts were stable at >1.0 /nL for at least 3 days. The time to platelet reconstitution over 20/nL was defined as the number of days for platelets to be stably above 20/nL without any transfusion. No patients received G-CSF after high-dose chemotherapy and transplantation.

Statistical Analysis

The results are presented as median values, ranges, and correlation coefficients, where applicable. The relationships between different hematologic parameters of the PB and leukapheresis products were analyzed by correlational statistics. Pearson's sample correlation coefficient and the corresponding P value for the null hypothesis of no correlation were calculated.

On multivariate logistic regression analysis we studied the impact of age, sex, b.w., cycles of previous chemotherapy cycles, and radiation therapy on stem cell mobilization, and time to hematologic recovery after transplantation.

RESULTS

Incidence of PM

Between January 2003 to December 2008, 868 consecutive patients with the following diagnoses were scheduled to undergo leukapheresis for autologous transplantations: MM (n = 602), NHL (n = 238), and Hodgkin disease (HD, n = 28). Because the number of patients with HD was small and only 2 of them were classified as PMs, patients with HD were not included in the final analysis, which was performed only on those with MM and NHL. The major indications for autologous transplantation have been focused on these 2 diseases in the past 6 years.

Our group and other authors have previously demonstrated the significant correlation between preleukapheresis CD34$^+$ levels in circulating blood and the absolute amount of CD34$^+$ cells collected in the leukapheresis products [19-22]. This correlation is confirmed in this retrospective analysis of altogether 840 leukapheresis procedures (see Figure 1A and B) for the first day of collection. Our data have provided convincing evidence that the level of CD34$^+$ counts is a reliable prospective parameter for estimation of success of mobilization. Based on our own observations and those reported in the literature, we have then defined PM as patients who had a peak CD34$^+$ concentration of <20/μL of CD34$^+$ cells upon stimulation
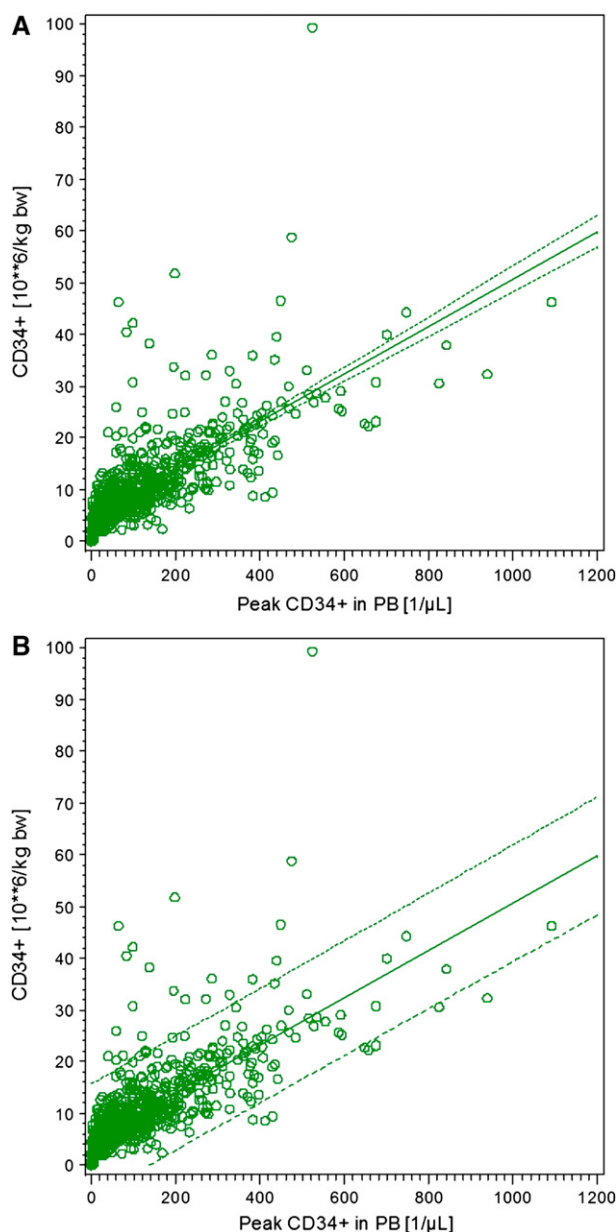
Figure 1. (A) Positive correlation between peak CD34^{+} values in peripheral blood and harvested CD34^{+} cells. The broken lines define the 95% confidence limits for mean predicted values. (B) Positive correlation between peak CD34^{+} values in peripheral blood and harvested CD34^{+} cells. The broken lines define the 95% confidence limits for individual predicted values.

with G-CSF subsequent to induction chemotherapy appropriate for the respective disease.

Of the 840 patients with MM and NHL scheduled to receive autologous transplants, 129 patients (15.3%) had CD34^{+} levels of $<20/\mu L$ at maximum stimulation and were therefore considered to be PM. This group was further subdivided: 38 patients (4.5%) had CD34^{+} levels of between 11 and $19/\mu L$, defined as "borderline" PM; 49 patients (5.8%) had CD34^{+} levels of between 6 and $10/\mu L$, defined as "relative" PM and 42 patients (5.0%) with levels of $<5/\mu L$

were defined as "absolute" PM. The clinical characteristics of these patients are shown in Table 1.

Leukapheresis Products and Autologous Transplantations

Among the 602 patients with MM, 516 patients (85.7%) had CD34^{+} levels of $\geq 20/\mu L$ at maximum stimulation and were therefore classified as good mobilizers. The goal of collecting 2.0×10^6 CD34^{+} cells/kg b.w. was achieved with 1 to 3 leukapheresis (median = 1) procedures in these 516 patients (see Table 2). Among the 29 borderline PM with MM, the target number of CD34^{+} cells could be collected with 1 to 4 leukapheresis (median = 2) procedures. For the 29 relative PM, the target CD34^{+} cell dose of 2.0×10^6 cells/kg b.w. could be achieved with 1 to 5 procedures (median = 3) in 25, that is, 86% of these patients, whereas for the 28 absolute PM, only 12 of them, that is, 43%, achieved the goal with 1 to 6 leukapheresis procedures (median = 4). Nevertheless 19 of these absolute PM received a transplant. The difference of 7 patients can be explained by the fact that we were able to collect between 1.5 to 1.9×10^6 cells/kg b.w. of CD34^{+} as an autologous graft in 4 patients and these levels of CD34^{+} cells were considered to be appropriate for the respective patients. In 3 further patients we were able to reach the collection goal in a second mobilization attempt. All of them engrafted.

Among the 238 patients with NHL, 195 patients (81.9%) were classified as good mobilizers and the goal of collecting 2.0×10^6 CD34^{+} cells/kg b.w. was achieved with 1 to 2 leukapheresis procedures (median = 1) in every everyone of the 195 patients. Among the 9 borderline PM with NHL, the target number of CD34^{+} cells could be collected with 1 to 3 leukapheresis procedures (median = 1) in all of the 9 patients. For the 20 relative PM, the target CD34^{+} cell number was collected with 1 to 5 procedures (median = 3) in 13, that is, 65% of these patients, whereas for the 14 absolute PM, only 5 of them, that is, 36% achieved the goal with up to 6 leukapheresis procedures (median = 4). Three of these absolute PM received ultimately a transplant (see Table 2).

Characterization of PMs

The percentage of patients classified as PMs for the whole group is 15%. As reported in the literature, it is controversial if a peak level of $10/\mu L$ or $20/\mu L$ of CD34^{+} cells should be considered as the threshold. If a peak level of $10/\mu L$ is considered as threshold, 11% instead of 15% were PMs. The correlation between CD34^{+} cell concentrations in peripheral blood with the PBSC collection is depicted in Figure 1A and B. Hence, we again confirm the previous observation that pre-apheresis CD34^{+} count predicts reliably the

Table 1. Clinical Features of Poor and Good Mobilizers

	Poor mobilizer		Good mobilizer	
Total	129	15%	711	85%
- MM	86	14%	516	86%
- NHL	43	18%	195	82%
Sex (male : female)	81 : 48		422 : 289	
Median age (years; range)	61	(19-75)	58	(12-74)
Previous irradiation	37	44%	23*	22%*
No. of previous chemotherapy cycles (median; range)	6	(1-20)	3*	(1-6)*

MM indicates multiple myeloma; NHL, non-hodgkin lymphoma. only a subgroup of "good mobilizer" (n=103) was analyzed.
*only a subgroup of "good mobilizer" (n=103) was analyzed.

quality and quantity of collection. The distribution of the preleukapheresis CD34$^+$ counts in peripheral blood samples among the patients with MM and with NHL is summarized in Figure 2A and B. Hence, close to 70% of the patients had a level of 20/μL to 250/μL of CD34$^+$ cells at the peak of stimulation. Levels as high as >500/μL of CD34$^+$ cells have been recorded.

We have analyzed the relationship between poor mobilizations with types of disease (MM versus NHL), sex, age, b.w., previous irradiation, number of cycles of previous combination chemotherapy, and pretreatment with Mel among the 840 patients. The results of univariant analysis are shown in Table 3. There was no difference in the incidence of PM between patients with MM versus those with NHL. Sex, age, b.w. and previous irradiation therapy did not make any difference. Only the number of cycles of previous chemotherapy ($P = .0034$), and previous treatment with Mel ($P = .0078$) had a significant impact on the ability to mobilize. For example, PM received a median of 6 cycles of chemotherapy, whereas good mobilizers received a median of 3 cycles.

Secondary Mobilization after Initial Failure

Secondary strategies to mobilize HSC from the 36 who failed to achieve an adequate collection included: (1) Administration of another cycle of induction chemotherapy and G-CSF: the goal of harvesting 2.0×10^6 CD34$^+$ cells/kg b.w. could be accomplished in 7 of 21 of these patients. (2) G-CSF alone for 4 days (up to 8 days of stimulation) after hematopoietic

recovery from previous induction chemotherapy: the goal could be achieved in 2 of the 9 patients thus mobilized. (3) Plerixafor within the compassionate use program: the goal was accomplished in 7 of 8 patients. In 1 patient only 1.78×10^6 CD34$^+$ cells/kg b.w. could be harvested, but these cells were considered as sufficient and appropriate for autologous blood stem cell transplantation (ABSCT) for this patient, so that all 8 patients were transplanted successfully. (4) BM harvest in lieu of collection of peripheral HSC in 5 patients: seven of the 36 PMs underwent more than 1 secondary attempt. The results are summarized in Figure 3.

Engraftment Data

For comparisons in time to engraftment of leukocytes and platelet counts between PMs and good mobilizers, we have chosen 305 patients, age and sex matched, from the "good mobilizers" group. We have retrospectively retrieved their reconstitution data and have compared them to those derived from the PMs described in this study. After autologous transplantation G-CSF was never administered in any case. The correlation between peak concentration of CD34$^+$ cells/μL before the first leukapheresis and the reconstitution time among these patients (305 good mobilizers and 89 PMs transplanted) is shown in Figures 4A and B.

For the good mobilizers, the median time to recovery of the WBC to 1.0/nL was 13 days, with a range of 7 to 22 days, whereas for the PM group it was 14 days, with a range of 8 to 37 days. This difference was

Table 2. Degrees of Poor Mobilizations and CD34$^+$ Cells Collected

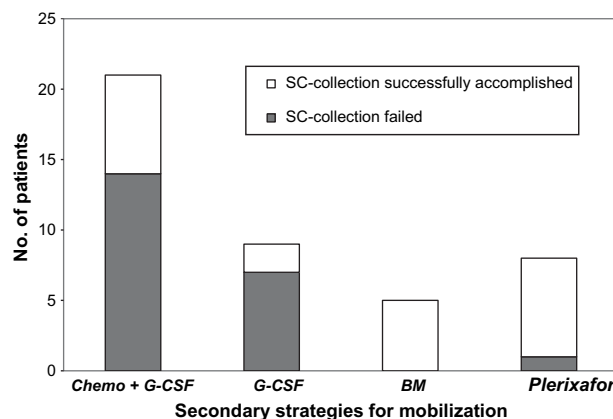|		Total no. of patients		Patients achieving collection goal of 2.0 ×10exp6 CD34$^+$ cells /kg bw		Patients transplanted	
MM (n=602)	**Peak level of CD34$^+$ cells**	**n**	**%**	**n**	**%**	**n**	**%**
- Good mobilizer	>=20 /μL	516	86%	516	100%	484	94%
- Borderline poor mobilizer	11-19 /μL	29	5%	29	100%	25	86%
- Relative poor mobilizer	6-10 /μL	29	5%	25	86%	24	83%
- Absolute poor mobilizer	0-5 /μL	28	5%	12	43%	19	68%
NHL (n=238)	**Peak level of CD34$^+$ cells**	**n**	**%**	**n**	**%**	**n**	**%**
- Good mobilizer	>=20 /μL	195	82%	195	100%	150	77%
- Borderline poor mobilizer	11-19 /μL	9	4%	9	100%	5	56%
- Relative poor mobilizer	6-10 /μL	20	8%	13	65%	11	55%
- Absolute poor mobilizer	0-5 /μL	14	6%	5	36%	3	21%

MM indicates multiple myeloma; NHL, non-hodgkin lymphoma.
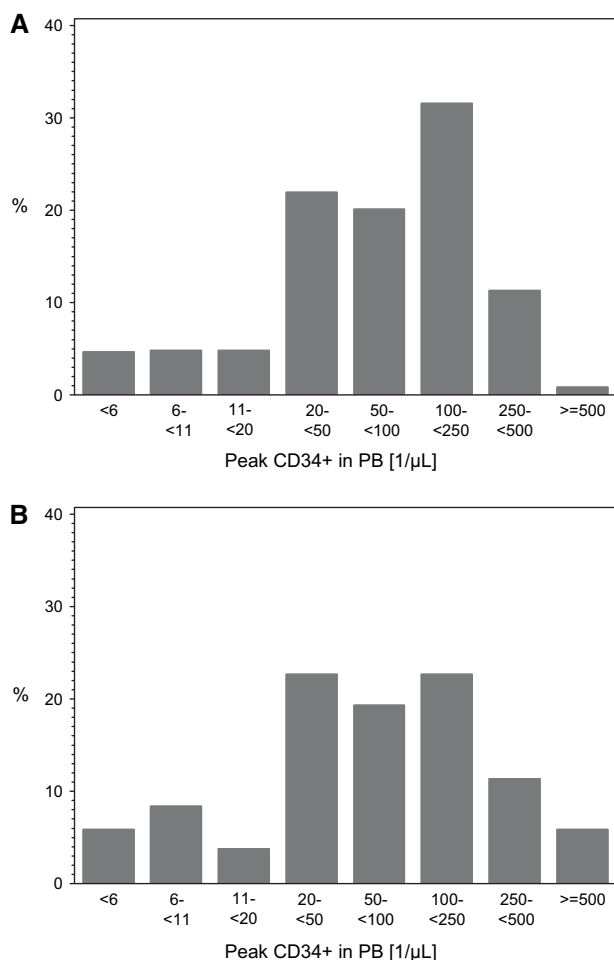
Figure 2. (A) Peak CD34$^+$ values in peripheral blood at the time of leukapheresis in patients with MM. (B) Peak CD34$^+$ values in peripheral blood at the time of leukapheresis in patients with NHL.

Figure 3. Efficacy of secondary strategies for mobilization of stem cells. Target cell dose: $\geq 2.0 \times 10^6$ of CD34$^+$ cells per kg body weight.

statistically not significant. The median time to recovery of the platelet counts to an unmaintained level of >20/nL was 11 days with a range of 6 to 17 days for the good mobilizers, whereas for the PMs it was 11 days with a range of 7 to 32 days. Again, this difference was statistically not significant. The corresponding reconstitution times, analyzed separately for the subgroups borderline PM, relative PM, and absolute PM are summarized in Table 4.

In summary the reconstitution data suggest that as long as 2.0×10^6 of CD34$^+$ cells/kg b.w. could be harvested, poor mobilization was not associated with poor engraftment.
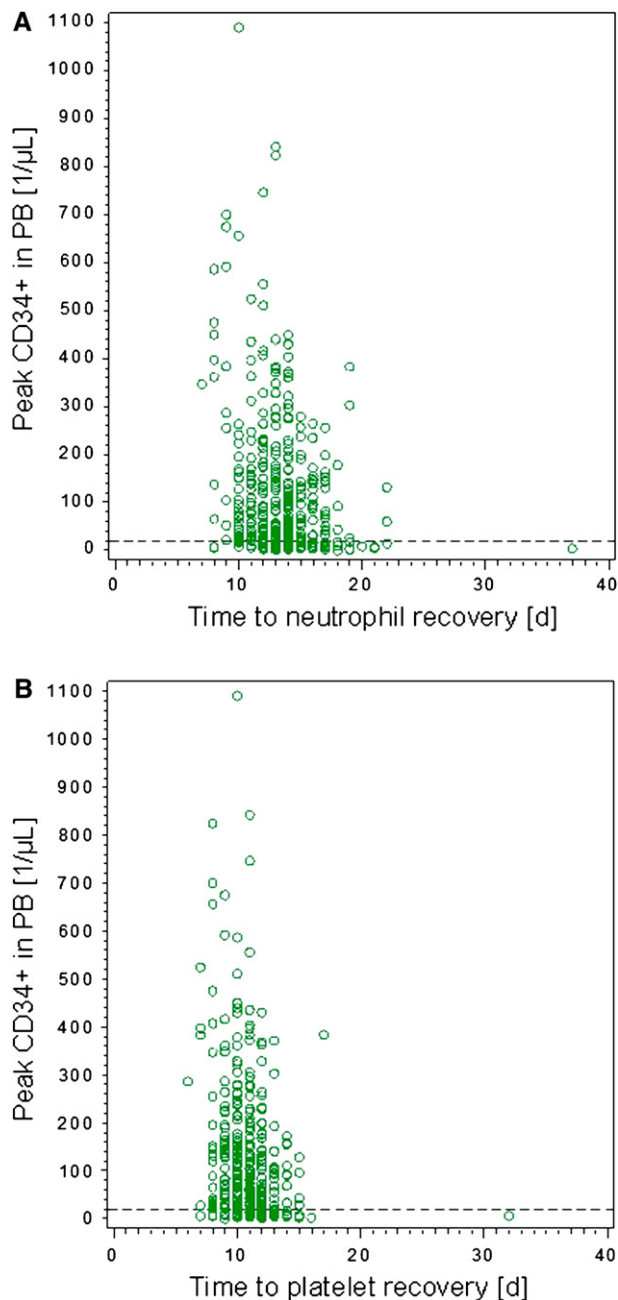
DISCUSSION

The development of strategies to promote release of HSC and progenitors from the BM into the circulating blood for clinical transplantation has evolved from a highly experimental procedure in the mid-1980s to standard of care for autologous transplantations (review in [23]). The main advantage of PBSC transplantation is the accelerated reconstitution compared to BM transplantations (BMTs) [1-5]. The major disadvantage is that HSCs reside in the BM and have to be mobilized into the circulation prior to being collected by apheresis [1,23]. The number of apheresis procedures needed and the success of transplantation are determined by the efficiency of stem cell mobilization.

Before hematopoietic growth factors were developed in the late 1980s, stem cell mobilization was achieved with chemotherapeutic drugs. After myelosuppressive chemotherapy, a significant increase in the number of PBSCs at the time of recovery was observed [1-5]. The yield was unpredictable, and many patients had to undergo 4 to 6 leukapheresis procedures before an adequate among was collected. The availability of GM-CSF and G-CSF has greatly revolutionalized the mobilization of PBSCs for autologous transplantations (review in [23]).

There were numerous reports on patients who failed to mobilize sufficient numbers of PBSCs for transplantation [36-43]. The incidence has been reported to be between 5% and 40% [37-43]. There is thus far also no consensus on the definition of PMs. Some reports used a value of 1.0×10^6 CD34$^+$ cells/kg b.w., whereas others reported a threshold of 2.0×10^6 CD34$^+$ cells/kg b.w. [37-43] as the parameter for poor mobilization. Using 2.0×10^6 CD34$^+$ cells/kg b.w. as the cutoff value, Pusic et al. [42] reported a failure rate of 18.6% in patients who received

Table 3. Factors Affecting Quality of Mobilization

	Wald Chi-Square	Pr > Chi Sq
Sex	1.0211	0.3123
Age	2.2328	0.1351
Body weight	1.9791	0.1595
Mobilization with plerixafor	2.0911	0.1482
Previous chemotherapy with melphalan	7.0688	0.0078
Previous irradiation therapy	0.0002	0.9878
No. of previous chemotherapy cycles	8.6057	0.0034

Figure 4. (A) Median time to recovery of the WBC to 1.0/nL in correlation to peak CD34$^+$ cells/uL in peripheral blood at the time of leukapheresis. (B) Reconstitution of platelets after ABSCT (ie, platelet count ≥20/nL without transfusions for 3 days) in correlation to peak CD34$^+$ cells/µL in peripheral blood at the time of leukapheresis.

Table 4. Engraftment Data in Relationship to Mobilization

	Median	Minimum	Maximum
Reconstitution of leukocytes	*(days after ABSCT)*		
- Good mobilizer	13	7	22
- Borderline poor mobilizer	15	10	22
- Relative poor mobilizer	13	8	21
- Absolute poor mobilizer	15	12	37
Reconstitution of platelets			
- Good mobilizer	11	6	17
- Borderline poor mobilizer	11	9	14
- Relative poor mobilizer	11	7	32
- Absolute poor mobilizer	12	9	16

ABSCT indicates autologous blood stem cell transplantation.

ing et al. [43] found that 29 (14.1%) of 206 patients failed to mobilize adequately on first attempt. The definition of poor mobilization was failure to collect a minimum of 2.0×10^6 cells/kg b.w. of CD34$^+$ cells in 4 leukapheresis procedures in this study.

Thus, most recent studies have adopted the definition of poor mobilization as inability to collect 2.0×10^6 cells/kw b.w. of CD34$^+$ cells. However, this can only be estimated retrospectively after leukapheresis has been performed. There was also no clear-cut stipulation on the number of leukapheresis procedures required to achieve this goal as a parameter to distinguish good mobilizers from PMs. Fruehauf et al. [19,20] have shown that there is a highly significant correlation between the CD34$^+$ concentration in peripheral blood and the potential to collect an adequate amount of CD34$^+$ cells within 1 or up to 3 leukapheresis procedures. We [34,44,45] have demonstrated that the optimal window for collection of CD34$^+$ cells after stimulation with G-CSF, with or without previous chemotherapy, was just 3 to maximally 4 days after reaching optimal stimulation. Given the present day advances in development of standards and guidelines for quality control of the PBSC products, there is an increasing need for a better definition of poor mobilization. Based on our previous reports [17,19,20,34, 44-46], and based on this retrospective analysis of 840 patients at our center who were mobilized with chemotherapy and growth factors with the intent of autologous transplantation, we propose a more precise definition of poor mobilization and PMs. In the light of recent development of new classes of mobilization agents such as CXCR4 inhibitors, predictive factors are urgently needed to discern those patients who might likely derive benefit from the more efficient mobilization agents.

According to the criteria that we have defined, of the 840 patients with MM and NHL scheduled to receive autologous transplants, 129 patients (15.3%) were considered to be PMs. Among them, 38 patients (4.5%) had CD34$^+$ levels of between 11 and 19/µL at maximum stimulation, defined as "borderline" PM, 49 patients (5.8%) had CD34$^+$ levels of between 6 and 10/µL,

G-CSF plus chemotherapy and a rate of 18.8% in those who received G-CSF for the initial mobilization. These authors have also defined 20/µL of CD34$^+$ cells as the minimum threshold needed for a successful day 1 collection. They concluded that current mobilization regimens are associated with a substantial failure rate irrespective of underlying disease.

In an analysis of PMs among patients with recurrent or relapsed lymphoma at a single center (M.D. Anderson Cancer Center, Houston, Texas, USA) Hos-

defined as "relative" PM, and 42 patients (5.0%) with levels of <5/μL, defined as "absolute" PM.

In contrast to the reports of Pusic et al. [42] and Hosing et al. [43], our retrospective analysis showed that the majority of the patients were able to mobilize readily. The median concentration of CD34+ cells/ μL among the patients included in this study was 81/μL. A total of 2.2% of patients had a concentration of >500 CD34+ cells/μL at the peak of stimulation and 85% of the patients had >20 CD34+ cells/μL at the time of maximum stimulation before leukapheresis. We have also demonstrated unequivocally that there was no significant difference between patients with NHL or MM. Moreover, once an adequate amount has been stimulated, CD34+ cell numbers above 2.0×10^6 cells/kw b.w. of CD34+ cells did not confer more advantage in terms of engraftment of leukocytes and platelets. The present data have confirmed our previous observation that although higher doses of CD34+ cells (i.e. (or, that is), $>6.5 \times 10^6$/kg b.w.) might marginally, but significantly, shorten the time to leukocyte and platelet recovery, stable engraftment was achieved with transplantation of 2.0×10^6 CD34+ cells /kg b.w. [47].

Similar to many others, our group has previously shown that autologous BMT or PBSCT might be associated with long term side effects such as myelodysplasia [48,49]. We have not been able to analyze the differences in the long-term side effects between PMs and good mobilizers yet. The present analysis is also not powered to address these issues and they also need to be addressed after longer follow-up period.

Secondary strategies to mobilize HSCs from the 36 patients included administration of another cycle of induction chemotherapy combined with G-CSF, G-CSF alone for 4 to 9 days upon hematopoietic recovery from previous induction chemotherapy, Plerixafor, and G-CSF within the compassionate use program, and BM harvest. The best results were accomplished with Plerixafor and G-CSF, and the goal of collecting 2.0×10^6 CD34+ cells/kg b.w. was accomplished in 7 of 8 patients. All 8 patients who were remobilized with Plerixafor and G-CSF received an autologous transplantation and all 8 have achieved stable engraftment. In the 1 patient who failed to reach the goal by a narrow margin, 1.78×10^6 CD34+ cells/kg b.w. could be harvested and he was transplanted successfully.

In the past 8 to 10 years, advances in stem cell research has led to a better understanding of the significance and the molecular mechanisms underlying the adhesion of the primitive HSCs and progenitor cells to the BM niche. Albeit many adhesion molecules have been shown to play a role in maintenance of the stem cells in the niche, interactions between SDF1α, which is expressed by BM stromal cells, and CXCR4, which is expressed on CD34+ cells, have been identified as a significant pathway for homing and mobilization of

CD34+ cells [24,25,27,28]. In the meantime, a specific inhibitor of CXCR4− SDF1α has been approved both by the Food and Drug administration (FDA) and the European Medicines Agency (EMEA) for mobilization of HSCs. In clinical trials, mobilization of CD34+ cells from the bone marrow into the peripheral blood has been reported to be augmented by single-dose administration of Plerixafor after 4 days of G-CSF [50-54]. Studies in NHL and MM patients showed that the combination of G-CSF and Plerixafor (G + P) resulted in a significant increase in CD34+ cell yield after apheresis compared to the administration of G-CSF alone. Moreover, G + P administration resulted in a rapid and sustained neutrophil and platelet engraftment of the mobilized HSCs [53,54]. In addition to an absolute increase in overall CD34+ cells upon Plerixafor mobilization, the proportion of CD34+/CD38− subset, a more primitive and potent progenitor cell population, was also significantly elevated [50,51].

Whereas the relative role and the significance of Plerixafor in the combinatorial mobilization will need to be defined in the future, it is important to define which group of patients will derive the most benefit from these novel agents. The first group of patients that will benefit are the absolute PMs. Albeit 40% of the absolute PM in this study were able to reach the collection goal, they required a median of 4 leukapheresis procedures. Thus, administering Plerixafor in addition to G-CSF in these patients will offer a much higher chance of successful collection for these patients with fewer leukapheresis procedures, and hence, reduced resource utilization. Therefore, patients who fail to reach CD34+ levels of >5/μL at maximum stimulation with G-CSF should benefit from the addition of Plerixafor, and leukapheresis could be performed 10 to 12 hours after administration of Plerixafor.

Other PMs (borderline, relative PM) might also benefit under certain circumstances, as collection of an adequate amount of CD 34' cells for tandem and even triple transplantations need to be considered within the overall treatment strategy of specific subgroups of patients. Our experimental data indicated that the combinatorial approach might induce a more primitive and eventually a different stem cell population that possesses higher potentials [55,56]. Whether these additional populations have an impact for the long-term outcome must be addressed in future studies.

ACKNOWLEDGMENTS

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