Subsets of CD34+ hematopoietic progenitors and platelet recovery after high dose chemotherapy and peripheral blood stem cell transplantation

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ABSTRACT

Background and Objective. Randomized clinical trials have shown that peripheral blood stem cell transplantations (PBSCT) with appropriate doses of CD34+ cells are associated with rapid, complete and sustained recovery of marrow functions. Nevertheless, in a minority af patients delayed platelet recovery may occur and it remains to be established whether analysis of transplanted CD34+ cell subsets may demonstrate correlation with this phenomenon. We studied a series of 80 consecutive transplanted patients with the aim of evaluating the effect of CD34+ stem cell numbers and, in a subgroup of 32 patients, the effect of the lineage specific subset numbers on time to platelet engraftment (i.e. time to platelet counts higher than 20×10^9/L for two consecutive days without the need for platelet transfusions).

Design and Methods. Different clinical and paraclinical factors were examined in a multivariate analysis for effect on platelet engraftment in 80 patients.

Results. The number of CD34+ cells/kg infused was the most important factor predicting the time to platelet engraftment. Patients receiving more than 10×10^6 CD34+ cells/kg had prompt platelet engraftment. The majority of the patients (78%) received fewer than 10×10^6 CD34+ cells/kg and 17/62 (27%) of these patients experienced delayed platelet engraftment. In 32 patients receiving fewer than 10×10^6 CD34+ cells/kg we focused on the content of different lineage specific CD34+ subsets in the PBSC products. The most significant correlation was recognized for CD34+/CD61+ megakaryocytic cell number and platelet engraftment. An inverse correlation between the CD34+/CD38− subset and platelet engraftment was found, indicating that a high number of CD34+/CD38− in the PBSC product might increase the risk for delayed engraftment. These results were further confirmed by the observation that patients who experienced platelet engraftment after day 20 had significantly more CD34+/CD38− cells/kg infused than patients with fast engraftment.

Interpretation and Conclusions. The number of total CD34+ cells/kg infused was the most important factor predicting time to platelet engraftment. CD34+

subset analysis in a subgroup of patients suggests that a high number of uncommitted progenitors may be associated with slower platelet recovery than transplantation with a higher fraction of more committed peripheral blood stem cells.
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High dose chemotherapy supported by infusion of autologous hematopoietic stem cells has been shown to be a feasible and safe treatment modality for a variety of malignant diseases. Hematologic recovery seems to be more rapid after infusion of peripheral blood stem cells (PBSC) than after bone marrow derived stem cells.[1,2]

Several studies have focused on various factors influencing engraftment after peripheral blood stem cell transplantation and all have found that the number of CD34+ cells infused is of significant importance to engraftment although the number of CD34+ cells resulting in fast and safe engraftment vary in the different studies.[3-6]

The CD34+ cells represent a heterogenous cell population consisting of both committed progenitors as well as primitive uncommitted and pluripotent stem cells.[7] The majority of the CD34+ cells are already committed to either erythroid, megakaryoid, myeloid or lymphoid cell lineages.[8,9] The most primitive hematopoietic stem cells have been shown to lack expression of the CD38 antigen (CD34+CD38−), whereas more differentiated committed progenitors express both CD34 and CD38 antigens as well as other lineage markers.[8,10-11]

One major clinical problem in autologous stem cell transplantation is the risk of late platelet engraftment which is seen in 10-20% of patients transplanted.[12,13] In an attempt to predict the risk before transplantation we performed the present study. The purpose was to evaluate the influence of different clinical factors as well as the number of lineage specific progenitor subsets on engraftment.

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Design and Methods

Patients

Patients referred consecutively between September 1992 and September 1996 to the Department of hematology and Department of Oncology, Herlev Hospital, University of Copenhagen and Department of Hematology and Oncology, Odense University Hospital for high dose chemotherapy with autologous stem cell support were included in this study. The characteristics of the 80 patients are given in Table 1.

Mobilization and stem cell collection

Different priming procedures were used as shown in Table 1. rhG-CSF alone was given at a dose of 10 µg/kg/day subcutaneously to 17 patients. In 38 patients stem cells were mobilized with high dose (4 g/m²) cyclophosphamide (HDCy) supported by MES-NA and hydration as described by To *et al.*[14] At the end of the nadir period, rhG-CSF was added at a dose of 10 µg/kg/day given as subcutaneous injections. In 18 breast cancer patients stem cells were mobilized with CEF (cyclophosphamide 1200 mg/m², epirubicin 60 mg/m², 5-fluorouracil 600 mg/m²) administered on day 1 followed by rhG-CSF 10 µg/kg/day from day 2 until leukapheresis was completed. In 7 cases stem cells were harvested after priming with other chemo-

Table 1. Characteristics of 80 patients

Gender	
F/M	51/29
Age	
median (range)	44 years (15 -65)
Diagnosis	
Hodgkin's disease	13
NHL	24
Multiple myeloma	12
Breast cancer	29
AML	2
Disease status at transplant	
Remission	25
Relapse	55
Stem cell source	
PBSC alone	66
PBSC + BM	14
G-CSF post transplant	
Yes	37
No	43
Priming procedure	
rhG-CSF alone	17
HDCy* + rhG-CSF	38
CEF° + rhG-CSF	18
Other	7

HDCy = high dose cyclophosphamide (4 g/m²); °CEF = cyclophosphamide, epirubicin, 5-fluouracil.

therapeutic regimens such as mini-BEAM (BCNU 60 mg/m² on day 1, etoposide 75 mg/m² on days 2 to 5, cytarabine 200 mg/m² on days 2 to 5, melphalan 30 mg/m² on day 6) in two cases, ABVD (adriamycin 25 mg/m², bleomycin 10 mg/m², velbe 6 mg/m², dacarbazine 375 mg/m² all administered on day 1) in one case, VP16 (800 mg/m² for four days) in one case, MIME (mitoguazone 400 mg/m² on day 1, ifosfamide 750 mg/m² on days 1 to 5, methotrexate 25 mg/m² on day 3, etoposide 100 mg/m² on days 1 to 3) in one case and cisplatin (75 mg/m² on day 1) and taxol (225 mg/m² on day 1) in two cases. In all seven cases rhG-CSF 10 µg/kg/day was administered from day 1 until leukaphereses were completed.

In all priming procedures blood levels of CD34+ cells were monitored daily during priming and mobilization and leukapheresis was performed when blood levels of CD34+ cells exceeded 20×10^3/mL blood.[15]

Leukaphereses continued until a total yield of at least 2×10^6 CD34+ cells/kg was obtained. Leukapheresis was performed on a Fenwal CS 3000 Plus blood cell separator (Fenwal Laboratories, Deerfield, IL, USA). A total blood volume of 10 L per apheresis was processed at a flow rate of 50-70 mL/min. If a sufficient yield ($\geq 2 \times 10^6$ CD34+ cells/kg) was not obtained within 5 days of leukapheresis an additional bone marrow harvest was performed. Bone marrow harvest was performed under general anesthesia 5 days after the first day of treatment with rhG-CSF 10 µg/kg/day. A total volume of 1000 mL bone marrow was harvested.[16]

Leukapheresis and bone marrow products were cryopreserved in DMSO using a controlled rate liquid nitrogen freezer (Planer Ltd. Biomed, Sunbury-on-Thames, United Kingdom) and stored in liquid nitrogen until reinfusion.

Progenitor cell estimations

In each leukapheresis product the number of CD34+ cells was enumerated before freezing in accordance with previously described recommendations from the Nordic Stem Cell Laboratory Group.[17,18]

Briefly: a sample containing $0.5-1.0 \times 10^6$ mononuclear cells in a volume of 50 µL were incubated with test and control antibody (anti-CD34 = HPCA-2-PE, control antibody = IgG1PE both from Immuno-Systems Becton Dickinson [BDIS]) in a final dilution 1:10. The samples were incubated for 15 minutes at room temperature in the dark, lysed with 2 mL Ortho Lysing solution for 10 min and washed 2-3 times with and resuspended in 0.3 mL PBS containing 10% newborn calf serum. Flow cytometry analysis was performed on a FACScan (BDIS) using CellQuest 1.1 software (BDIS) by collection of 50,000 events in list mode with debris eliminated. The estimation of CD34 positive cells was done after SSC/FL-2 marking of one positive population. In quadrant statistics the population was identified in the upper left quadrant, among cells with a side scatter profile as lymphocyt-

ic cells. The negative control was substracted if it exceeded 0.05%.

Subsets of CD34+ cells expressing various lineage specific and adhesion antigens were quantified from thawed samples of each frozen leukapheresis product. The subset analysis of CD34+ cells was performed on a minimum of 1000 cells acquired in a CD34 SSC/fluorescence gate. Cells were incubated and double-stained with anti-CD34 PE or FITC conjugated moAbs plus one of the following conjugated moAbs: anti-CD33 FITC (My 9, Coulter Clone), anti-CD38 PE (Leu 17 PE, BDIS), anti-GlyA FITC (DAKO A/S), anti-CD61 FITC (BDIS), anti-CD54 FITC (ICAM-1, Immunotech) or anti-CD11a FITC (LFA-1, Immunotech).

The absolute number of the various CD34+ subsets in each days' leukapheresis product was calculated by multiplication of the total number of CD34+ cells/kg enumerated at the day of harvest with the percentage of the mentioned subsets analyzed on a frozen/thawed sample from that days leukapheresis product. In patients transplanted with more than one leukapheresis product the number of CD34+ subsets from each product was added to find the total number of CD34+ subsets infused. The calculation was valid because we have not found selective loss of CD34+ subsets during the freezing and thawing procedure (own unpublished results).

Conditioning regimens

Patients with non-Hodgkin lymphomas and Hodgkin's disease received BEAM (BCNU 300 mg/m² on day –7, etoposide 1600 mg/m² on days –6 to –3, cytarabine 800 mg/m² on days –6 to –3, melphalan 140 mg/m² on day –2) except for two cases. They were treated with total body irradiation (TBI) (8 Gy) and cyclophosphamide 60 mg/m².

Breast cancer patients received *5FU,A,C,Cis* (5-fluouracil 1000 mg/m² on days –14 to –10, adriamycin 125 mg/m² on day –5, cyclophosphamide 2500 mg/m² on days –5 to –3, cisplatin 55 g/m² on days –5 to –3) in 7 cases, *Cis, C, BCNU* (cisplatin 55 mg/m² on days –6 to –4 , cyclophosphamide 1875 mg/m² on days –6 to –4, *BCNU* 600 mg/m² on day –3) in 2 cases, *Ca, No, C* (carboplatin 200 mg/m², novantrone 15 mg/m², cyclophosphamide 1500 mg/m² on days –7 to –3) in 5 cases, *Ca, Th, C* (carboplatin 200 mg/m², thiotepa 125 mg/m², cyclophosphamide 1500 mg/m² on days –7 to –3) in 15 cases.

Twelve patients with multiple myeloma received melphalan 200 mg/m² on day –2.

Finally, 2 patiens with AML received a total dose of busulphan 16 mg/kg on days –7 to –3 and cyclophosphamide 120 mg/kg on days –7 to –5.

Supportive care

A total of 37 patients received subcutaneous injections of rhG-CSF at a dose of 10 μg/kg daily starting 24 h after transplantation until an ANC of 0.5×10⁹/L was reached for 2 consecutive days. Prophylactic or therapeutic antibiotic treatments were given according to local routines. Platelets and red blood cells were also transfused according to the policy of each participating center.

Engraftment

The day of PBSC infusion was designated as day 0. Platelet engraftment was defined as the first of two consecutive days on which the platelet count exceeded 20×10⁹/L unsupported by transfusions.

Only half of the patients received rhG-CSF after transplantation. Growth factor treatment post-transplant has been shown to reduce time to neutrophil engraftment by 3-6 days but not to influence platelet engraftment.[3-4,6,19] Nevertheless, the influence of post-transplantation rhG-CSF on platelet engraftment was evaluated.

Statistical analysis

The time to platelet engraftment was compared by the log rank test. Patients who died before engraftment was achieved were censored on the date of death.

A multiple regression analysis was performed to identify variables influencing platelet engraftment. The following variables were analyzed for effect on platelet engraftment: age by decade, gender, diagnosis, disease status at transplant (remission or active disease), priming procedure ([1]rhG-CSF alone, [2]HDCy plus rhG-CSF, [3]CEF plus rhG-CSF, [4]other regimens), number of PBSC collections infused (harvested on 1 day, 2 days, 3 days or more), previous chemotherapy (mildly treated defined as one induction chemotherapy course despite disease, moderately treated defined as induction chemotherapy plus radiotherapy, heavily treated defined as more than the two other groups), bone marrow function evaluated by blood counts (abnormal function defined as low leukocyte count (white blood cell count < 3.0×10⁹/L) and/or low platelet count (platelets < 250×10⁹/L), normal function defined as normal leucocyte as well as platelet count). The speed of mobilization was evaluated by the number of days from the start of the mobilization regimen to the day the CD34+ blood level exceeded 20×10³/mL. Based on the distribution of days in the different priming procedures two groups could be identified; slow and fast mobilizers, which were included in the multivariate analysis. The CD34+ blood level at the first day of leukapheresis and finally the number of CD34+ cells/kg infused at transplant assigned as a continous variable were also included in the multivariate analysis.

In all tests a *p* value < 0.05 was considered significant.

Results

The overall median time to platelet engraftment was 15 days (range = 9-65 days). Seven patients died before achieving platelet engraftment: on days +6, +9, +15, +37, +56, +62 and +65, respectively. One patient
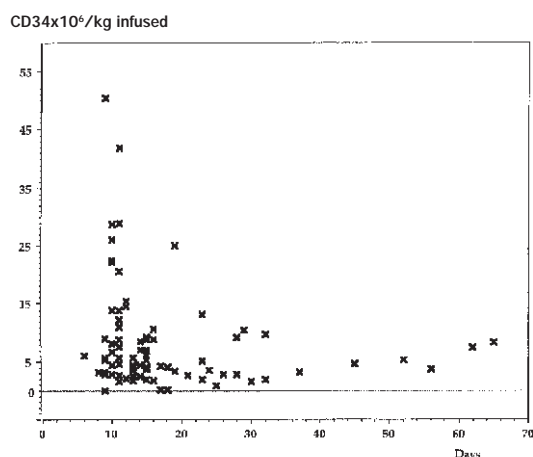
CD34x10⁶/kg infused

**Figure 1. Correlation between the number of CD34⁺ cells ×
10⁶/kg infused and time to platelet count more than 20×
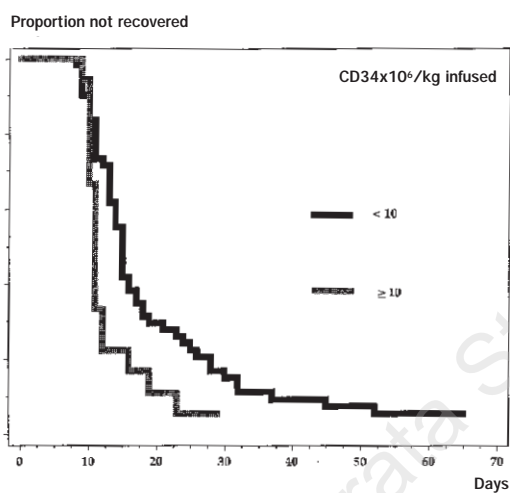10⁹/L unsupported by transfusions.**

Proportion not recovered

**Figure 2. The probability of achieving platelet count ≥ 20×
10⁹/L (unsupported) for CD34 cell dose < 10×10⁶/kg
(black line) and ≥ 10×10⁶/kg (grey line).**

had not achieved engraftment on day +29 and
received PBSC back-up on that day and achieved sub-
sequently stable platelet function. That patient was
censored with regard to platelet engraftment on day
+29.

A median of 5.26×10^6 CD34⁺ blood derived
cells/kg (range: 0.03-50.33) were infused. Fourteen
patients, 11 of whom had less than 2.5×10^6 PBSC/kg
infused were given additional bone marrow. The
remaining 3 patients had had sufficient PBSC har-
vested but additional bone marrow was reinfused as
part of the safety procedure when our center changed
from a policy of bone marrow to PBSC transplanta-
tion. In multivariate analysis the number of CD34⁺
cells/kg infused (Chi-square = 9.133, $p = 0.0025$) was
found to be the only factor significantly influencing
time to platelet engraftment.

The correlation between days to platelet engraft-
ment and number of CD34⁺ cells infused is given in
Figure 1.

The median time to platelet engraftment in 37
patients receiving rhG-CSF post-transplantation was
15 days (range: 6-45 days) compared to 13 days
(range 8-65 days) in 43 patients without rhG-CSF
treatment post-transplantation. The difference was
not statistically significant ($p = 0.36$).

To analyze further at which dose rapid and safe
engraftment would occur, a sequential dose analysis
of CD34⁺ cells infused was performed. A significant
difference in time to platelet engraftment was found
at a cell dose $\geq 10 \times 10^6$ CD34⁺ cells/kg infused ($p =
0.0199$). The Kaplan-Meier probability for platelet
engraftment depending on the CD34⁺ cell dose being
above or below 10×10^6 CD34⁺ cells/kg infused is giv-
en in Figure 2. This figure illustrates that more than
30% of the patients transplanted with a CD34⁺ cell
dose below 10×10^6/kg experienced late platelet
engraftment (i.e. thrombocytopenia after day 20).
The patients receiving fewer than 10×10^6 CD34⁺
cells/kg did not differ with regard to clinical charac-
teristics from patients receiving more than 10×10^6
CD34⁺ cells/kg.

CD34⁺ subset analysis and engraftment

Leukapheresis products were available for CD34⁺
subset analysis in 46 patients. A total of 89 leuka-
pheresis products were analyzed. There was no dif-
ference in the distribution of subsets between leuka-
pheresis products collected on the first, second or
third day (results not shown). Patients receiving more
than 10×10^6 CD34⁺ cells/kg achieved prompt and
rapid platelet engraftment. Consequently, the group
of interest is the 62 patients into whom fewer than
10×10^6 CD34⁺cells/kg were infused. In 17/62 (27%)
of these patients delayed platelet engraftment,
defined as platelet engraftment beyond day 20, was
seen. For 32 of the patients with fewer than 10×10^6
CD34⁺ cells/kg infused, frozen samples were avail-
able and CD34 subset analysis was performed.

Spearman Rank correlations were performed to
correlate the numbers of calculated CD34⁺ subsets
reinfused in the 32 patients with time to platelet
recovery (Table 2). Surprisingly, the total number of
CD34⁺ cells (i.e. below 10×10^6/kg) did not correlate
with platelet engraftment in this subgroup of
patients. However, the analysis identified a signifi-
cant correlation with the CD34⁺ CD61⁺ cell number
($r = -0.36$, $p = 0.04$). Interestingly, an inverse corre-
lation was found between the uncommitted CD34⁺
CD38⁻ subset and time to platelet engraftment ($r =
0.53$, $p = 0.003$), indicating a high risk of delayed
engraftment when a high number of CD34⁺ CD38⁻
cells/kg was infused (Figure 3). In Figure 3 a single
outlier can be identified (upper right side) and a fur-
ther analysis was performed with this outlier omit-
ted, but the same correlation was still found ($r =

Table 2. Correlation of subsets of CD34+ cells with time to platelet engraftment in 32 patients who had fewer than 10×10⁶ CD34+ cells/kg infused.

	Correlation coefficient	*p*
All CD34+ cells	-0.05	NS
Subsets of CD34+ cells		
CD34+CD61+	-0.36	0.04
CD34+GlyA+	-0.06	NS
CD34+CD33+	0.07	NS
CD34+CD33-	-0.06	NS
CD34+CD38+	-0.11	NS
CD34+CD38-	0.53	0.003
CD34+CD54+	0.13	NS
CD34+CD11a+	-0.09	NS

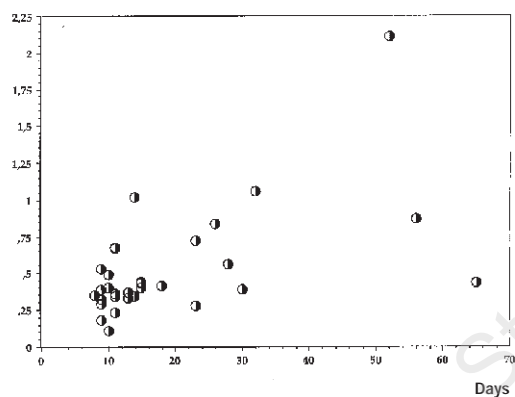**CD34+ CD38- cells x10⁶/kg infused**

Figure 3. Correlation between the number of CD34+CD38- cells ×10⁶/kg infused and time to platelet count more than 20×10⁹/L unsupported by transfusions.

0.51, *p* = 0.007). Subsets defined and enumerated by expression of other lineage or adhesion antigens did not correlate with platelet engraftment.

Patients with slow platelet engraftment (after 20 days) had a significantly higher number of uncommitted progenitors (CD34+CD38- cells) infused than patients with fast engraftment (before day 20) (Table 3), confirming the results from the Spearman Rank correlation. There was no significant difference between the numbers of other CD34+ subsets infused and fast and slow engraftment (Table 3).

Discussion

One major clinical problem in PBSC transplantations is delayed platelet engraftment manifested as prolonged thrombocytopenia. There may be several explanations for this phenomenon, including severe post-transplantation infections, regimen related toxicity or inadequate quality of the stem cell graft. In an

Table 3. Median number and range of CD34+ subsets ×10⁶/kg infused in patients with slow and fast platelet engraftment.

	Platelet engraftment (platelets > 20×10⁹/L)		
	Fast (within 20 days)	*Slow (after 20 days)*	*p*
N	23	9	
CD34+CD61+	0.09 (0.02–0.27)	0.09 (0.14–0.83)	NS
CD34+CDGlyA+	1.52 (0.29–3.44)	1.80 (0.29–4.17)	NS
CD34+CD33+	0.84 (0.07–2.30)	0.61 (0.10–2.79)	NS
CD34+CD33-	3.58 (2.23–8.02)	2.89 (1.07–9.28)	NS
CD34+CD38+	4.24 (2.06–16.16)	3.19 (1.16–8.72)	NS
CD34+CD38-	0.39 (0.11–1.02)	0.72 (0.28–2.11)	0.02
CD34+CD54+	1.09 (0.24–4.55)	1.48 (0.28–2.61)	NS
CD34+CD11a+	2.53 (1.44–7.91)	3.38 (0.88–5.00)	NS

attempt to identify the group of patients at risk of prolonged thrombocytopenia we studied a series of transplanted patients with the aim of evaluating the effect of CD34+ progenitor and stem cell numbers and lineage specific subset numbers on time to platelet engraftment.

The present study confirmed that the number of CD34+ cells/kg infused at transplant was the most important factor influencing the time to platelet engraftment. This dose-response relationship is in accordance with previous studies.[3-6,20] The largest study including 692 patients found the numbers of mobilization procedures to achieve sufficient numbers of CD34+ cells of significant influence on engraftment after myeloablative therapy.[6] We, too, evaluated the mobilization *tempo* i.e. time to obtain a sufficient CD34+ cell blood level during the priming procedure and the number of mobilization procedures but did not find that these factors had any influence on engraftment.

The relationship between CD34+ cell dose and time to platelet engraftment is non-linear (Figure 1).[4,6,20,21] Several investigators have tried to define a threshold above which prompt engraftment occurs. We found this threshold to be 10×10⁶ CD34+ cells/kg infused which is somewhat higher than that found in other studies. In two studies the threshold was found to be 5×10⁶ CD34+ cells/kg,[4,6] whereas others have found

the threshold to be 4×10^6 CD34+ cells/kg[5] or 3×10^6 CD34+ cells/kg.[3] The different threshold found in different studies may be explained by the method used for enumeration of CD34+ cells. Without doubt there are major differences in the flow cytometry techniques used for identification and enumeration of stem cell products.[12,21] This problem points to the need for each laboratory to compare its actual technique with others.[23]

Patients receiving more than 10×10^6 CD34+ cells/kg had prompt platelet engraftment in our study. However, the majority of the patients (78%) received fewer than 10×10^6 CD34+ cells/kg and 17/62 (27%) of these patients experienced delayed platelet engraftment. It has previously been shown that peripheral blood CD34+ cells display heterogenous surface menbrane differentiation antigens analogous to bone marrow stem cells.[24] In those patients receiving fewer than 10×10^6 CD34+ cells/kg we focused on the content of different lineage specific CD34+ subsets in the PBSC products. The most significant correlation was recognized for CD34+CD61+ cell number and platelet engraftment. The CD61 antigen is known as a megakaryocytic lineage marker[25] identifying early progenitors which are expected to give rise to regeneration of megakaryopoiesis after transplantation.[26] This confirms the findings of Dercksen et al. who found a correlation between CD34+ CD41+ cell dose and platelet engraftment; CD41 being another megakaryocytic marker.[20] These findings are in accordance with an earlier study showing an increase in peripheral blood megakaryocyte progenitors (CFU-Meg) following administration of high-dose cyclophosphamide plus hematopoietic growth factors.[27]

In our analysis of uncommitted progenitors we found an inverse correlation between the CD34+ CD38- subset and time to platelet engraftment, indicating that high numbers of CD34+ CD38- cells might increase the risk of delayed engraftment. These results were further confirmed by the observation that patients who experienced delayed platelet engraftment (after day 20) had significantly more CD34+ CD38- cells/kg infused than patients with fast engraftment. This is the first time an inverse relationship betweeen primitive progenitors CD34+ CD38- cells) and late platelet engraftment has been recognized. This contrasts with results from Buscemi et al.[28] who reported a significant positive correlation between CD34+ CD38- cells and platelet engraftment, concluding that a high proportion of CD34+ CD38- cells infused would produce fast engraftment. This correlation was, however, found in only a small series of 7 patients all with fast engraftment.

The CD38 antigen is expressed on more differentiated cells and is not present on primitive progenitors.[8,29] The CD34+ CD38- cell population produces colonies later than more mature and differentiated cells CD34+ CD38+ cells).[30,31] CD34+ CD38+ cells are highly responsive to proliferative signals, unlike CD34+ CD38- cells which remain relatively dormant even after exposure to cytokines.[32,33] Hematopoietic stem cells responsible for rapid engraftment consist of cells that most likely are highly proliferative and short-lived, while cells capable of long-term engraftment comprise primarily a non-cycling population.[34] On this background it may be reasonable to speculate that the CD34+ CD38+ subpopulation may be responsible for rapid engraftment whereas the CD34+ CD38- subpopulation may be responsible for long-term engraftment in humans as has been shown to be the case in a sheep model.[11] Consequently, reinfusion of stem cell products with a high number of CD34+ CD38- cells would be expected to delay engraftment as indicated in this study. Whether high numbers of CD34+ CD38- cells in the reinfused stem cell product are associated with better long-term hematopoiesis could not be answered in this study, and will have to await gene marking studies in the future.

It is still an open question why some patients mobilize high numbers of uncommitted progenitors into blood during the priming regimen. One very simple explanation could be that in the patient population in question the hematopoietic progenitor compartment may have undergone a shift from a normal distribution to a high content of CD34+ CD38- cells. Such a shift could be the consequence of chemotherapy with a selective inhibitory effect on late CD34+ CD38+ progenitors. Very recently we have found such a selective shift in the bone marrow of multiple myeloma patients after treatment with melphalan,[35] an agent which is known to influence the mobilization capacity and engraftment quality of PBSC products (own unpublished observation).[36]

No other lineage committed subsets of CD34+ cells correlated with the time to platelet engraftment. The GlyA antigen, being a erythroid lineage marker, and the CD33 antigen, being a myeloid marker, would not be expected to affect platelet engraftment. Reticulocyte and neutrophil recovery may have been affected by CD34+GlyA+ and CD34+CD33+ subsets[37] respectively but, they were not evaluated in this study.

CD54 (ICAM-1) is a ligand for CD11a (LFA-1).[38] Both CD11a and CD54 are expressed to varying degrees on CD34+ progenitor cells as well as on differentiating cells of several lineages.[39] The LFA-1–ICAM-1 interactions are supposed to be important in the adhesion of stem cells to bone marrow stromal matrix.[40] However, neither the CD34+CD54+ nor the CD34+ CD11a+ subsets correlated with platelet engraftment which is in accordance with results from a previous study.[21]

The conclusion from this analysis is that infusion of a PBSC product with fewer than 10×10^6 CD34+ cells/kg containing a high number of uncommitted progenitors (CD34/CD38- cells) very likely increases the risk of late platelet engraftment. This model of

predicting prolonged thrombocytopenia in PBSC transplantations needs to be evaluated in co-operative studies between many clinical centers and their laboratories.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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