Determining the Time of Harvesting Peripheral Blood Stem Cells Using the HPC, for Monitoring Hematopoietic Progenitor Cells, of the Automated Hematology Analyzer XE-2100

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We have been using the CD34$^+$ cell count or the Hematopoietic progenitor cell (HPC) count to determine the adequate time to harvest peripheral blood stem cells. HPC counting is convenient way, using the IMI channel of the Automated Hematology Analyzer XE-2100 (Sysmex, Japan), with samples taken for CBC. In this study, we analyzed 73 patients of autologous stem cell transplantations or allogenic peripheral blood stem cell donors. Stem cells were harvested from 53 subjects, using a combination of chemotherapy and G-CSF, at a time when the HPC count was 20/μL or more. A total of 77 harvests were performed, sufficient number of CD34$^+$ stem cells could be obtained in 69 (89.6%) of these harvests. On an average, 6.04×10^6/kg CD34$^+$ could be obtained per stem cell harvest, which was sufficient for tandem transplantation in myeloma cases. There was positive correlation between the number of CD34$^+$ cells harvested and the peripheral blood HPC count. Mobilization was done through G-CSF administration alone in the case of donors. Harvesting was done when the peripheral blood CD34$^+$ cell count was 10/μL or more, and the HPC count was also taken simultaneously. Twenty six harvests were done from 16 donors, 1 million or more CD34$^+$ cells per kg body weight could be harvested in 23 (88.5%) harvests. There was no significant correlation between the number of harvested CD34$^+$ cells and the peripheral blood HPC count (r=0.346, P=0.0836). The peripheral blood CD34$^+$ cell count became 10/μL or more in 4 donors on the 4th day of stem cell mobilization, but HPC remained at less than 10/μL, and although their HPC count exceeded 20/μL on the 5th day. Although the time of harvesting could be determined in autologous stem cell harvest using a combination of chemotherapy and G-CSF, it could not be decided based on the HPC count alone in case of allogenic donors.

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Key Words Automated Hematology Analyzer, XE-2100, Stem Cell Harvest, Transplantation, Donor, HPC

INTRODUCTION

Autologous stem cell transplantation is performed for saving the bone marrow of patients who are likely to be cured, or their survival extended, by high-dose chemotherapy or radiotherapy. In such cases, peripheral blood is generally chosen at present in preference to bone marrow as the source of the stem cells. Peripheral blood stem cells are harvested as an alternative for bone marrow cells in allogenic hematopoietic stem cell transplantation also. In the harvesting of autologous peripheral blood stem cells, the bone marrow is usually suppressed first by chemotherapy and G-CSF is administered around the recovery period before harvesting the stem cells. The stem cells are mobilized through administration of G-CSF alone for harvesting allogenic peripheral blood stem cells. The feasibility of stem cell transplantation is decided on the basis of the CD34$^+$ cell count per kg body weight, and the time of harvesting the stem cells is normally decided on the basis of the CD34$^+$ cell count in the peripheral blood. Conventionally, a flow cytometer that uses a fluorescent antibody is generally used for counting the CD34$^+$ cells, which requires at least 2 hours even when done by an experienced technician. There is therefore a need for a much simpler and faster method of determining the suitable time of harvest. The IMI channel of Sysmex automated hematology analyzer is very useful for obtaining the hematopoietic progenitor cell (HPC) count because the procedure is very simple and samples for CBC can be used[1-5]. We had determined the time of harvest on the basis of the HPC count measured with the Sysmex Automated Hematology Analyzer XE-2100 (XE-2100; Sysmex, Japan) after mobilizing stem cells with G-CSF post-chemotherapy. There are some reports that claim that there is no correlation between the HPC count and the CD34$^+$ cell count when peripheral blood stem cells are harvested for allogenic transplantation. We therefore examined, in the present study, the relationship between the HPC count and the CD34$^+$ cell count when stem cells are mobilized by giving G-CSF only for autologous or allogenic stem cell transplantation.

Table 1 *Patients' Characteristics*

Patient Diagnosis	n	Age (mean±SE)	Sex M/F	Stem Cell Auto	Stem Cell Allo	Chemotherapy Yes	Chemotherapy No
leukemia	10	38.2±13.9	7/3	1	9	1	9
lymphoma	29	44.3±11.9	18/11	25	4	25	4
myeloma	20	51.7±9.9	14/6	17	3	17	3
others	10	32.9±8.4	9/1	11	0	10	0
total	69	43.3±13.1	49/24	57	16	53	20

MATERIALS AND METHODS

Study subjects

Patients who were assessed fit for autologous or allogenic peripheral stem cell transplantation and stem cell donors were included in the study, which was conducted during the 3 years from September 6, 2002 to September 5, 2005. There were 69 patients with leukemia (n=10), lymphoma (n=29), multiple myeloma (n=20), or other solid tumors (n=10) (***Table 1***). When both chemotherapy and G-CSF are given before harvesting the stem cells, 400μg/m^2 of filgrastim was subcutaneously injected at the recovery stage from chemotherapy for mobilizing the stem cells. Fifty three patients received this combination mobilization treatment. Chemotherapy before harvesting stem cells consisted of high-dose AraC therapy (cytarabine 24g/m^2, mitoxantrone 14mg/m^2) for acute leukemia, ESHAP therapy (etoposide 160 mg/m^2, mPSL 2,500mg, cytarabine 2g/m^2, CDDP 100mg/m^2) or ACES therapy (where the CDDP of ESHAP is replaced by CBDCA 400mg/m^2) for non-Hodgkin's lymphoma, high-dose cyclophosphamide (4g/m^2) treatment for multiple myeloma, and mini-ICE therapy (ifosphamide 5g/m^2, CBDCA AUC=5, etoposide 300mg/m^2) for germ cell tumor and neuroblastoma. For the 16 allogenic peripheral blood stem cell donors, 400μg/m^2 of filgrastim was administered subcutaneously every day, and harvesting was done 1 or 2 times during the 4th to the 6th day of filgrastim administration.

HPC counting

In HPC counting, sometimes, the most stable results are obtained when the measurement is made within 4 hours of sampling with EDTA-2K[5]. Therefore, we made measurements with an XE-2100 using the sample left over after CBC, within 1 hour of sampling. The method of measurement was the same as in the earlier study[1]. Briefly, the principle of measurement is as follows: Mature cells and erythrocytes are lysed with a hemolytic reagent; STOMATOLYSER IM (Sysmex). The immature leukocytes remaining are classified two dimensionally using direct current (DC) and radio frequency (RF), to count the HPC.

CD34 counting

The percentage of CD34$^+$ cells was determined with a flow cytometer; FACSCalibur (Beckton Dickinson [BD], USA), using an FITC-labeled CD34 antibody (HPCA-2, BD) and a PerCP-labeled CD45 antibody (H-Lel, BD).

Harvesting of stem cells

A CD34$^+$ cell count of 10/μL or more was used as the criterion for suitability for harvesting stem cells. An HPC count of 20/μL or more was taken as the criterion because it is known that the HPC count is generally double that of CD34$^+$ cells[5-7]. G-CSF was administered 4 hours before the harvest because it has been reported that the harvesting efficiency is reduced when the time between its administration and the harvest is short[8]. COBE Spectra Apheresis System (Gambro BCT Inc, USA) were used for harvesting. Basically using the Auto-PBSC program (version 6.1), the number of harvests was adjusted when the monocyte count was high. Normally, 1-3 stem cell harvests are done in a series. It is considered that if more than a million CD34$^+$ cells per kg body weight could be obtained in a harvest, enough stem cells for transplantation can be obtained in one harvest series. For this reason, a harvest of a million CD34$^+$ cells per kg body weight was defined as an effective harvest and one that yielded fewer than a million cells as an ineffective harvest.

RESULTS

HPC count and CD34$^+$ cell count

A correlation trend was seen between the peripheral blood HPC count and peripheral blood CD34$^+$ cell count when these were measured at the same time initially in a few patients from whom the stem cells were to be harvested (***Table 2***). There are reports that there is a correlation between the HPC count and CD34$^+$ cell count[1, 2, 4]. So, only one of these cell counts was taken in the subsequent screening for stem cell harvests after chemotherapy followed by G-CSF administration.

Stem cell harvesting after chemotherapy and G-CSF administration

Table 3 shows the result of mobilization with G-CSF at

Table 2 *CD34 and HPC in peripheral blood*

No	HPC before harvest (/μL)	$CD34^+$ before harvest (/μL)	HPC in harvest bag (/μL)	$CD34^+$ in harvest bag (/μL)	stem cell transplantation
AU1	160	148	1580	8008	Y
AU2	205	215	10900	10954	Y
AU3	164	68	7770	6982	Y
AU4	25	96	700	7221	Y
AU5	242	203	4640	13631	Y
AU6	99	28	9590	2065	Y

Table 3 *Stem cell harvest mobilized with chemotherapy and G-CSF*

Patient		Harvest			Regression		HPC(/μL)#	CD34(10^6/Kg)##
diagnosis	n	n	E*	L**	r	p	Mean±SE	Mean±SE
leukemia	1	2	1	1			64	1.05
lymphoma	24	40	34	6	0.447	0.0034	187.58±24.93	5.19±1.22
myeloma	18	23	23	0	0.701	0.0001	220.26±34.55	8.61±1.38
others	10	12	11	1	0.100	0.7521	227.71±79.61	4.30±1.00
total	53	77	69	8	0.400	0.0003	208.87±22.05	6.04±0.79

#: HPC count in pheripheral blood
##: $CD34^+$ cell count per donor body weight in collected bag
E*: effective apheresis CD34≧1.0×10^6/Kg
L**: less effective apheresis CD34<1.0×10^6/Kg

the stage of recovery from bone marrow suppression caused by treating the primary disease. The results of 77 peripheral blood stem cell harvests from 53 patients (1 with leukemia, 24 with lymphoma, 18 with multiple myeloma, and 10 with other solid tumors) showed that 69 (89.6%) of them were effective harvests. Eight of the harvests were ineffective. However, 2 million or more $CD34^+$ cells per kg body weight could be obtained from all the patients in subsequent harvests. The mean $CD34^+$ cell count per kg body weight per stem cell harvest was 5.19×10^6/kg in malignant lymphoma patients, 8.61×10^6/kg with multiple myeloma, and 4.30×10^6/kg with other tumors. Thus, the harvest tended to be bigger in multiple myeloma patients. The overall mean harvest was 6.04×10^6/kg, and the mean number of harvests was 1.45, one harvest being sufficient in most cases. Linear regression analysis showed significant correlation between the peripheral blood HPC count and the number of $CD34^+$ cells harvested (r=0.400, P=0.0003), the correlation being strongest (r=0.701, P=0.0001) in the multiple myeloma patients. No correlation was observed with other solid tumors (r=0.100, P=0.7521).

Harvesting of allogenic peripheral blood stem cells

Table 4 shows the results of stem cell harvests with subcutaneous injection of 400μg/m^2 filgrastim from allogenic peripheral blood stem cell donors (n=16) and patients scheduled for autologous peripheral blood stem cell transplants (n=4). A total of 26 harvests were made from the donors out of which 23 (88.5%) were effective and 3 ineffective. The number of $CD34^+$ cells obtained from the donors per harvest was 2.68×10^6/kg body weight. Two million or more $CD34^+$ cells could also be obtained per kg body weight of the recipients in all but one case. In one case, two harvests yielded 1.97 million $CD34^+$ cells per kg body weight of recipient. So, the case was therefore considered transplantable, and no further harvesting was done from this person. There was no correlation between the peripheral blood HPC count and the number of $CD34^+$ cells harvested (r=0.346, P=0.0836). In some cases, the HPC count was low in spite of the large number of $CD34^+$ cells present. ***Table 5*** lists the cases where the HPC count was less than 20/μL in spite of the $CD34^+$ cell count being 10/μL or higher, in the peripheral blood. The HPC count remained low in all cases up to the 4^{th} day of G-CSF administration, and it rose on the 5^{th} day.

DISCUSSION

There are various reports about using the HPC count of the peripheral blood as a criterion for suitability for harvest when mobilizing stem cells by administering G-CSF after chemotherapy. For instance, Suh et al.[9] have reported a minimum requirement of 5/μL, Pollard et a[l6] 10/μL, Karaai et al.[3] 20/μL, and Lee et al.[10] 50/μL. In our present study, 60/69 (89.6%) harvests were found to be effective when the minimum requirement of HPC was set at 20/μL. Considering that the lowest level of peripheral blood HPC count among the effective harvests was 25/μL

Table 4 *Stem cell harvest mobilized with G-CSF alone*

Patient		Harvest			Regression		HPC(/μL)#	CD34(10^6/Kg)##
Stem cell	n	n	E*	L**	r	p	Mean±SE	Mean±SE
allogenic	16	26	23	3	0.346	0.0836	60.00±10.84	2.68±.32

#: HPC count in pheripheral blood
##: $CD34^+$ cell count per donor body weight in collected bag
E*: effective apheresis CD34≧1.0×10^6/Kg
L**: less effective apheresis <1.0×10^6/Kg

Table 5 *Discrepancies between HPC<20/μL and CD34+ cells>10/μL*

No	Age	Sex	Day*	Peripheral Blood		Harvest Bag		
				HPC (/μL)	CD34 (/μL)	HPC (/μL)	CD34 (/μL)	CD34 ($\times10^6$/Kg)
AL12	24	F	4	6	16	470	1741	2.306
			5	71	34	3720	2453	4.243
AL17	31	M	4	4	14	40	837	1.089
			5	33	16	670	735	1.353
AL20	50	F	4	1	20		968	0.691
			5	29	22		858	0.784
AL21	45	M	4	4	18	90	666	1.389
			5	27	24	420	1718	3.911

* mobilization day

and that the harvest was ineffective in some case even when the HPC count was 22-24/μL, a peripheral blood HPC count of 25/μL seems to be appropriate as a lower limit for stem cell harvesting, for our facility. One thing worth mentioning is that there was no case where the HPC count led to underestimation of the $CD34^+$ count. Therefore, all cases that met the criterion were considered to be fit for harvesting. On the other hand, 5 patients who had HPC counts of less than 20/μL and had their $CD34^+$ cell counts determined for the sake of confirmation, all had $CD34^+$ counts of less 10/μL and were indeed found to be not suited for stem cell harvesting (data not shown).

Nevertheless, this does not mean that an HPC count of 25/μL or more would always enable effective harvesting. Apart from the mobilization of stem cells into the peripheral blood, the conditions of harvesting are also major factors that affect the result of stem cell harvests. There are many factors in the harvest conditions. Conditions like inability to process properly because of difficulty in access to blood, not achieving the planned number of harvests because of inadequate centrifuge facilities, etc can be listed. The average HPC count in the ineffective harvests of the present study was 89.9/μL (22-280/μL), which was comparatively high although lower than the mean HPC count of 280/μL (25-1046/μL) in the effective harvests. Though there was no statistically significant difference, there was a tendency for the harvests to become ineffective when the percentage of multinucleated cells in the peripheral blood was far higher than that of mononucleated cells. The definition of effective harvest here was that a sufficient number of stem cells could be obtained in 1 or 2 harvests. The key point is whether a large number of stem cells can be harvested in a short time. The fact that transplantable amounts of stem cells could be obtained in all the eligible cases in our study indicates that the method of determining the suitable time of harvest based on the HPC count is useful.

There is a report that says that HPC count is not useful for harvesting allogenic peripheral blood stem cells[7]. Therefore, we used the $CD34^+$ cell count to determine the suitable time of transplantation. In some patients, the HPC count was fairly low despite the $CD34^+$ cell count being considerably high. ***Table 5*** lists the cases where the HPC count was less than 20/μL in spite of there being 10/μL or more of $CD34^+$ cells in the peripheral blood. In all the patients, the HPC count remained low up to the 4^{th} day of G-CSF administration, and increased from the 5^{th} day. On the contrary, the HPC count tended to be higher than the count of $CD34^+$ cells in the mobilization for harvesting autologous peripheral blood stem cells. This suggests the possibility of there being some difference between healthy donors and the patients, who had undergone more than one treatment, in the cells that are measured as HPC. There have been reports of the

possibility of $CD34^-$ human hematopoietic stem cells being counted as HPC[11]. The possibility of the HPC count becoming higher than that of $CD34^+$ cells in the quantitative determination of human hematopoietic stem cells cannot be ruled out. Whether the current method of determining the suitability for transplantation on the basis of the $CD34^+$ cell count needs to be changed can be decided only after further study.

The time needed for taking HPC measurement at our facility is about 2 minutes, which is far less than the approximately 2 hours required for the CD34 cell counting. One can inform the patient and medical staff well in advance whether stem cell harvesting would be done on a given day. The harvesting equipment can also be prepared well ahead of time. These are the advantages of HPC counting. In spite of this, if we recall the basic rule of keeping a 4 hours time gap from G-CSF administration to stem cell harvest, the aforesaid difference in time requirement may not be all that significant. From the point of view of cost of the measurements, HPC counting is advantageous because it can be done along with CBC. For these reasons, at our center, we rely on the results of HPC measurements for deciding the time of harvesting stem cells in the case of G-CSF administration post-chemotherapy. Nevertheless, it is necessary to keep in mind that in some cases, the number of $CD34^+$ cells actually harvested may not be as high as one might expect from the HPC count.

There is no doubt that the direct counting of $CD34^+$ cells is the most reliable method for determining the time of harvest when the feasibility of transplantation is decided on the basis of the $CD34^+$ cell count. The shortcomings of CD34 measurement are the long time required for the measurement and the high cost of the test. The biggest advantages of HPC measurement are that the test is convenient, no special treatment is required, and the cost is very low. The usefulness of stem cell transplantation as a method of correcting the damage caused to the bone marrow by therapy has been recognized. Its usefulness as a therapy for autoimmune diseases is also being established. Against this background, there would be an increasing requirement of stem cell harvesting in the future. It would be very advantageous if the suitable time of harvesting stem cells can be determined through a simple measurement.

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