Study of Collection Efficacy of Cell Separator Machine and Engraftment outcome of Peripheral Blood HSCT

Dr. Rima Kusumgar[1], Dr Sandip Shah[2], Dr. Manoj Shah[3], Dr.Pragnesh Shah[4]

Abstract

To evaluate collection Efficacy of cell separator machines and post transplant outcome of stem cell therapy collected from peripheral blood in various haematological conditions. Present study of 170 patients who underwent autologus and allogenic stem cell transplant collected from peripheral blood of both adult and paediatric age groups from year 1999 to 2012. Total 308 procedures were done among which 72(23%) were on Cs 3000 plus and rest on Amicus cell separator. 218 procedures were autologus and 90 were allogenic procedures. Mononuclear cell collection protocols on cell separators were adopted for harvesting PBSCs with the help of aphaeresis catheter. Mean collection efficacy of the Amicus aphaeresis machine is 75% and median yield is $6.6x10^8$ MNC/Kg body weight. A median of $5.6x10^6$ CD 34+ cells per kg recipient body weight were collected. On CS 3000 plus average collection efficacy was 50 % and yield $5.54x10^8$ MNC/Kg body weight. $2.1x10^6$ CD 34+ cells per kg body weight were harvested in a median product volume 50 ml. Post transplant median time for neutrophilic and platelet recovery is 12 days and 16 days respectively. Only 2 patients of Aplastic anemia and 2 patients of thalassemia had primary graft rejection. 6 patients died due to GVHD grade IV. Other patients had low grade reversible GVHD. Collection of PBSCs using the Amicus cell separator allows adequate yields of MNCs and CD34+ cells. Though the values of MNC count and CD 34+ cells are lower in CS3000 plus, outcome of engraftment was not affected. Engraftment takes place in more than 95% of the patients. Stem cell transplant is boon to the patients where other treatment is not responding or available.

Key Words: Engraftment, graft versus host disease, mononuclear cell (MNC), Peripheral blood stem cell

[1]Assistant Professor and In-charge Blood bank, [2]Consultant Hematologist, [3] Professor & Head, dept. of Pathology, GCRI Medical college, Ahmedabad, [4]Associate Professor and In-charge Blood bank, Government Medical College, Bhavnagar, Gujarat, India.

Corresponding author mail: rima_27@rediffmail.com

Introduction: In the recent times, peripheral blood stem cells (PBSCs) have increasingly become a major source of hematopoietic stem cells for transplantation in patients with various hematological and oncological conditions and are being preferred over stem cells obtained from the bone marrow.[1]

Traditionally, bone marrow was always the source of choice for stem cells in the treatment of various clinical conditions primarily because there are more progenitor cells, and presumably stem cells in the small volume of bone marrow than in the 5 litres of circulating blood in a human.[2] During the early attempts at using peripheral blood stem cells for transplantation, the challenge was to address the very low number of hematopoietic stem cells present in the circulating peripheral blood.[2,3]

Later it was established that the number of circulating hematopoietic stem cells dramatically increased under various conditions, especially during the recovery from ablative phase following chemotherapy. This made the process of mobilization of these cells into circulation and their subsequent collection for transplantation more feasible.[2,3]

Clinical results of peripheral blood stem cell transplantation (PBSCT) have shown benefits of earlier hematopoietic recovery, faster engraftment, lower morbidity, and greater cost-effectiveness compared with the conventional bone marrow transplantation.[4] In addition, the relative ease of obtaining large amounts of stem cells has made stem cell transplantation a viable option in the treatment of malignancies. The method also provides the flexibility of further increase of chemotherapy dose intensity in certain conditions.[3]

In recent years, there has been an increase in PBSCT performed for malignant conditions, both hematological and solid tumors. The number of PBSCTs has rapidly surpassed the number of bone marrow transplants (BMTs) performed in the autologous setting and PBSCT is also increasingly used in the allogenic setting.[5]

Optimum mobilization of stem cells into the peripheral circulation is the key to a successful PBSCT. The higher number of progenitor cells in mobilized compared with steady-state peripheral blood enables sufficient cell harvest with fewer collection sittings. Also, the benefits of enhanced hematopoietic recovery in PBSCT are only seen using mobilized collections. Clinically, mobilization

regimes consist of chemotherapy or hematopoietic growth factors (such as G-CSFs) or both.[2,3]

After mobilization, PBSCs are collected by multiple apheresis procedures on automated cell separators.[6] PBSCs represent a diverse group of cells with different functional capacities, but a majority of them express CD34 antigen that is usually used for their detection.[6] A significant increase in the number of PBSCTs and technological advancements has led to the development of new and more efficient apheresis devices, improving PBSC collections.[6]

Since the advent of automated cell separators, PBSC collection has been well established on the first generation of Cell Separators – CS-3000plus (Baxter-Fenwal) and Spectra (Cobe).[1,3,7] The machines target collection of mononuclear cells (MNCs) from mobilized donors and patients. The MNCs include the CD34+ stem cells, which are the ones needed for the transplantation.[3]

As in the developed nations, PBSCT has been slowly gaining wider acceptance in India. Various Cancer Institute's and Hematology-Oncology set-ups in the country have recognized the benefits of using stem cells in various malignant and non-malignant conditions.

For long, CS-3000plus has been consistently and successfully used in many centres in India for PBSC collection and harvesting.[5,7] Controlled product volume with high MNC and CD34+ yield as well as superior collection efficiency has always been achieved and documented through PBSC collection on CS-3000plus.[6,7] Technology has led to the introduction of second generation cell separators designed to provide faster, comfortable and efficient collections(Ex. Amicus).[6]

Various international studies have demonstrated that the PBSCs collected by the Amicus device are viable after transfusion and capable of inducing a durable engraftment that was comparable with those published for other cell separators.[5,6,7,8]

Additionally, the mean Amicus CD34+cell collection efficiency was better and platelet content in the stem cell products significantly lower than that of the CS-3000plus.[6,7]

We have been using CS-3000plus since over 10 years for PBSC collection and have started using the Amicus since last 5 years. In the current paper, we compared

the CD34+cell collections by Amicus and CS-3000plus both in autologous and allogeneic settings for adult and pediatric patients. The collection efficiencies and the engraftment outcomes of both the machines were studied.

Materials and Methods:

Patients:

The study included 170 patients of diverse hematological conditions of both adult and pediatric age groups from year 1999 to 2012. During first 6 years only 46 procedures (26 patients) were done. All were using CS 3000 plus only. Total 308 procedures were done among which 218(70.8%) were autologus from 109(64%) patients and 90(29.2%) were allogenic procedures from 71(41%) patients. 72(23%) of the procedures were done using CS 3000 plus (Baxter) and rest of the procedures were done on AMICUS^{TM} (Baxter/Fenwal). Procedures were started only after taking informed consent and performing serological tests (HIV,HBsAg,HCV) of either patients or donors. Diagnosis wise defferentiation is shown in **Chart I.**

34(20%) patients of allogenic transplant were of paediatric age group.

The patients were mobilized with G-CSF (10ug/kg/day) following various chemotherapy regimens. Collections were begun at the discretion of the attending physician.

PBSC collection:

MNC protocols on both the cell separators were adopted for harvesting PBSCs with the help of apheresis catheter. In case of Pediatric age group, priming was done by anticoagulated cross-matched blood. Blood volume processed three times higher than the body volume. Per cycle volume kept 1000 ml if WBC count is more than 35000/ul and 1400 ml if WBC count is less than 35000/ul. Two pints of Normal Saline with calcium gluconate and magnesium sulphate running during process.

For collection on the CS-3000plus and Amicus cell separators, parameter settings as per the manufacturer's recommendations were used.

The PBSC product was analyzed for volume, WBCs, MNCs, and CD34+cell yields.

WBC, MNC and hematocrit were determined using an automated cell counter. MNC count was confirmed using a Leishman stain and manual count.

CD34+cell determination:

CD34 +cells were enumerated in the peripheral blood and apheresis component by flow cytometry.

In case of allogenic mismatched ABO group transplant RBCs were removed by adding HES (ratio 1:8).

Autologous stem cells were preserved by adding cryoprotectant DMSO and stored at -80 ^{0} C till transplantation. CD 34 count was done by Flow Cytometer.

Engraftment was reported by periodical check of blood counts and chimerism study. Days of engraftment were considered after infusion of CD34 + cells when absolute neutrophil count $\geq$ 5oo/ul and platelets $\geq$ 20000/ul for 3 consecutive days.

Results:

Results were studied for product out come in the form of volume, MNC/Kg body weight and CD 34+ cells/Kg body weight, mean collection efficiency of equipments, average processing time and engraftment results in the form of platelet and neutrophilic recovery and are shown in **Table 1.**

No severe adverse effects were observed during or after the PBSC collection procedures other than mild hypocalcemic symptoms. Only 9(6%) of the total aphaeresis collections were interrupted due to venous access problems but it was solved after changing the inlet to out let and vice versa.

Only 2(1.17%) patients of Aplastic anemia and 2(1.17%) patients of thalassemia had primary graft rejection. 6 patients died due to GVHD grade IV. Other patients had low grade reversible GVHD.

Table 2 shows comparison of patient and collection variables of two aphaeresis equipments used in our study.

Chart 1: Diagnosis of Patients

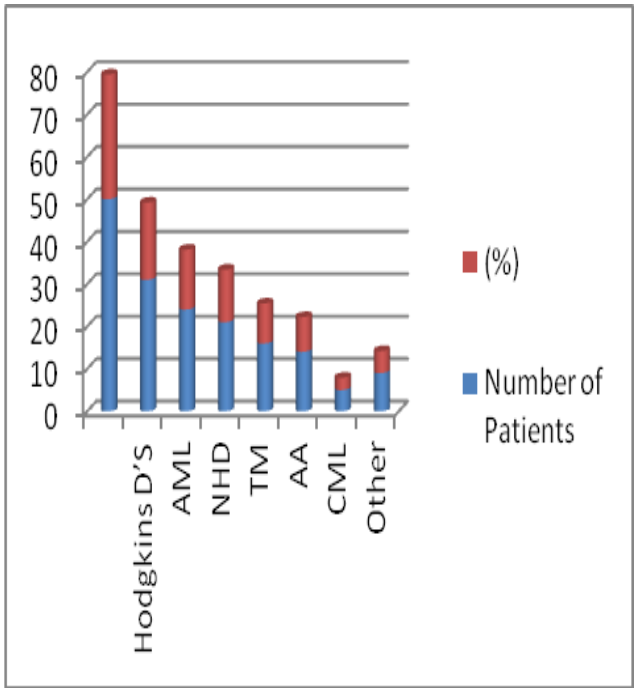

Table 1: Mean of Apheresis Equipment Product Data

		Amicus	**CS**
Volume Processed	Adult	12,500 (9000-15000)	11,600(8000-13000)
	Pediatric	8300(4300-9000)	81,00(4200-10000)
Auticogulant Consumption (ml.)		948(612-1148)	840(600-1250)
Flow rate (ml/min)		50(38-62)	60(50-70)
Separation time (hrs)*		5(4.2-5.6)	4.5(2.5-5)
Product Volume	Allo	205(88-275)	50(45-55)
	Auto	220(95-265)	
MNC * 10^8/Kg*		6.6(1.17-8.7)	5.6(1.5-6.5)
CD 34 * 10^6/Kg*		5.54	2.1

NOTE: * p value < 0.05

Table 2: Comparison of Patient & Collection Variables

		CS 3000	AMICUS
Patients (number)		72	98
Age (years)	Adult	53.1(32-68)	53.4(32-68)
	Pediatric	6.2(2.5-14)	6.5(3-14)
Gender	Male	40(23.5%)	47(27.6)
	Female	32(18.8%)	51(30%)
Body Weight/ Kg	Adult	66	65.2
	Pediatric	18.5	20.2
Peripheral WBC (*10^3/ ul)		38.2	40.0
Platelets (*10^3/ul)		2.05	1.48
Peripheral Hct (%)		28.4	33.2

Table 3: Comparison with Other Studies

Parameters			p-Value
MNC*10^8 /Kg (Amicus)	Our study	6.6(n=124)	0.02
	Snyder et al.	7.8(n=36)	
Neutrophilic Recovery (Days)	Our Study	12(n=170)	0.01
	Snyder et al.	8.7(n=31)	
	our study		0.015
	Yale et al.	9.3(n=400)	
Platelete Recovery (Days)	Our Study	16(n=170)	0.009
	Snyder et al.	9.7(n=31)	
	our study	10.9(n=400)	0.002
	Yale et al.		
Collection Efficacy of Amicus	Our Study	75(n=124)	0.001
	Yale et al.	50(n=46)	

Discussion: A successful engraftment of the collected hematopoietic cells in a patient is based on optimum mobilization of stem cells into the peripheral blood and an efficient collection of CD34+ cells during the harvest. A superior CD34+ collection efficiency improves CD34+ harvest yields and ensures sufficient cells to go ahead with the infusion and transplantation. The CD34+efficiency depend on various factors; significant among them is the use of the appropriate cell separator. In this study, we evaluated the performance of the Amicus Cell Separator of Fenwal for the first time in our institution. At the same time, we compared product yields and CD34+efficiency of two separators. The CS-3000plus, which had been using effectively since a long time and Amicus (both of Fenwal, Baxter) on two groups of donors – Allogeneic and Autologous, that did not differ significantly in terms of their age, gender, diagnosis, body weight, blood indices and initial CD34+ cell concentrations.

Our study has shown that AMICUS cell separator effectively collects MNCs. Successful collections were seen in adult as well as paediatric patients. The results are comparable and similar to various studies published earlier.[3,4,5] The CD 34+ yields per harvest and of cumulative harvests in allogeneic donors were higher than autologous harvests.[9] Collection of PBSCs using the Amicus cell

separator allows adequate yields of MNCs and CD34+ cells. Though the values of MNC count and CD 34+ cells are lower in CS 3000 +, outcome of engraftment was not affected. Though the Amicus procedure takes little longer than the one on CS-3000plus, the platelet contamination in the product is lower than the CS-3000plus.[5,9] The comparative results are in line with other comparative studies published in literature.[4,6,7,10]

Engraftment times for allogenic and autologous PBSCs were also equivalent to data published in the literature.[1,3,7] Because it is more efficient than the CS-3000plus, the Amicus separator would be more useful for collecting PBSCs from patients who are poor mobilizers and who have marginal circulating CD34+ cells.[6,7]

As far as GVHD is concerned, the results are equivalent to stem cells collected from bone marrow. Engraftment takes place in more than 95% of the patients.

Table 3 shows comparison with various studies. In our study although the CD 34+ cells/Kg was slightly lower than other studies, our engraftment results were quite acceptable.

Conclusion: The CS 3000+ and Amicus separator are effective and efficient in the collection of a good yield of PBSCs though Amicus collects CD34+ cells with greater efficiency and collects MNCs with higher yield than does the CS-3000 Plus, the engraftment outcome results are equivalent to stem cells collected from bone marrow. Stem cell transplant is a boon to those patients for whom other treatments are not effective or available.

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