1.0 INTRODUCTION

Peripheral blood stem cells (PBSC) have been used for transplantation since the early 1990s, and they are increasingly replacing bone marrow as a main source of stem cells. PBSC apparently have a number of advantages such as ease of collection without anesthesia and a more rapid engraftment following high dose chemotherapy. Moreover, tumour contamination is reduced and a sufficient number of PBSC can be harvested for sequential high dose chemotherapy cycles (Beyer *et al*., 1995).

Although PBSC have been harvested and transplanted for more than 10 years, more information about the release of progenitor cells from the bone marrow into the peripheral blood and their collection efficiency during apheresis is needed. It is well accepted that there is a small number of circulating progenitor cells in the peripheral blood in normal healthy individuals and that this number can be increased dramatically by a wide variety of mobilization stimuli, meaning that progenitor cells can be recruited into the peripheral blood during PBSC collection (Zingsem *et al*., 1992; Hillyer *et al*., 1993; Murea *et al*., 1996; Fliedner, 1998; Humpe *et al*., 1999; Smolowicz *et al*., 1999; Rowley *et al*., 2001). However, there were still patients that mobilized only low amounts of progenitor cells following the mobilization stimuli, which were insufficient for transplantation (Rick *et al*., 2000)

For the identification and quantification of these haematopoietic progenitor cells, a surface CD34 marker is used. This is mainly because almost all hematopoietic progenitor cells express CD34 antigen. The CD34 antigen is a family of differentially glycosylated type 1 transmembrane single chain glycoproteins. The levels of $CD34^{+}$ cells are very low in the peripheral blood of unmobilized individuals, approximately 0.1% of total nucleated cells in the

peripheral blood. This CD34+ cells are markedly increased after being mobilized by chemotherapy and/or growth factors. However, the levels of peripheral blood $CD34^+$ cell counts for pre- and post-apheresis and the numbers of collected $CD34^+$ cells are variable between patients (Rowley, 2001). The higher peripheral blood $CD34^+$ cell counts following apheresis as compared to pre-apheresis peripheral blood $CD34^+$ cell counts had been observed in a number of patients despite the fact that a significant amount of PBSC have been collected. This finding may support the hypothesis that the apheresis procedure itself may help in the recruitment and mobilization of progenitor cells from the bone marrow.

Therefore, in this study, the analysis of pre and post apheresis peripheral blood $CD34^+$ cells as well as the harvested $CD34^+$ cells count have been carried out, to compare and correlate these result in order to have better understanding of the correlation of peripheral blood $CD34^+$ cell counts and harvested stem cell count. The results obtained may help to provide information for further improvement of the collection efficiency and thus the PBSC yield for successful transplantation.

1.1 OBJECTIVE

The general objective of this study is to determine the collection efficiency of peripheral blood stem cell harvesting using peripheral blood $CD34^+$ cells quantification.

The specific objectives of this study were:

1. To determine the peripheral blood $CD34^+$ cell counts pre and post apheresis and the stem cell yield.
2. To correlate the PBSC yield and pre-apheresis peripheral blood $CD34^+$ cell counts.
3. To evaluate the cut-off value of pre-apheresis peripheral blood before performing PBSC harvesting.
4. To correlate the day 1 post-apheresis peripheral blood $CD34^+$ cells and the day 2 pre-apheresis peripheral blood $CD34^+$ cell counts.
5. To correlate the day 1 post-apheresis peripheral blood $CD34^+$ cells and day 2 stem cell yield.
6. To determine the collection efficiencies of $CD34^+$ cells.

2.0 LITERATURE REVIEW

2.1 STEM CELL

2.1.1 Introduction

The stem cell is the origin of life. “All cells come from cells”, as stated first by the great pathologist Rudolph Virchow. Stem cells have been found in most of the multi-cellular organisms. Stem cell is a special kind of cell that has a unique capacity. Although most cells of the body, are committed to conduct a specific function such as heart cells or skin cells, stem cell are uncommitted and remains uncommitted, until it receives a signal to develop into specialized cells. Their proliferative capacity combined with the ability to develop into specialized cells makes stem cells unique (Joanna *et al*., 2004).

The classical definition of a stem cell requires that it possess two properties which are;

i) self-renewal that means the ability to go through numerous cycles of cell division while maintaining the undifferentiated state and potency.

ii) the capacity to differentiate into specialized cell types. The stem cells have varying degrees of differentiation potential. This ranges from totipotent (ability to form the embryo and the trophoblast of the placenta of the fertilised oocyte), pluripotent (ability to differentiate into almost all cells that arise from the three germ layers of embryonic stem cells), multipotent (capability of producing a limited range of differentiated cell lineages appropriate to their location of most tissue based stem cells) and unipotent (only able to generate to one cell types of cells such as the epidermal stem cells and the spermatogonial cells of the testis (Preston *et al.*, 2003).

2.1.2 Types of Stem Cell

The two major types of mammalian stem cells are embryonic stem cell and adult stem cell.

i) Embryonic Stem Cell

An embryonic stem cell is derived from embryos at a developmental stage before the time that implantation would normally occur in the uterus. Fertilization normally occurs in the oviduct, and during the next few days, a series of cleavage divisions occur as the embryo travels down the oviduct and into the uterus. Each of the cells (blastomeres) of these cleavage-stage embryos are undifferentiated. They do not look or act like the specialized cells of the adult, and the blastomeres are not yet committed to becoming any particular type of differentiated cell. Indeed, each of these blastomere has the potential to give rise to any cell of the body (Thomson *et al.*, 1998).

The first differentiation event in humans occurs at approximately five days of development, when an outer layer of cells committed to becoming part of the placenta (the trophectoderm**)** separates from the inner cell mass (ICM). The ICM cells have the potential to generate any cell type of the body, but after implantation, they are quickly depleted as they differentiate to other cell types with more limited developmental potential. However, if the ICM is removed from its normal embryonic environment and cultured under appropriate conditions, the ICM-derived cells can continue to proliferate and replicate themselves indefinitely and still maintain the developmental potential to form any cell type of the body (Thomson *et al.*, 1998).

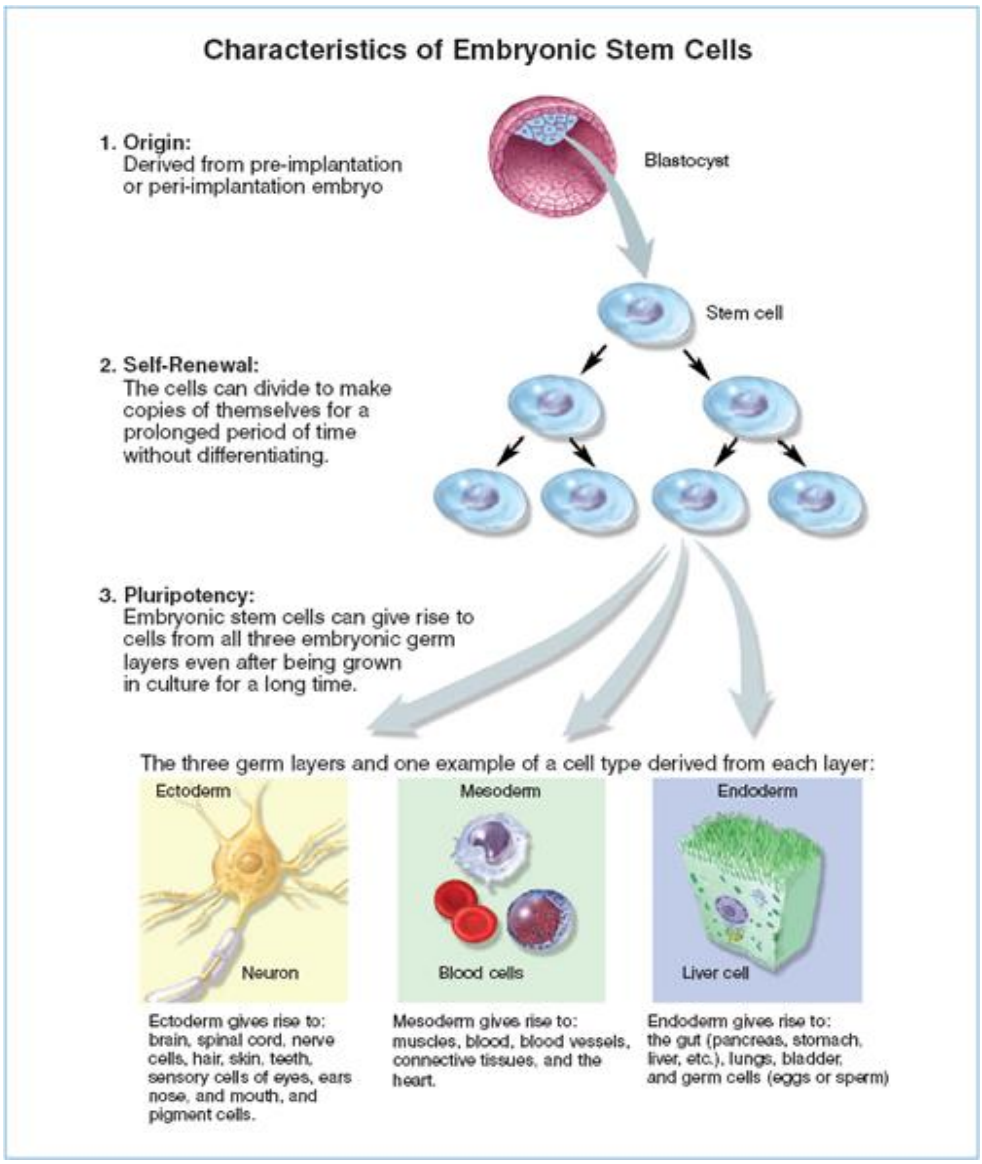

Figure 1: Characteristics of Embryonic Stem Cells (adopted from Terese, 2001)

In a developing embryo, stem cells can differentiate into all of the specialized embryonic tissues. In other words, they can develop into each of the more than 200 cell types of the adult body when given sufficient and necessary stimulation for a specific cell type.

ii) Adult Stem Cell

The term adult stem cell refers to any cell which is found in a developed organism that has two properties, which are the ability to self-renew and make identical copies of itself for the lifetime of the organism and also to differentiate into mature cell types that have characteristic, shapes and specialized functions. It is also known as somatic stem cells (from Greek word which means "of the body").

The primary functions of adult stem cells are to maintain the steady state of a cell called homeostasis, to replace cells that died because of injury or disease (Holtzer, 1978 and Leblond, 1964). For example, only an estimated 1 in 10,000 to 15,000 cells in the bone marrow is a hematopoietic (bloodforming) stem cell (Weissman, 2000).

Furthermore, adult stem cells are dispersed in tissues throughout the mature animal and behave very differently, depending on their local environment. For example, stem cells in the small intestine are stationary, and are physically separated from the mature cell types they generate but in contrast, hematopoietic stem cells **(**HSC**)** are constantly being generated in the bone marrow where they differentiate into mature types of blood cells.

HSC give rise to all the blood cell types including myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T-cells, B-cells, NK-cells). Indeed, they are ultimately responsible for the constant renewal of blood; the production of billions of new blood cells each day (Domen and Weissman, 1999) as shown in Figure 2.

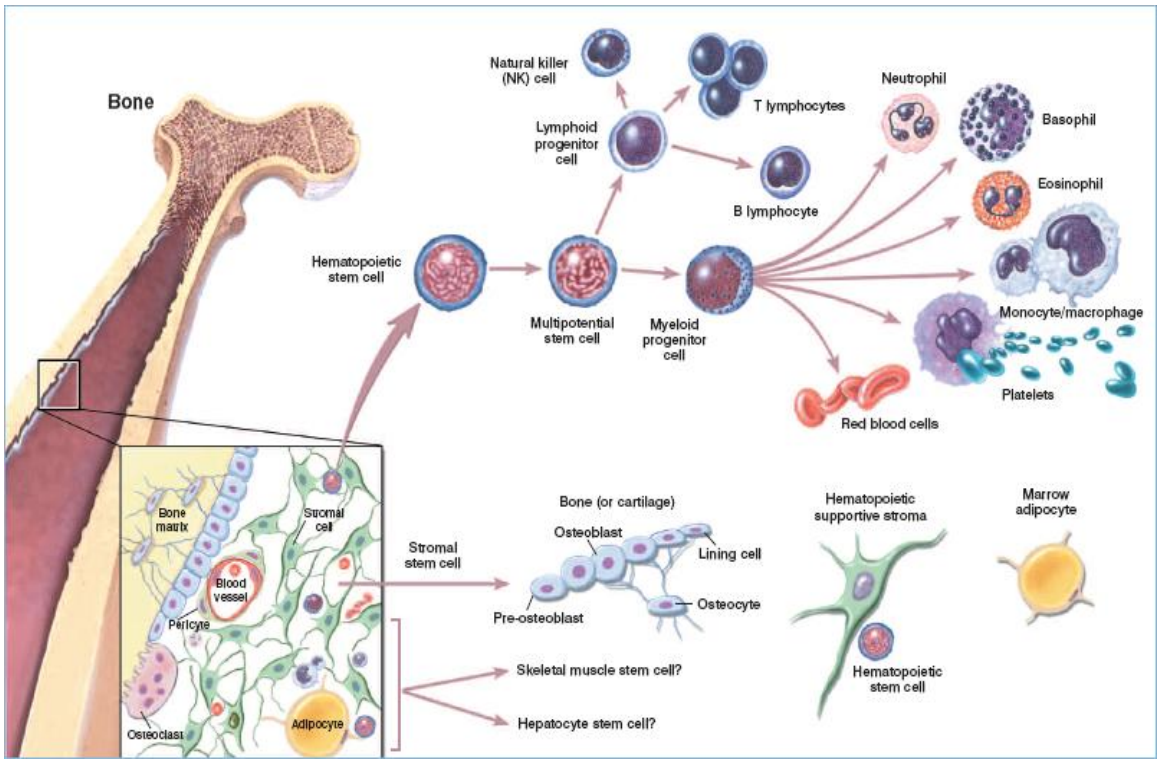

Figure 2: Hematopoietic and stromal cell differentiation (adopted from Terese, 2001)

Adult stem cells have been successfully used for many years to treat leukemia and related blood cancers through hematopoietic stem cell transplantation with either bone-marrow-derived stem cell or mobilised peripheral blood stem cell.

2.2 HEMATOPOIETIC STEM CELL TRANSPLANTATION

2.2.1 Introduction

Hematopoietic stem cell transplantation (HSCT) is the transplantation of pluripotential hematopoietic stem cell that derived from bone marrow, umbilical cord blood or peripheral blood stem cells. HSCT was pioneered using bone-marrow-derived stem cells by a team at the Fred Hutchinson Cancer Research Center led by E. Donnall Thomas. They showed that the destroyed bone marrow may be repopulated by intravenous infusion of cellular suspensions of marrow taken from healthy donors (Thomas *et al*., 1957). HSCT has evolved rapidly over recent decades and widely applied in a variety of haematological malignancies and non-malignant diseases.

2.2.2 Types of Hematopoietic Stem Cell Transplantation (HSCT)

i) Autologous HSCT

Autologous HSCT means transplantation using the patient's own stem cells. The stem cells were collected from the patient through a bone marrow harvest or apheresis procedure when the patient has achieved minimal residual disease or in remission. The stem cell product is then cryopreserved before storage in the cryogenic freezer to preserve the integrity of the stem cells and to maintain the optimum viability after thawing for transplantation.

The patient is then treated with high-dose conditioning chemotherapy with or without radiotherapy with the intention of eradicating the patient's malignant cell population. The patient's own stored stem cells are then reinfused into his/her body, where these stem cells will home to the bone marrow and proliferate to resume the patient's normal blood cell production.

Autologous HSCT have several advantages such as low risk of infection during the immune-compromised portion of the treatment since the recovery of immune function is rapid and graft versus host disease (GVHD) is very rare due to the donor and recipient being the same individual. These advantages have enabled autologous HSCT to be established as one of the standard second-line treatment for lymphoma (Canellos and George, 1997).

ii) Allogeneic HSCT

Allogeneic HSCT means that the stem cells were obtained from a donor. Allogeneic HSC donors must have the same human leukocyte antigen (HLA) as the recepient to limit the risks of transplanted stem cell rejection or severe GVHD. Allogeneic HSC donors may be a closely HLA matched sibling, an identical twin of the patient (*syngeneic*) or an unrelated donor but found to have very close degree of HLA matching. Usually an identical twin is a source of perfectly HLA matched stem cells.

Matching is performed on the basis of variability at three or more loci of the HLA loci, and a perfect match at these loci is preferred. The HLA antigens fall in two categories; Class I and Class II. In general, mismatches of the Class I antigen such as HLA-A, HLA-B, or HLA-C will increase the risk of graft rejection. A mismatch of an HLA Class II antigen for example HLA-DR, or HLA- DQB1 will increase the risk of graft-versus-host disease.

In addition, a genetic mismatch as small as a single DNA base pair is significant so perfect matches require knowledge of the exact DNA sequence of these genes for both donor and recipient. Furthermore, race and ethnicity are also known to play a major role in donor selection for unrelated donor as members of the same ethnic group are more likely to have matching genes, including the genes for HLA (Cutler and Antin, 2001).

In general, by transplantation of healthy stem cells to the recipient, allogeneic HCST appear to improve the chances for cure or long-term remission once the immediate transplant-related complications are resolved (Russell *et al.*, 2000). Therefore, the allogeneic treatment may be preferred for Acute Myeloid Leukemia (Bruno *et al.*, (2007) but the risk of acute and chronic GVHD is much more higher compared to autologous HSCT.

2.2.3 Sources of HSC

i) Bone Marrow

Bone marrow is the standard source of HSC for transplantation. The HSC are aspirated from the bone marrow of the donor, typically at the pelvis bone. This is done by using a large needle and syringe to draw out the bone marrow cells and the donor has to be under general anesthesia. This technique is referred to as a bone marrow harvest and shown in Figure 3.

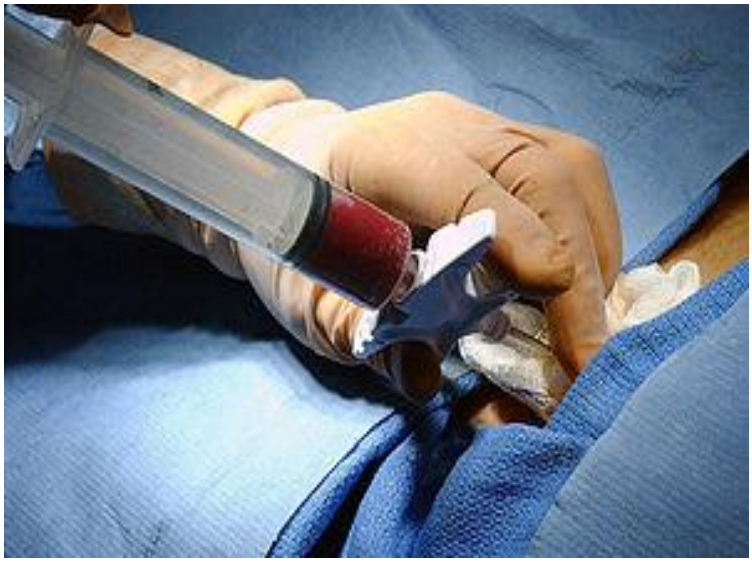

Figure 3: Bone Marrow Harvest

About 1 in every 100,000 cells in the marrow is a long term blood-forming stem cell and the other cells present include stromal cells, stromal stem

cells, blood progenitor cells and mature and maturing white and red blood cells (Liliane *et al.*, 2001).

ii) Umbilical Cord Blood

In the late 1980s, physicians began to recognize that blood from the human umbilical cord as an important clinical source of HSC. The umbilical cord supports the developing fetus during pregnancy and is delivered along with the baby and it is normally discarded. The collection and therapeutic use of these cells has grown since the first successful umbilical cord blood transplant in a child with Fanconi anemia (Barker and Wagner, 2003).

The concentration of HSC in umbilical cord blood (UCB) is lower than in adult blood due to the limited number of cells that may be harvested (typically about 50 ml) and makes it more suitable for transplantation into small children than into adults. Several approaches have been tested to overcome the cell dose issue, including newer techniques using ex-vivo expansion of cord blood units and the use of double cord blood units for single transplant in adults.

UCB was also found to delay the immune reconstitution following transplant, which leaves patients vulnerable to infections for a longer period of time. But, UCB has the advantages of its easy availability, ease of harvest, and the reduced risk of GVHD. Furthermore, HSC from cord blood have been noted to have a greater proliferative capacity than adult HSC (Koh *et al*., 2004).

iii) Peripheral Blood Stem Cells

Peripheral blood stem cells are now the most common source of stem cells for allogeneic HSCT. It has been known for decades that in adults, under steady-state conditions, the majority of HSCs reside in bone marrow and a small number of stem and progenitor cells circulate in the bloodstream. However, in the past 10 years, researchers have found that by injecting the donor with a cytokine, such as granulocyte-colony stimulating factor which can induce the progenitor cells to migrate from the bone marrow into peripheral blood in greater numbers.

PBSC is preferable for donor as harvesting from peripheral blood is easier with minimal pain, no anesthesia, shorter period of hospital stay and better yields for transplants. The peripherally harvested cells contain twice as many HSC as stem cells taken from the bone marrow and it was also result in faster engraftment (Childs *et al*, 2000).

2.3 PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

2.3.1 Introduction

Peripheral blood stem cells (PBSC) have been used for transplantation since the early 1990s, and they are increasingly replacing bone marrow as a source of stem cells. Peripheral blood stem cell transplants (PBSCT) are procedures that restore the stem cells that have been destroyed by treatment of high dose chemotherapy. Chemotherapy delivered in high doses will destroy all the cancer cells but at the same time, it will also destroy the stem cells that are present in the bone marrow. Therefore, PBSCT will restore all the cells that have been destroyed by the chemotherapy.

2.3.2 Peripheral Blood Stem Cell Mobilization

It is well accepted that there is a small number of circulating progenitor cells in the peripheral blood and that this number can be dramatically increased by a wide variety of mobilization stimuli (Link, 2001). As in adults, under steady-state, the majority of the HSC reside in the bone marrow and only a very low number is present in peripheral blood (Hong *et al.*, 1994 and Stadmauer *et al.*, 1995). However, it was noted that there was a dramatic increase in the numbers of circulating early hematopoietic cells during recovery from cytotoxic chemotherapy. Thus, the chemotherapeutic agents used were shown to be able to mobilize stem cells (Reiffers *et al*., 1986, Gianni *et al*., 1989, Tavassoli, 1992 and Richman *et al*., 1996).

Later, it was found that certain hematopoietic growth factor can augment the mobilization of circulating progenitor cells when given after chemotherapy (Duchrsen *et al*., 1988; Sheridan *et al*., 1992; Hong *et al.*, 1994 and Stadmauer

et al., 1995). Recently, growth factor alone has been found to increase the percentage of progenitor cells in blood. Several growth factors including granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL) -1, IL-3, IL-8, IL-11 and stem cell factor (SCF) have been shown to mobilize progenitor cells into the circulation (Fibbe *et al*., 1992; Andrew *et al*., 1992; Guillaume *et al*., 1993; Eaves, 1993; Peter *et al*., 1993; Smith, 1994; Reisner and Segall, 1995; Laterveer *et al*., 1995 and Mauch *et al*., 1995).

Combinations of cytokines have been studied in animals, and these early studies demonstrated that certain cytokines, for example G-CSF+ SCF, G-CSF + GM-CSF or IL-11+SCF, may be superior to single cytokines in mobilizing progenitor cells into the circulation (Briddell *et al*., 1993; deRevel *et al*., 1994 and Mauch *et al*.,1995). However, G-CSF is the most widely used cytokine for mobilization of stem cells at present (Pusic and DiPersio, 2008*)*. Daily administration of G-CSF permits the PBSC harvest at day 5 after the initiation of the G-CSF in healthy donor. While in autologous patient setting, the use of chemotherapy and G-CSF, the optimal day for harvesting varied from patient to patient, ranging from days 10 to 15 after the initiation of chemotherapy.

Several factors such as long therapeutic history, prior exposure to alkylating agents or radiation therapy and bone marrow infiltration had been reported to affect the stem cell mobilisation negatively (Drake *et al*., 1997; Ketterer *et al*., 1998 and Perea *et al*., 2001).

2.3.3 Peripheral Blood Stem Cell Harvesting

2.3.3.1 Introduction

Although PBSC harvest and transplantation have become routine procedures, more is to be known about the release of progenitor cells from the bone marrow into the peripheral blood. It has been shown that progenitor cells can be recruited into the peripheral blood during PBSC collection (Zingsem *et al*., 1992; Hillyer *et al*., 1993; Murea *et al*., 1996; Fliedner, 1998; Smolowicz *et al*., 1999; Humpe *et al*., 1999, Rowley *et al*., 2001). PBSC are collected from the blood through a process known as apheresis.

Apheresis is a Greek word meaning taking away, which can be performed by several devices available such as Cobe Spectra, Haemonetics, Baxter Amicus and Fresenius. The donor or patient blood is passed through an apparatus that separates out the stem cells constituent and the remainder is returned to the circulation. Centrifugation is the most common method of separation and it can be divided into two types; continuous flow centrifugation and intermittent flow centrifugation.

Continuous flow centrifugation required two venous access for collecting and returning the blood to patient/donor. 'Continuous' means the blood is collected, spun, and returned simultaneously. The main advantage of this system is the low extracorporeal volume used in the procedure, which is of benefit to the elderly and children. However, the intermittent flow centrifugation works in cycles, taking blood, spinning/processing it and then giving back the necessary parts to the donor in a bolus. The main advantage of this is a single venipuncture site.

2.3.3.2 Principle of PBSC Harvesting (Apheresis) using COBE Spectra ™ Apheresis System (COBE BCT, Inc., Lakewood, CO, USA)

PBSC harvesting is the procedure for collection of PBSC contained in the mononuclear cell layer after mobilization of stem cell into the peripheral circulation of autologous patients. The Cobe Spectra Apheresis System is the automated centrifuge based blood cell separator that provides the function necessary to control and monitor the extracorporeal circuit during apheresis procedures.

The Cobe Spectra Apheresis System consists of spectra disposables and spectra apheresis system. Spectra disposables consists of a separation channel that spins in centrifuge to separate blood into it components and blood tubing that routes blood and replacement fluids through the system.

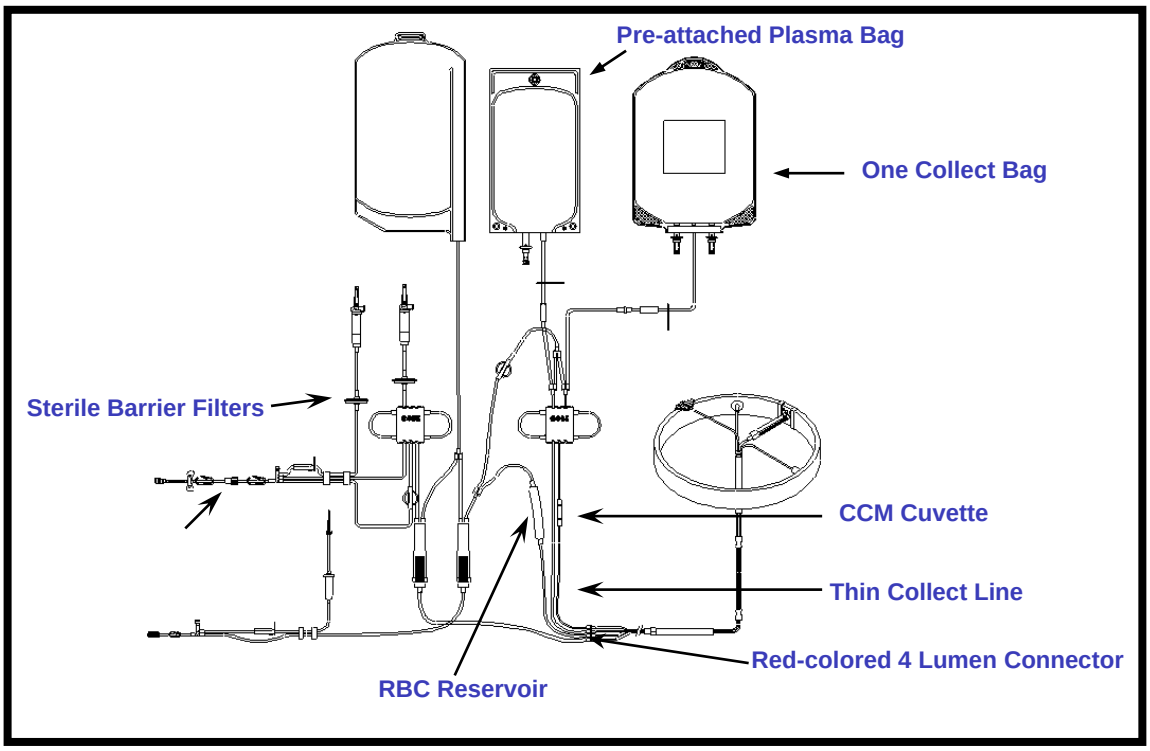

Figure 4: Diagram of Disposable Set of the COBE Spectra ™ Apheresis System

This system uses continuous flow centrifugation type of separation. The centrifugation will separate the cells to the cell layers according to cell size and specific gravity as shown in Figure 5 and Figure 6.

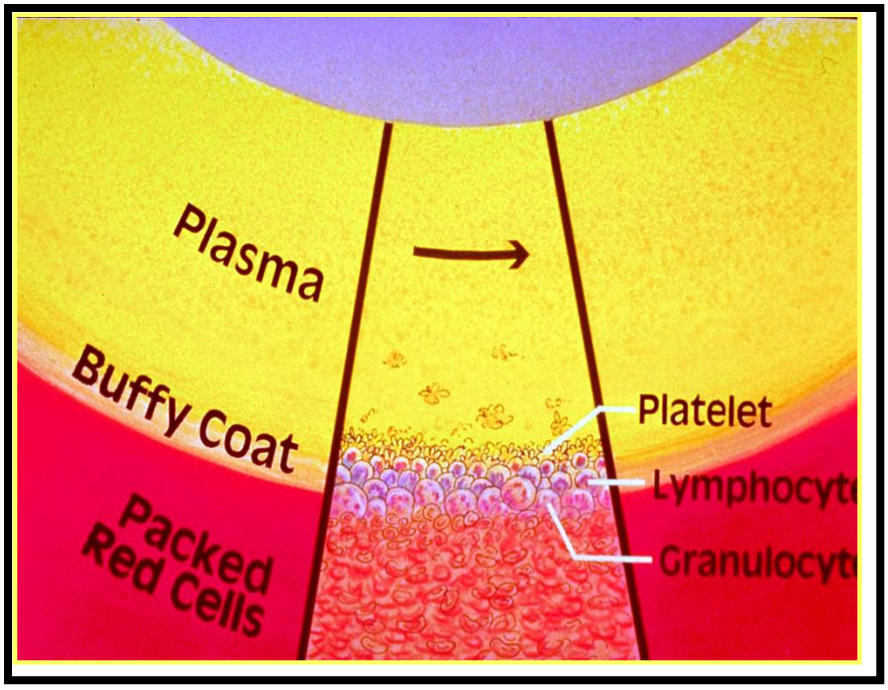

Figure 5: The Diagram Show the Layers of Cells Distributed in the Centrifuge of COBE Spectra Apheresis System

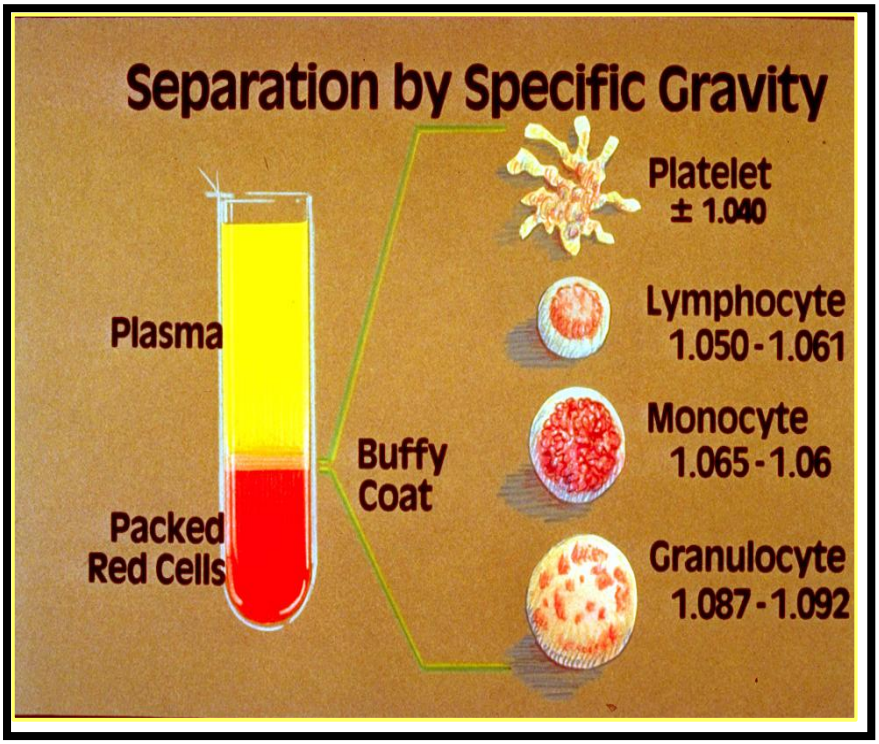

Figure 6: The Cells Separation by Specific Gravity

During the apheresis procedure, the anticoagulant is automatically mixed with the blood as it is pumped out from the body into the apheresis machine. The purpose of this mixing procedure is to prevent coagulation of the blood.

2.4 $CD34^+$ CELLS

2.4.1 Introduction

The use of monoclonal antibodies against CD (cluster of differentiation) antigens has excellently facilitated the precise characterization of different haemotopoietic lineages. Monoclonal antibodies against the same antigens are grouped together based on the reactivity and biochemical and/or genetic characterization of the corresponding antigen (Schlossman *et al*., 1995).

The CD34 antigen is a family of differentially glycosylated type 1 transmembrane single chain glycoproteins that is expressed on virtually all hematopoietic progenitor cells. $CD34^+$ cells which represent the most immature 1 to 4 percent of bone marrow mononuclear cells including more primitive haematopoietic progenitor cells and the levels of $CD34^+$ cells are very low in unmobilized blood approximately 0.1% of total blood mononuclear cells (Smeland *et al*., 1992).

After mobilization with cytokines, the level of $CD34^+$ cells increases markedly and the percentage of $CD34^+$ cells in the apheresis products may approach the percentages in bone-marrow grafts (Siena *et al*., 1993; Smith, 1994; Fielding *et al*., 1994 and Stadmauer *et al.*, 1995). The $CD34^+$ cell numbers in the peripheral blood can be used to determine the optimal time for apheresis and according to previous studies a cut off peripheral blood $CD34^+$ cells with a varied range of 8 to 40 cells/ul is an indicator for stem cell harvesting (Yu *et al*., 1999; Fantao *et al*., 1999; Heuft et al., 2000; Kudo *et al.,* 2003 and Moncada *et al*., 2003).

2.4.2 $CD34^+$ Cells and Engraftment

Several studies have shown that there is a direct relation between the time of engrafment and the number of $CD34^+$ cells in the apheresis product infused to the patient during transplant after high dose therapy. The studies showed that more than 2 $x10^6$ $CD34^+$ cells/kg body weight of patient are needed for timely multilineage engraftment (Dercksen *et al*., 1995; Bensinger *et al*., 1995 and Mavroudis *et al*., 1996). Furthermore, in autologous transplantations using mobilized peripheral blood $CD34^+$ cells, it has been found that doses of $CD34^+$ cells higher than $5x10^6$ $CD34^+$ cells/kg are associated with early trilineage engraftment reduced transfusion needs and shortened the hospital stay (Bender *et al.*, 1992; Bensinger *et a*l., 1995; Weaver *et al*., 1995 and Remes *et al*., 1997).

Transplantation of $CD34^+$ cells of more than $2x10^6$/kg has led to faster recovery than doses below $2x10^6$ $CD34^+$ cells/kg. It has also been shown that, the recovery of platelets often was delayed when fewer $CD34^+$ cells were transplanted to patients even though the recovery of neutrophil remained fast (Mavroudis *et al*., 1996), whereas in patients given doses below $1x10^6$ $CD34^+$ cells/kg, they had a higher transplantation related mortality than patients who received more than $1x10^6$ $CD34^+$ cells/kg (Moore, 1991). These data suggests that the dose of $CD34^+$ cells given to patient can predict the rate of survival post-transplantation as well as the speed of engraftment.

2.4.3 Quantification of CD34 by Flow Cytometry

2.4.3.1 Introduction

Enumeration of $CD34^+$ cells has become a reality with the development of monoclonal antibodies and flow cytometer. It is possible to determine the percentage or absolute number of $CD34^+$ cells in routine clinical use nowadays. Currently, the most widely used guidelines for stem cell identification is proposed by International Society of Hematotherapy & Graft Engineering, ISHAGE (Sutherland *et al.,* 1996).

Cytometry is a combination of two words which are cyto meaning cell and metry meaning measurement. In other words, cytometry is a measurement of physical and chemical characteristics of cells. It allows simultaneous measurement of single cells at a rapid rate up to thousands of particles per second through an optical and electronic apparatus (Chapman, 2000).

Figure 7: Flow Cytometer; Becton Dickinson FACSCalibur

2.4.3.2 Principle of Flow Cytometry

A flow cytometer consist of three main components which are fluidics, optics and electronics as shown in Figure 8.

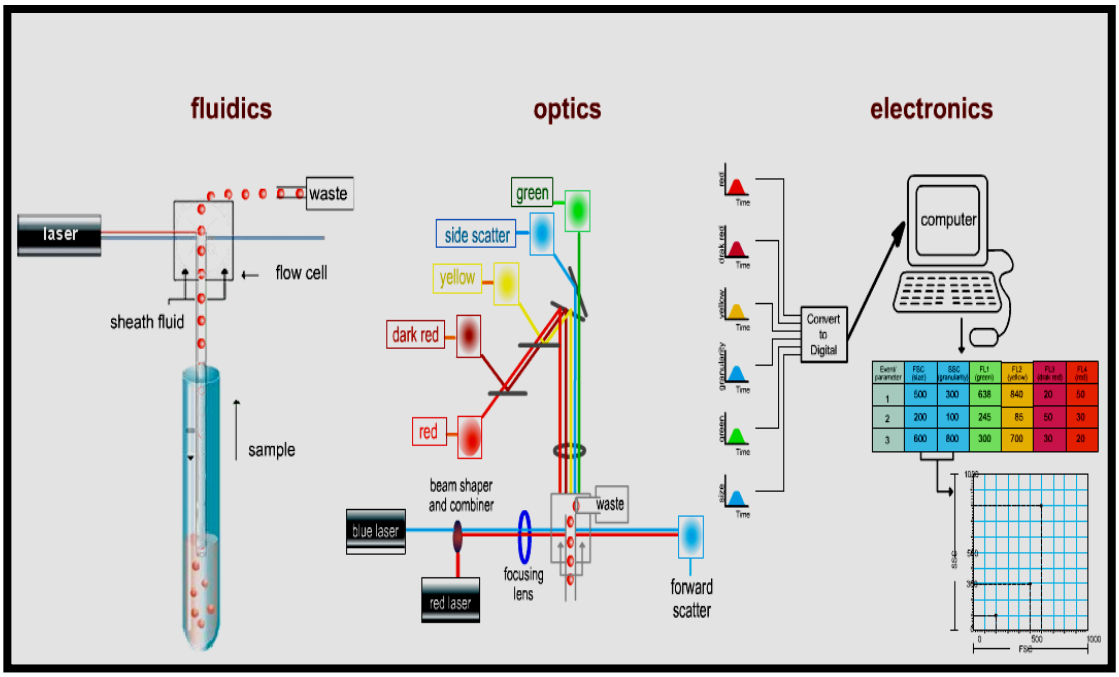

Figure 8: Components of Flow Cytometer

A beam of laser light of a single wavelength is directed onto a hydrodynamically-focused stream of fluid. A number of detectors are aimed at the point where the stream passes through the light beam: one in line with the light beam (Forward Scatter or FSC) and several perpendiculars to it (Side Scatter or SSC).

Each suspended particle from 0.2 to 150 micrometers passing through the beam scatters the ray, and fluorescent chemicals found in the particle or attached to the particle may be excited into emitting light at a longer wavelength than the light source. This combination of scattered and fluorescent light is picked up by the detectors. Then fluctuations in brightness at each detector were analyzed to derive various types of information about the physical and chemical structure of each individual particle. FSC correlates with the cell volume and

SSC depends on the inner complexity of the particle such as the shape of the nucleus, the amount and type of cytoplasmic granules or the membrane roughness.

The process of collecting data from the flow cytometer is termed as ‘acquisition’. Acquisition is mediated by a computer that is connected to the flow cytometer and the software which handles the digital interface with the cytometer. The software is capable of adjusting parameters such as voltage, compensation and threshold for the specimen being tested. The data generated by the flow cytometer can be plotted to produce histogram, two or three dimensional dot plot. The regions on this dot plot can be sequentially separated based on the fluorescent intensity, by creating a series of subset extraction terms as ‘gates’ (Ormerod, 2008). Specific gating protocol exists for diagnostic and clinical purposes especially in hematology.

3.0 METHODOLOGY

3.1 PATIENTS

This is a cross-sectional and retrospective study from January 2007 to December 2009. All autologous patients undergoing PBSC harvesting were recruited. The inclusion criteria are all autologous patients who have successfully undergone PBSC harvest procedures. The exclusion criteria are patients who have undergone PBSC harvesting but with incomplete data and those who experienced failed procedures. For this study, one patient was excluded because of failed procedure due to unstable condition of the patient.

Data were retrieved from the UKM Medical Centre Integrated Laboratory Management System (ILMS) and also from the records of the Stem Cell Laboratory.

3.2 METHODS

3.2.1 PBSC Mobilisation

For patients undergoing autologous PBSC harvesting, chemotherapy and G-CSF were given for stem cell mobilization. The use of G-CSF(Neupogen) continue after the initiation of chemotherapy regime, and it varied from patient to patient, ranging from days 10 to 15. Once peripheral blood $CD34^+$ count $\geq$ 15 cells/ul, the PBSC from patient will be harvested until achieve $\geq 2 \times 10^6$ $CD34^+$ cells/kg body weight.

In the case of good mobilizer, PBSC harvesting will be continued until the collection yield achieves $\geq 5 \times 10^6$ $CD34^+$ cells/kg body weight even though the collection yield on day 1 had achieved 2×10^6 $CD34^+$ cells/kg body weight as high stem cell dose may be associated with improved hematopoietic recovery and survival in patients undergoing HSCT. In some patients, mobilization may

be ineffective to allow collection of this threshold dose. Poor mobilization is defined as collection of $\leq 2 \times 10^6$ $CD34^+$ cells/kg body weight.

3.2.2 PBSC Harvesting (Apheresis)

All aphereses were performed using a COBE Spectra ^{TM} Apheresis System (COBE BCT, Inc., Lakewood, CO, USA). Stem cell collections in all these patients were performed with the semiautomated MNC program (software Version 5.1). The materials and methodology performing apheresis are as in Appendix I. Figure 9 shows patients during the PBSC harvesting procedure. The patients were connected to the apheresis machine using a femoral catheter.

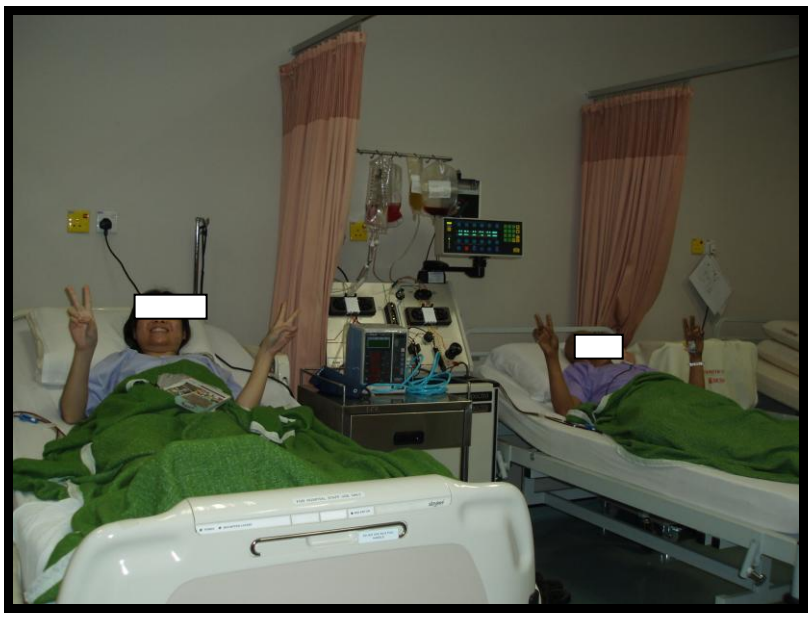

Figure 9: PBSC Harvesting Procedure

Total blood volume (TBV) processed was calculated using the COBE Spectra software based on sex, height and weight of the patient. The machine will process approximately 2x TBV of the patients. The apheresis procedure usually will take about three to four hours to complete and the collection volume ranging from 50 ml to 200ml. The length and volume of collection varied

between patients depending on their own TBV and also their condition throughout the procedure. The targeted collection yield was to achieve $\geq 2 \times 10^6$ $CD34^+$ cells/kg body weight, the minimum dose required for transplantation. PBSC harvesting will be performed on the next day if the stem cell yield count for day 1 was $\leq 2 \times 10^6$ $CD34^+$ cells/kg body weight. PBSC harvesting will continue until the minimum targeted doses of $\geq 2 \times 10^6$ $CD34^+$ cells/kg body weight is achieved.

3.2.3 Sampling

The peripheral blood for $CD34^+$ cells count taken on the day of harvesting is named as the 'pre-apheresis peripheral blood $CD34^+$ cells sample'. Pre-apheresis samples of peripheral blood for $CD34^+$ cells analysis were drawn about 2 hours after G-CSF administration on the day of PBSC harvesting.

The peripheral blood sample taken after harvesting was completed is named as 'post-apheresis $CD34^+$ cells count'. The stem cell product samples of harvested material were taken from the collection bags after gentle mixing before processing, and this sample is named as the 'stem cell yield'. All these samples were collected into EDTA anticoagulant tubes and analyzed by flow cytometry within 2 hours of collection.

The peripheral blood for $CD34^+$ cells count sent on the second day of harvesting is named as the 'pre-apheresis peripheral blood $CD34^+$ cells day 2'. The stem cell product sample for second day harvesting is named as the 'stem cell yield day 2'. If the harvesting still continue, The third day peripheral blood sent for $CD34^+$ cells count is named as the 'pre-apheresis peripheral blood $CD34^+$ cells day 3'. 'Stem cell yield day 3' referred to the stem cell product sample for third day of harvesting.

3.2.4 $CD34^+$ Cells Enumeration

Daily White Blood Cell (WBC) count was monitored daily before performing the $CD34^+$ Cells Enumeration. Once WBC count was ≥ 1.0 $x10^9$/L, peripheral blood $CD34^+$ cells enumeration was performed daily until the peripheral blood $CD34^+$ ≥ 15/ul. The patient's PBSC was harvested once the peripheral blood $CD34^+$ ≥ 15/ul.

The pre- and post-apheresis peripheral blood $CD34^+$ cells and PBSC product samples were stained with BD combined antibody reagents which consist of PE-conjugated anti-CD34 and FITC-conjugated anti-CD45 in a BD Trucount Tube. Exclusion of dead $CD34^+$ cells from viable $CD34^+$ cells enumeration is achieved using the 7-Amino actinomycin (7-AAD) viability Dye where the viable cells remain unstained (negative).

The materials and methods for performing $CD34^+$ cells enumeration is described in the Appendix II. The steps involved in $CD34^+$ cells staining before quantification by flow cytometer is shown in Figure 10. The staining procedure normally takes about 30 minutes.

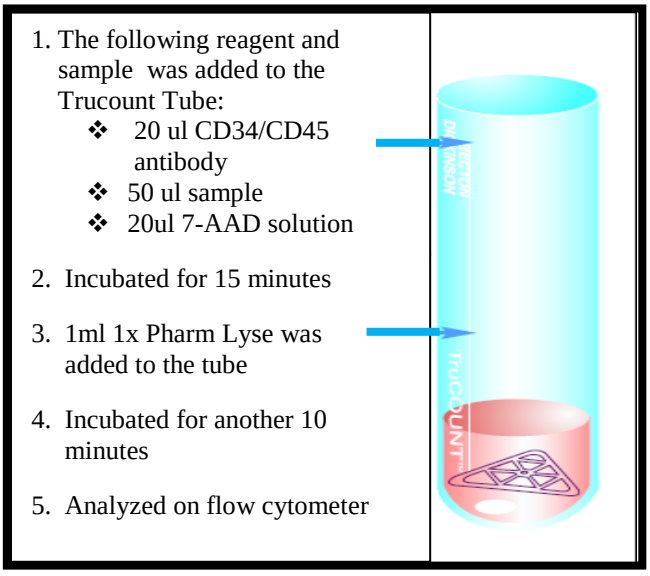

Figure 10:The Diagram Showing the Steps in $CD34^+$ Cells Staining

3.2.5 Acquisition and Analysis of $CD34^+$ Cells by Flow Cytometer

Flow cytometric analysis of $CD34^+$ cells in pre- apheresis, post-apheresis peripheral blood and apheresis yield was performed by FACScalibur (Becton Dickinson, USA) with Becton Dickinson Stem Cell kit. The acquisition protocol is described in the Appendix III. The analysis is performed using the ISHAGE protocol for true $CD34^+$ HSC criteria;

i. **Positive CD34 expression**
ii. **Dim CD45 expression**
iii. **Low to intermediate FSC**
iv. **Low SSC**

An example of dot plot obtained after acquisition by flow cytometer is shown below in Figure 11(a-f).

Plot 1

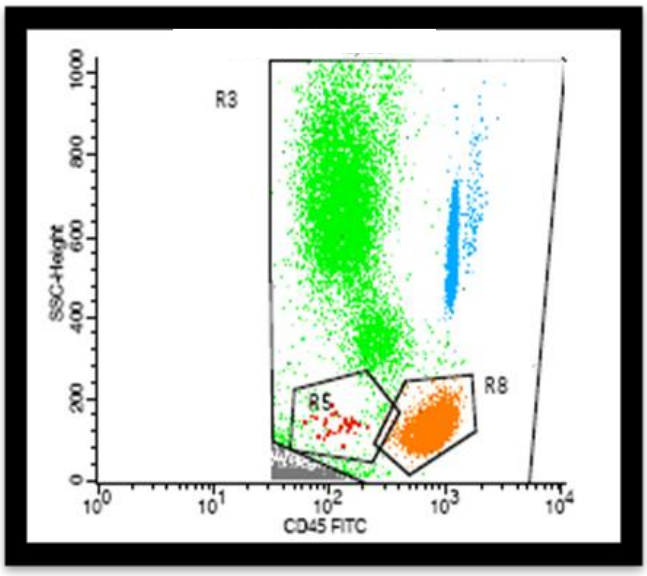

Figure 11(a): Plot 1; All events collected are shown in this plot. R3 defines all leucocytes (lymphocytes, monocytes and granulocytes) except blue coloured dots defined as beads. Region R5 defines $CD45^+$ events. Region R8 defines the lymphocyte population.

Plot 2

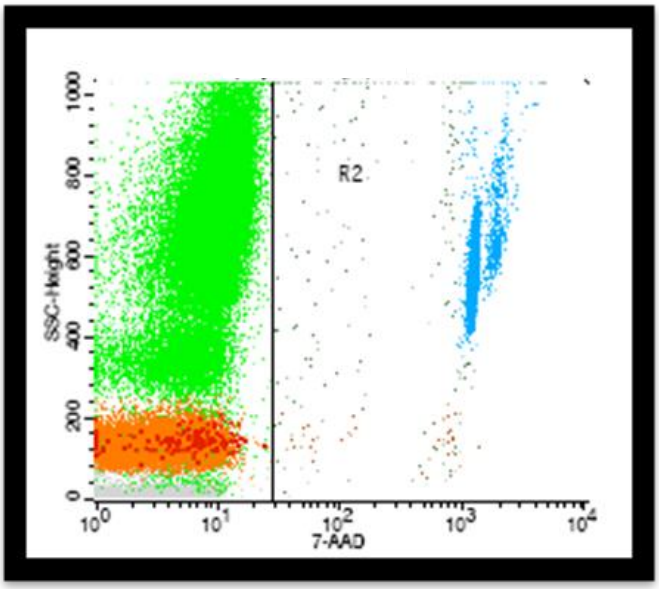

Figure 11(b): Plot 2; Discrimination of live cells from dead cells. Events falling in R2 (non-viable cells) were gated out from further analysis.

Plot 3

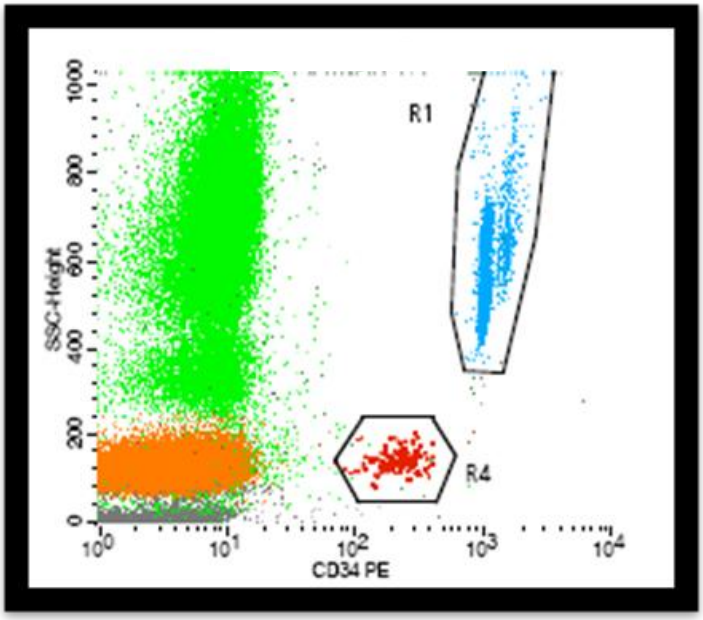

Figure 11(c): Plot 3; Gated on live $CD45^{+}$ leukocytes. R1 defines TruCOUNT beads. R4 defines as bright $CD34^{+}$ events.

Plot 4

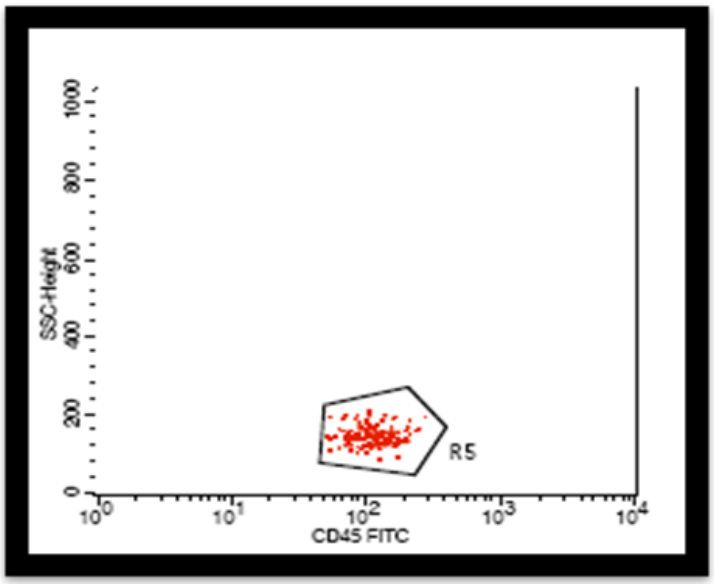

Figure 11(d): Plot 4; R5 defines clustering of live $CD45^+$ and $CD34^+$ events

Plot 5

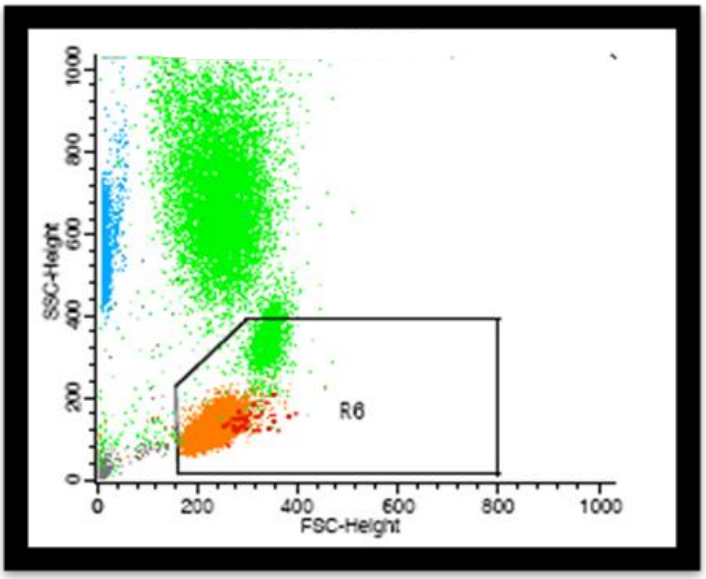

Figure 11(e): Plot 5; Live lymphocytes and monocytes. The left side of R6 is drawn to include cells no smaller than lymphocytes.

Plot 6

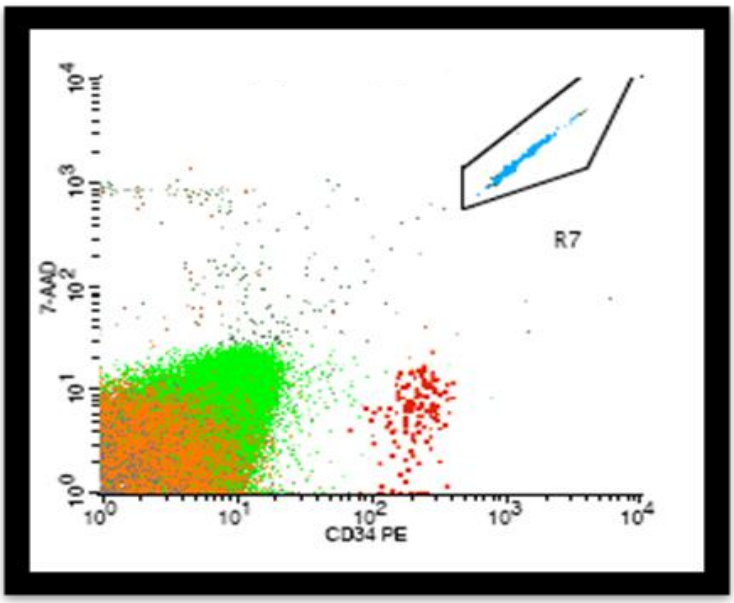

Figure 11(f): Plot 6; Region R7 defines the TruCOUNT beads

3.3 Statistical Analysis

The statistical analysis for this study was carried out using Statistical Package for Social Science (SPSS) software version 18 and MedCalc Statistic for Biomedical Research version 11 for windows.

3.3.1 Data Analysis

a) The following equation was used for calculation of collection efficiency:

$$\frac{\text{Total CD34}^{+}\text{ cells in Apheresis Product}}{\left[\frac{\text{Pre-apheresis CD34}^{+}\text{ cells} + \text{Post-apheresis CD34}^{+}\text{ cells}}{2}\right] \times \text{Total Blood Processed}} \times 100$$

b) Correlation study using Pearson Correlation analysis between:

i) pre-apheresis peripheral blood $CD34^{+}$ cells and stem cell yield

ii) day 1 post-apheresis peripheral blood $CD34^{+}$ cells and day 2 pre-apheresis peripheral blood $CD34^{+}$ cells

iii) day 1 post-apheresis peripheral blood $CD34^{+}$ cells and day 2 stem cell yield

c) Receiver Operating Characteristic (ROC) Curve Analysis was used to determine the cut-off point of pre-apheresis peripheral blood $CD34^+$ cells to achieve sufficient stem cell yield of $\geq 2 \times 10^6$/ kg body weight for PBSCT.

4.0 RESULTS

4.1 PATIENT DEMOGRAPHY

A) A total of 94 PBSC harvesting procedures have been performed for 48 patients (30 men and 18 women). These patients were diagnosed with non-Hodgkin's Lymphoma, NHL (n=24), Acute Myeloid Leukemia, AML (n=12), Multiple Myeloma, MM (n=8), and Hodgkin's Lymphoma (n=4). NHL cases include Follicular Lymphoma, Diffuse Large B-Cell Lymphoma (DLBCL), Waldenström's macroglobulinemia, Mediastinal NHL, Burkitt's Lymphoma, Mantle Cell Lymphoma, Primary Lung Lymphoma and Richter's Transformation of Chronic Lymphocytic Leukaemia (CLL). The median age is 58 years old (range 14-67) and the age ranges for the different disease groups are as shown in Table 1. The male:female ratio is 1.7:1 and the median body weight was 58 kg (range 34.6 – 101 kg).

Table 1: Characteristics of Patients (for age and weight, in range)

	NHL	AML	MM	HODGKIN'S LYMPHOMA	TOTAL
NO OF PATIENTS	24	12	8	4	48
MALE	20	4	5	1	30
FEMALE	4	8	3	3	18
AGE (RANGE)	17-67	14-51	40-63	26-29	14-67
PATIENT'S BODY WEIGHT, KG (RANGE)	34.6-94	44-64	58-101	38-65	34.6 - 101

B) For the peripheral blood stem cell harvesting the minimal targeted stem cell yield of $CD34^+$ cells is $\geq 2x10^6$ per kg recipient body weight. The median number of apheresis procedures per patient was 2 (range, 1-3) in which 13 patients have undergone one apheresis, 24 patients with two procedures and the

remaining 11 patients with three apheresis procedures with the intention to collect a sufficient amount of $CD34^+$ cells for transplantation.

C) These patients have been treated with the standard chemotherapy regimens and were in remission or minimal residual disease state. These patients were mobilized with chemotherapy and G-CSF (neupogen). 43/48 patients (89.6%) managed to produce $CD34^+$ cells yield of ≥ 2 $x10^6$/ kg body weight with 3 or less apheresis procedures. 34/48 (70.8%) of these patients achieved this minimal targeted yield with only one apheresis procedure, 9/48 patients (18.8%) with two procedures. The remaining 5 patients failed to obtained the stem cell yield of 2 $x10^6$/kg body weight. 16/48 patients (33.3%) were able to achieved $CD34^+$ cells collection yield of ≥ 5 $x10^6$/ kg body weight in one harvesting procedure.

Table 2: Apheresis Yield of $CD34^+$ Cell

$CD34^+$ Cells Yield	**No of patients**
≥ 2 $x10^6$/ kg body weight	**43**
With one apheresis	34
With two apheresis	9
≤ 2 $x10^6$/ kg body weight	**5**

4.2 PRE-APHERESIS BLOOD CELL COUNT AND COLLECTION CHARACTERISTIC

The characteristics of pre- apheresis blood cell count and the collection yield are shown in Table 3. The median WBC pre-apheresis was 19.5 $x10^9$/L (range 2.0-71.3 $x10^9$/L) and the median percentage of lymphocyte and monocyte were 4.5% and 6.7% with ranges (1.0-33.1% and 0.3-39.5%) respectively.

The median pre-apheresis peripheral blood $CD34^+$ cells were 33.6/ul (9.5-456.5/ul). For the PBSC harvesting procedures using Cobe Spectra performed with the semiautomated MNC program (software Version 5.1), an estimate of 2x total blood volume were processed. In these patients, the median blood volume processed was 8701 ml (range 4583 – 12918 ml), and the median PBSC volume collected was 153.5 ml (40-246 ml).

The median $CD34^+$ cells collected was $2.6x10^6$ /kg body weight, with the range 0.1 $x10^6$ /kg - 32.5 $x10^6$ /kg. Following the harvesting, the median post apheresis peripheral blood $CD34^+$ cells was 28.5/ul (range 3.58/ul - 240.5/ul) which was about 15% lower compared to pre-apheresis $CD34^+$ count.

Table 3: Pre-Apheresis Blood Cell Count and Collection Characteristic

	Median	Range
White Blood Cell (WBC) Count, $x10^9$/L	**19.5**	**2.0-71.3**
Pre-harvest Lymphocyte count, %	**4.5**	**1.0 - 33.1**
Pre-harvest Monocyte count, %	**6.7**	**0.3 - 39.5**
Pre-apheresis Peripheral Blood $CD34^+$ Cells/ul	**33.6**	**9.5 - 456.5**
Post-apheresis Peripheral Blood $CD34^+$ Cells/ul	**28.5**	**3.58 - 240.5**
Total CD34+ Cells Yield, $x10^6$/ kg body weight	**2.6**	**0.1 - 32.5**
Total Blood Volume Processed, ml	**8701**	**4583 - 12918**
Total Collection Volume, ml	**153.5**	**40 - 246**

4.3 CORRELATION STUDY

4.3.1 Pre-apheresis Peripheral Blood CD34$^+$ Cells and Product Yield

The correlation study between pre-apheresis peripheral blood CD34$^+$ cells and product yield using Pearson's Correlation analysis based on paired samples from 94 apheresis for day 1 to day 3 of apheresis procedures (Figure 12(a) has shown a positive correlation with r = 0.963. Based on all the 94 apheresis procedures, a cut off value of 33 cells/ul pre-apheresis peripheral blood CD34$^+$ cells count may predict a stem cell yield of ≥ 2 x10^6 CD34$^+$ cells/ kg body weight (r = 0.963, p < 0.001).

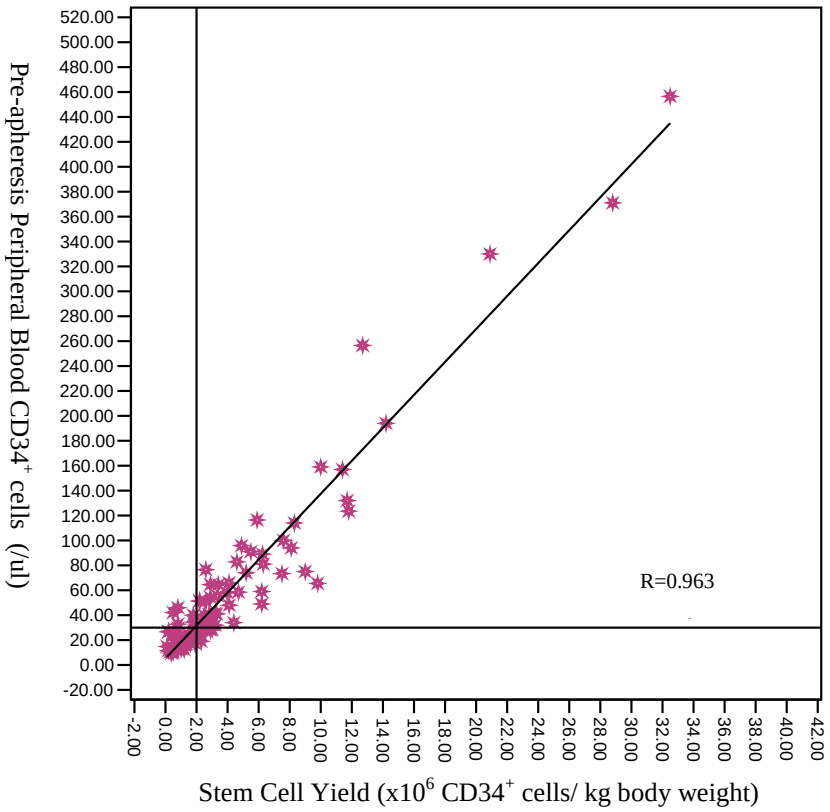

Figure 12(a): Correlation Analysis of Overall Pre-Apheresis Peripheral Blood CD34$^+$ Cells and Stem Cell Yield for Day 1, 2 and 3 PBSC Harvesting

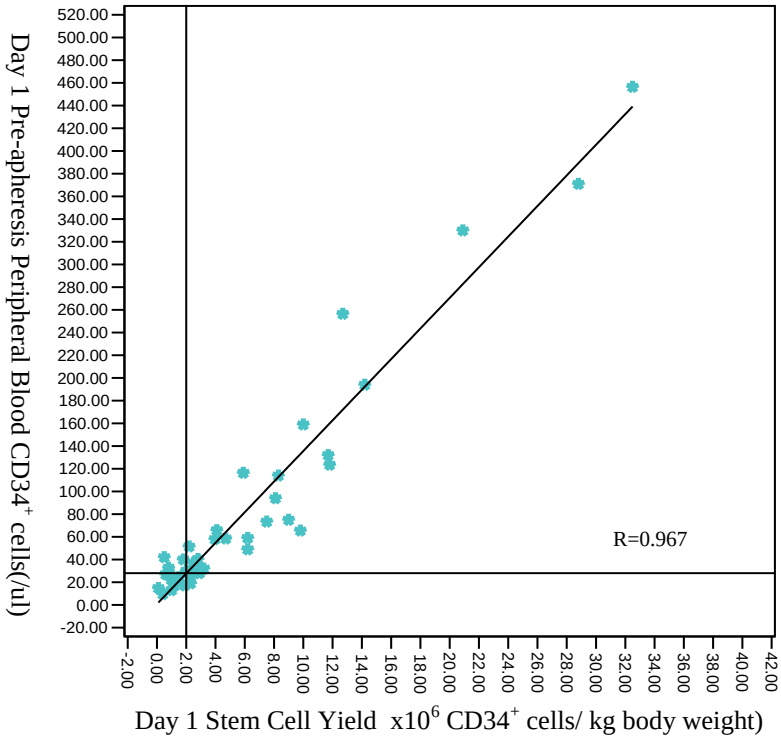

Figure 12(b): Correlation Analysis of Pre-apheresis Peripheral Blood $CD34^+$ Cells and Stem Cell Yield for Day 1 PBSC Harvesting

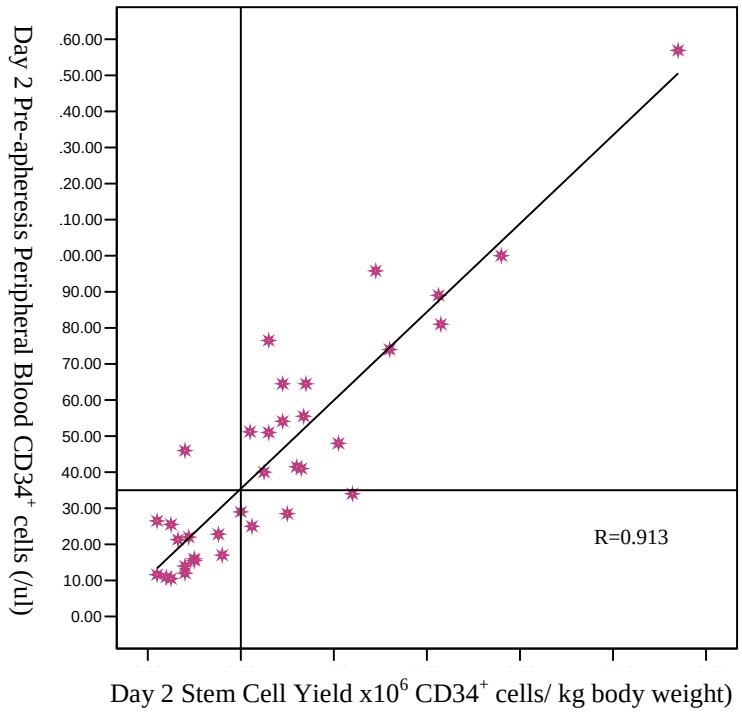

Figure 12(c): Correlation Analysis of Pre-apheresis Peripheral Blood $CD34^+$ Cells and Stem Cell Yield for Day 2 Harvesting

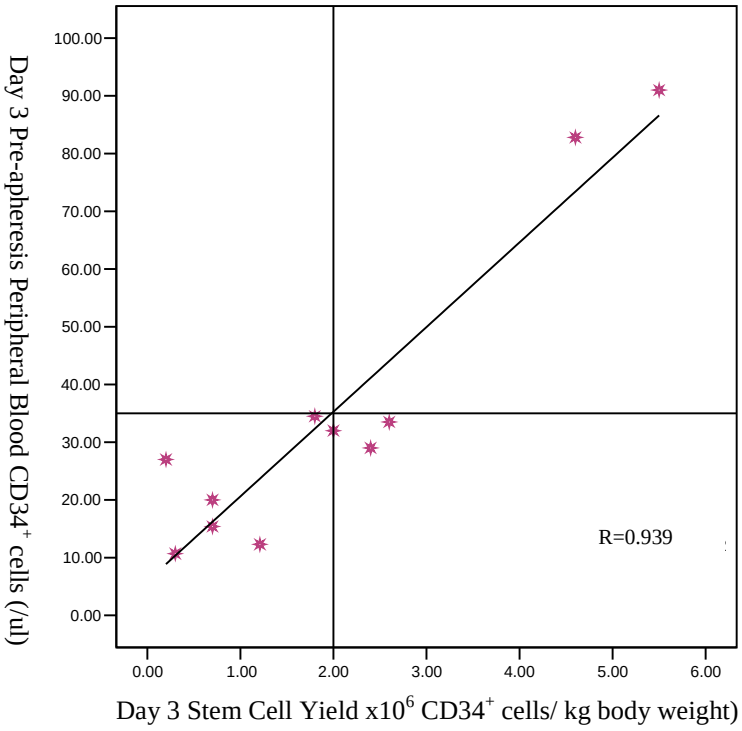

Figure 12(d): Correlation Analysis of Pre-apheresis Peripheral Blood $CD34^+$ Cells and Stem Cell Yield for Day 3 Harvesting

In Figure 12 (b-d), pre-apheresis peripheral blood $CD34^+$ cells for day 1, 2 and 3 correlated well with the respective stem cell yield for each day with r value of 0.967 (day 1), 0.913 (day 2) and 0.939 (day 3). In these correlation study, they showed that to predict the stem cell yield of $\geq 2 \times 10^6$ $CD34^+$ cells/ kg body weight with single apheresis, the pre-apheresis peripheral blood $CD34^+$ cells count should be ≥ 28 cells/ul, and to achieve the yield of $\geq 5 \times 10^6$ $CD34^+$ cells/ kg body weight, the pre-apheresis peripheral blood $CD34^+$ cells count of ≥ 70 cells/ul should be obtained. Whereas for day 2 and day 3 PBSC harvesting, the cut off value of peripheral blood $CD34^+$ cells count to achieve the stem cell yield of $\geq 2 \times 10^6$ $CD34^+$ cells/ kg body weight was approximately 35 cells/ul.

4.3.2 Post-apheresis Peripheral Blood CD34$^+$ Cells Day 1 and Day 2 Pre-apheresis Peripheral Blood CD34$^+$ Cells

The correlation between post-apheresis peripheral blood CD34$^+$ cells day 1 PBSC harvesting and pre-apheresis peripheral blood CD34$^+$ cells day 2 showed that there is a positive correlation between this two variables with the r value of 0.895, which means that the higher post-apheresis peripheral blood CD34$^+$ cells count day 1 predicted a higher count of day 2 pre-apheresis peripheral blood CD34$^+$ cells ($r = 0.895$, $p < 0.001$). Figure 13 showed a cut off value of ≥ 25 cells/ul post-apheresis peripheral blood CD34$^+$ cells count day 1 correlated with a 35/ul CD34$^+$cells for day 2 pre-apheresis peripheral blood. Referring to Figure 12 (c), to predict a day 2 stem cell yield of $\geq 2 \times 10^6$ CD34$^+$ cells/ kg body weight, a day 2 pre-apheresis peripheral blood CD34$^+$ cells count of 35/ul should be considered.

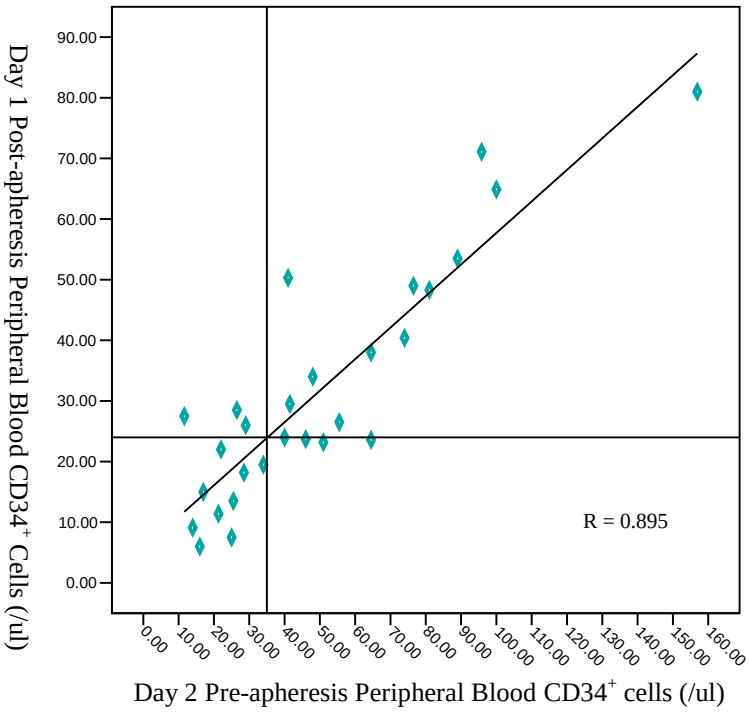

Figure 13: Correlation Analysis of Post-Apheresis Peripheral Blood CD34$^+$ Cells Day 1 and Day 2 Pre-Apheresis Peripheral Blood CD34$^+$ Cells

4.3.3 Post-apheresis Peripheral Blood CD34$^+$ Cells Day 1 and Day 2 Stem Cell Yield

Further analysis was carried out to determine a correlation between post-apheresis peripheral blood CD34$^+$ cells day 1 PBSC harvesting and day 2 stem cell yield in the 28 apheresis procedures (Figure 14). In this analysis, 7 apheresis procedures were excluded due to incomplete data. The scatter dot plot showed that post-apheresis peripheral blood CD34$^+$ cells day 1 PBSC harvesting correlated well with day 2 stem cell yield cells (r = 0.811, p < 0.001). A cut off value of 25 cells/ul post-apheresis peripheral blood CD34$^+$ cells count day 1 correlated well with the stem cell yield day 2 with r value of 0.811 though slightly lower as compared to day 2 pre-apheresis peripheral blood CD34$^+$ cells and stem cell yield day 2 with r = 0.913.

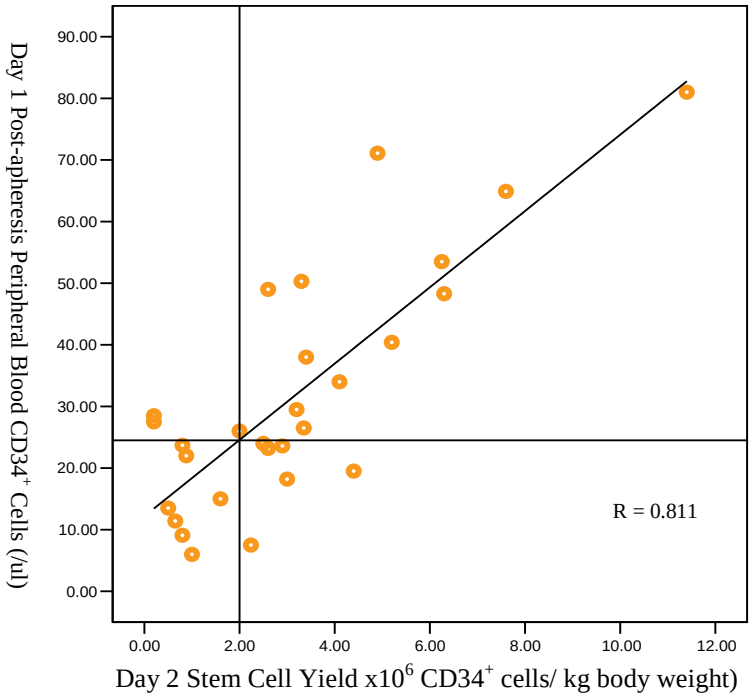

Figure 14: Correlation Analysis of Post-apheresis Peripheral Blood CD34$^+$ Cells Day 1 and Day 2 Stem Cell Yield

In terms of the number of procedures achieving stem cell yield of $\geq 2 \times 10^6$ $CD34^+$ cells/kg body weight with cut off values of 25 cells/ul day 1 post-apheresis and 35 cells/ul day 2 post-apheresis were 17 and 16 respectively.

With these results, day 1 post-apheresis peripheral blood $CD34^+$ cells count may be used to predict the day 2 stem cell yield without performing a day 2 pre-apheresis peripheral blood $CD34^+$ cells count which is time consuming and may delay the PBSC harvesting procedures.

4.4 RECEIVER OPERATING CHARACTERISTIC CURVE ANALYSIS

The Receiver Operating Characteristic (ROC) curve analysis was used to evaluate the cut-off value of pre-apheresis peripheral blood $CD34^+$ cells to achieve the stem yield of $\geq 2 \times 10^6$ $CD34^+$ cells/ kg body weight. Data from 94 apheresis for day 1 to day 3 of apheresis procedures were analyzed. Based on all the 94 apheresis procedures, a cut off (or criterion) value of > 27 cells/ul pre-apheresis peripheral blood $CD34^+$ cells count was the value with the highest average of sensitivity (95%) and specificity (82.4%) that may predict a stem cell yield of $\geq 2 \times 10^6$ $CD34^+$ cells/ kg body weight. Figure 15(a).

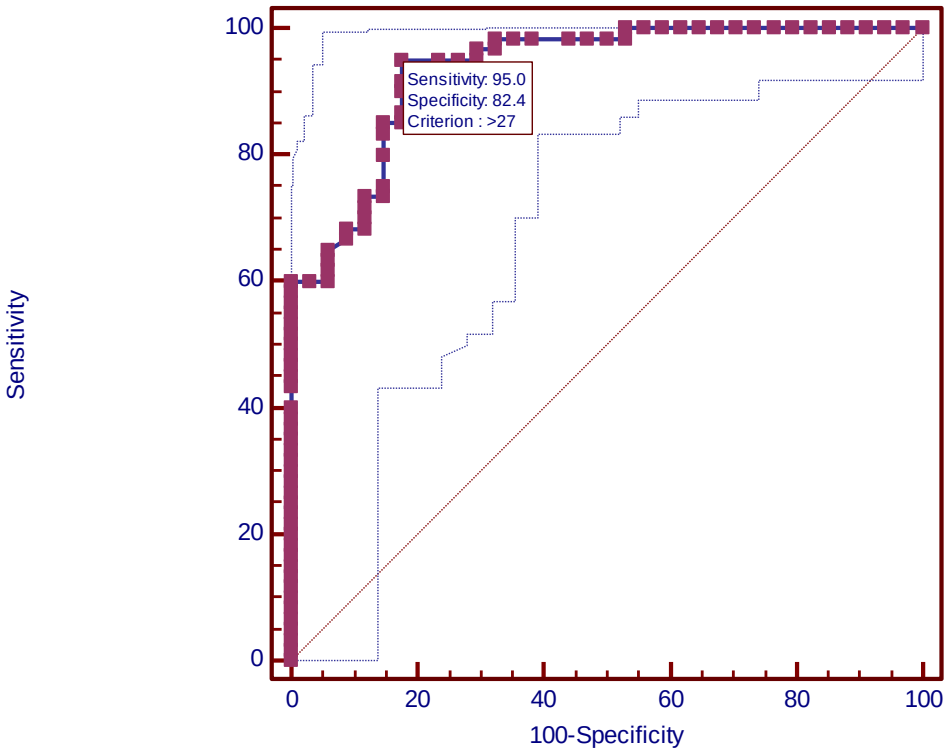

Figure 15(a): ROC Curve of Overall Pre-Apheresis Peripheral Blood $CD34^+$ Cells and Stem Cell Yield for Day 1, 2 and 3 PBSC Harvesting

Table 4(a): Criterion Values and Coordinates of the ROC Curve for Day 1, 2 and 3 PBSC Harvesting

Area under the ROC curve (AUC)	0.935
Standard Error [a]	0.0248
95% Confidence Interval [b]	0.864 to 0.975
z statistic	17.501
Significance level P (Area=0.5)	<0.0001

[a] Hanley & McNeil, 1982
[b] Binomial exact

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>27 *	95	86.1 - 99.0	82.35	65.5 - 93.2	5.38	0.061	90.5	90.3

Analysis from overall data showed an area of 0.935 under the ROC curve. This means that a randomly selected individual from the positive group (stem cell yield ≥ 2 x10^6 $CD34^+$ cells/ kg body weight) has a test value larger than that for a randomly chosen individual from the negative group (stem cell yield ≤ 2 x10^6 $CD34^+$ cells/ kg body weight) in 93.5% of the time. Area under the ROC curve (AUC) also lies with 95% confidence interval and was significantly different between the two groups (P <0.0001).

In Figure 15 (b-d), ROC curve showed a cut off value of pre-apheresis peripheral blood $CD34^+$ cells for day 1, 2 and 3 with the respective stem cell yield for each day with AUC of 0.895 (day 1), 0.969 (day 2) and 0.9 (day 3). All the data was significantly different with p value <0.001. For day 1, ROC curve analysis showed that to predict the stem cell yield of ≥ 2 x10^6 $CD34^+$ cells/ kg body weight with single apheresis, the pre-apheresis peripheral blood $CD34^+$ cells count should be ≥ 27 cells/ul. Whereas for day 2 and day 3 PBSC harvesting, the cut off value of peripheral blood $CD34^+$ cells count to achieve

the stem cell yield of ≥ 2 x10^6 $CD34^+$ cells/ kg body weight was 26.5 cells/ul and ≥ 27 cells/ul respectively.

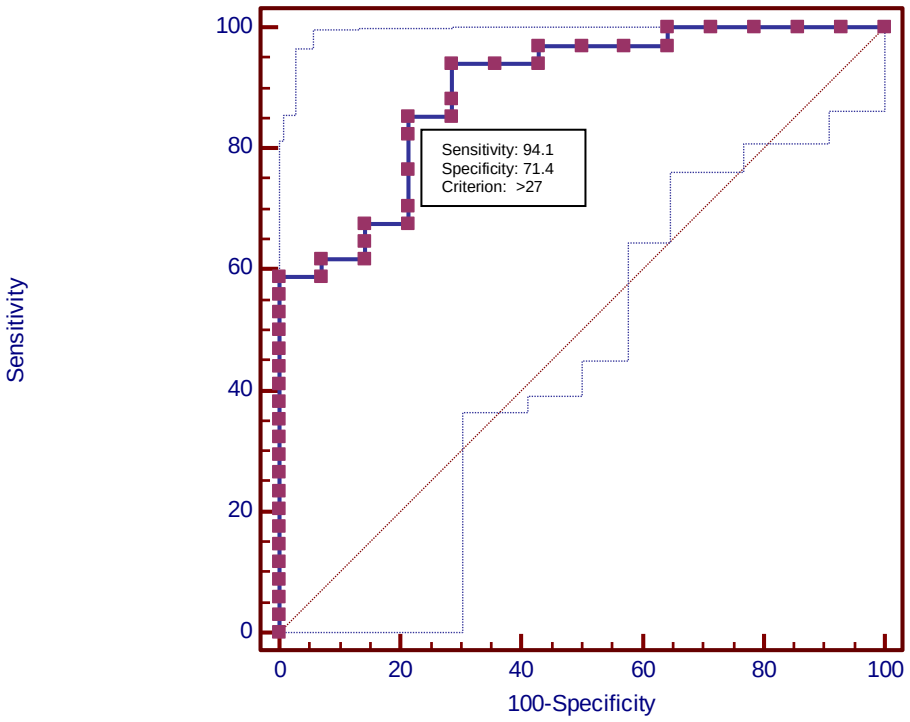

Figure 15(b): ROC Curve of Pre-apheresis Peripheral Blood $CD34^+$ Cells and Stem Cell Yield for Day 1 Harvesting

Table 4(b): Criterion Values and Coordinates of the ROC Curve for Day 1 PBSC Harvesting

Area under the ROC curve (AUC)	0.895
Standard Error [a]	0.0451
95% Confidence Interval [b]	0.772 to 0.965
z statistic	8.767
Significance level P (Area=0.5)	<0.0001

[a] Hanley & McNeil, 1982

[b] Binomial exact

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>27 *	94.12	80.3 - 99.3	71.43	41.9 - 91.6	3.29	0.082	88.9	83.3

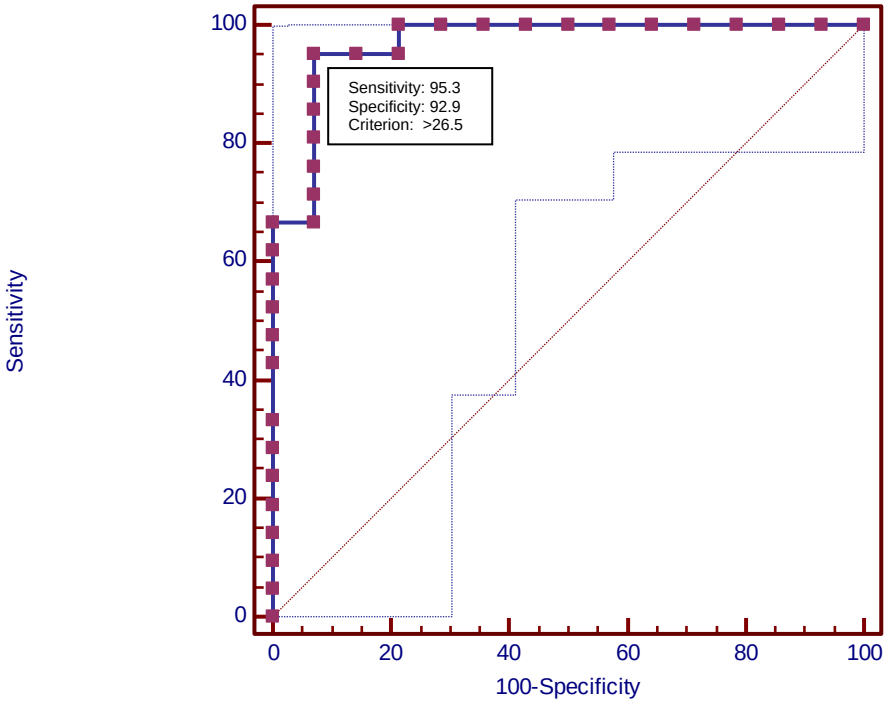

Figure 15(c): ROC Curve of Pre-apheresis Peripheral Blood $CD34^{+}$ Cells and Stem Cell Yield for Day 2 Harvesting

Table 4(c): Criterion Values and Coordinates of the ROC Curve for Day 2 PBSC Harvesting

Area under the ROC curve (AUC)	0.969
Standard Error [a]	0.0284
95% Confidence Interval [b]	0.848 to 0.999
z statistic	16.525
Significance level P (Area=0.5)	<0.0001

[a] Hanley & McNeil, 1982

[b] Binomial exact

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>26.5 *	95.24	76.2 - 99.9	92.86	66.1 - 99.8	13.33	0.051	95.2	92.9

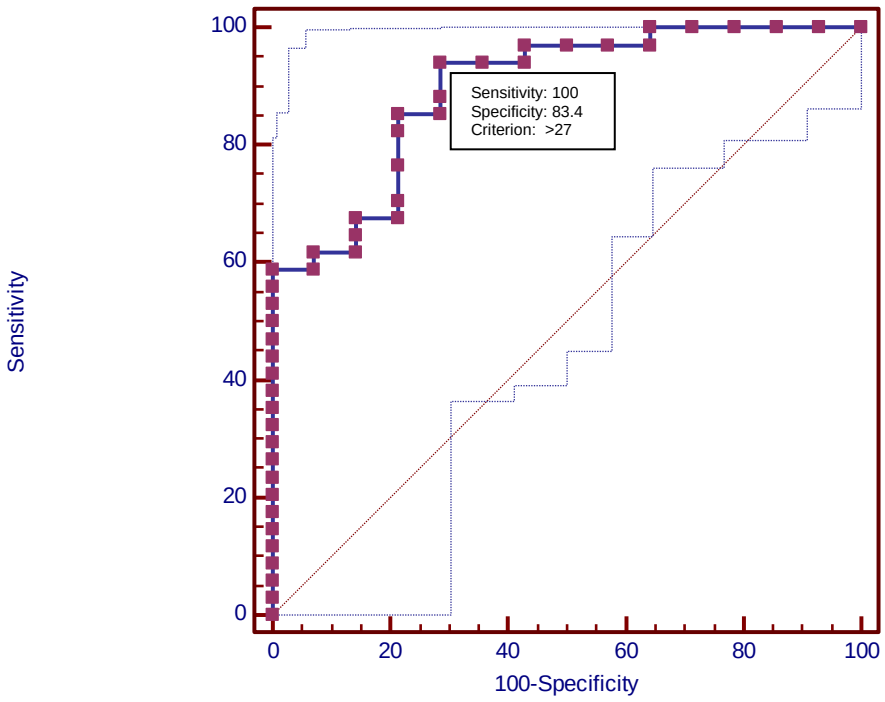

Figure 15(d): ROC Curve of Pre-apheresis Peripheral Blood CD34^{+} Cells and Stem Cell Yield for Day 3 Harvesting

Table 4(d): Criterion Values and Coordinates of the ROC Curve for Day 3 PBSC Harvesting

Area under the ROC curve (AUC)	0.9
Standard Error [a]	0.106
95% Confidence Interval [b]	0.576 to 0.997
z statistic	3.78
Significance level P (Area=0.5)	0.0002

[a] Hanley & McNeil, 1982

[b] Binomial exact

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>27 *	100	47.8 - 100.0	83.33	35.9 - 99.6	6	0	83.3	100

4.5 COLLECTION EFFICIENCY

A total of 77 apheresis data had been analyzed for collection efficiency of $CD34^+$ cells using Cobe Spectra Apheresis Machine with semiautomated MNC program. Calculation of collection efficiency for 17 apheresis could not be performed due to incomplete variables. The variables that are needed in the calculation of collection efficiency are total $CD34^+$ cells in apheresis product, pre-apheresis peripheral blood $CD34^+$ cells, post-apheresis peripheral blood CD34+ cells and total blood volume processed.

The following formula was used for the calculation of collection efficiency;

$$\frac{\text{Total } CD34^+ \text{ cells in Apheresis Product}}{\left[\frac{\text{Pre-apheresis } CD34^+ \text{ cells} + \text{Post-apheresis } CD34^+ \text{ cells}}{2}\right] \times \text{Total Blood Processed}} \times 100$$

The median values and range for all parameters that influenced the percentage of collection efficiency are shown in Table 5. The median overall collection efficiency of $CD34^+$ cells was 63.7%. This collection efficiency is comparable to the manufacturer's data which is in the range of 55-65%.

Table 5: Collection Efficiency and Contributing Variables

	Median	**Range**
Pre-apheresis Peripheral Blood $CD34^+$ Cells/ul	33.6	9.5 - 456.5
Post-apheresis Peripheral Blood $CD34^+$ Cells/ul	28.5	3.58 - 240.5
Total CD34+ Cells Yield, $x10^6$	137.7	4.4 - 2567
Total Blood Volume Processed, ml	8701	4583 - 12918
Collection Efficiency, %	63.7	5.5 – 116.2

5.0 DISCUSSION

A major challenge in PBSC harvesting from mobilized patients is to collect a sufficient number of $CD34^+$ cells for transplantation and the requirements are different between institutions. In UKM Medical Centre, a minimum amount of 2 $x10^6$ $CD34^+$ cells/kg body weight recipient is used. This study was performed on 48 patients with haematological diseases.

In the current practice, PBSC harvesting will be performed when the pre-apheresis peripheral blood $CD34^+$ cells count achieved 15cells/ul. From the analysis, 43 patients (89.6%) were successful and 5 patients (10.4%) failed to obtain stem cell yield of 2 $x10^6$/ kg body weight with 3 or less apheresis procedures. Only 34 patients (70.8%) achieved $\geq$ 2 $x10^6$/ kg body weight with one apheresis procedures.

In this study, the pre-apheresis peripheral blood $CD34^+$ cell count and product yield has shown a significant positive correlation with $r = 0.963$ and $p < 0.001$. The cut-off value of 33 cells/ul pre-apheresis peripheral blood $CD34^+$ cells count has shown to predict a stem cell yield of $\geq$ 2 $x10^6$ $CD34^+$ cells/kg body weight ($r = 0.963$, $p < 0.001$).

Meanwhile, the data also been evaluated for cut-off point using Receiver Operating Characteristic (ROC) curve analysis. Based on all the 94 apheresis procedures, a cut off (or criterion) value of > 27 cells/ul pre-apheresis peripheral blood $CD34^+$ cells count was the value with the highest average of sensitivity and specificity that may predict a stem cell yield of $\geq$ 2 $x10^6$ $CD34^+$ cells/ kg body weight. Both methods of evaluation showed different cut-off value. However, the use of ROC analysis was more appropriate statistical method when evaluating the cut-off values for clinical intervention.

ROC curve analysis was used to compare the diagnostic performance of two or more laboratory or diagnostic tests (Griner *et al*., 1981). The ability of a test to discriminate diseased cases from normal cases also can be evaluated using ROC curve analysis (Metz, 1978; Zweig and Campbell,1993). In determining the cut-off (or criterion) value, rarely there will be a perfect separation between the two groups. Indeed, the distribution of the test results will overlap. In ROC curve analysis, the criterion value selected had the highest average of sensitivity and specificity.

In a ROC curve, the true positive rate (Sensitivity) is plotted in function of the false positive rate (100-Specificity) for different cut-off points. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions) has a ROC plot that passes through the upper left corner (100% sensitivity, 100% specificity). The closer the ROC plot is to the upper left corner, the higher the overall accuracy of the test (Zweig and Campbell, 1993).

Therefore, a cut-off value of 27 cells/ul instead of 33 cells/ul pre-apheresis peripheral blood $CD34^+$ cell count could be implemented in order to get a minimum dose of $\geq 2 \times 10^6$ $CD34^+$ cells/ kg body weight in most patients. This result is comparable to the study done by Alessandro *et al*., 2005. They found that a value ≥ 66/μl was associated with a higher probability of collecting $\geq 4 \times 10^6$ /kg CD34+ cells, which is considered the optimal target for an autologous support after high-dose therapy.

Besides that, Heuft *et al*., 2000 has concluded that pre-apheresis peripheral blood $CD34^+$ cell count ≥ 40/ul is highly predictive of a single PBSC apheresis product with more than 2.5×10^6 /kg CD34+ cells. Not only that, the recommendations for the level of pre-apheresis peripheral blood $CD34^+$ cells

count that should be used to initiate apheresis has varied from 8 to 20/ul (Yu *et al*., 1999; Fantao *et al*., 1999; Kudo *et al.,* 2003 and Moncada *et al*., 2003). If harvesting is initiated at a lower pre-apheresis peripheral blood $CD34^+$ cells count, daily yield will be lower and more apheresis procedures are required to achieve an adequate harvest. If the target is set too high for the pre-apheresis peripheral blood $CD34^+$ cells count, only few patients will reach the targeted count or the peak of mobilisation may be missed in some heavily pre-treated patients.

Although the logic for having a target setting for pre-apheresis $CD34^+$ cells count was to avoid the inefficiency and cost of collections with poor yields, this also result in the exclusion of a number of patients from PBSC harvest and eventually from transplantation. Therefore, Gidron *et al.,* 2008 propose a formula to facilitate the decision of PBSC harvesting if performing apheresis whenever there are any circulating $CD34^+$ cells is an approach of practice. By collecting a small numbers of stem cells over several harvest, this may afford these patients an opportunity to undergo transplantation.

However, by using this approach or an absolute threshold as guideline before performing PBSC harvesting does not give 100% guaranteed result as there were also other factors that may affect the stem cell yield. Patient's age, diagnosis, preceding chemoradiotherapy, disease invasion of the bone marrow and mobilizing chemotherapy and cytokines, timing for apheresis, machines and operating software, length and volume of collection would affect the yield in PBSC collection for autologous transplants.

The present study also showed that there is a significant positive correlation between day 1 post-apheresis peripheral blood $CD34^+$ cells and day 2 pre-apheresis peripheral blood $CD34^+$ cells ($r = 0.895$, $p < 0.001$), and it also

correlated well with day 2 stem cell yield (r = 0.811, p < 0.001). A cut off value of $\geq$ 25 cells/ul post-apheresis peripheral blood $CD34^+$ cell count may be used to predict the day 2 stem cell yield of $\geq$ 2 $x10^6$ $CD34^+$ cells/kg body weight without performing a day 2 pre-apheresis peripheral blood $CD34^+$ cell count.

Therefore, PBSC harvesting procedure for the day 2 can be performed without waiting for the result of peripheral blood $CD34^+$ cells count of the day which may delay the second day apheresis procedure, thus leading to late processing and cryopreservation of stem cell products. Besides, by doing the day 1 post-apheresis peripheral blood $CD34^+$ cells count which parellel with the count of stem cell yield day 1, this may also save manpower as well as the reagent costs.

Up to this point of time, no previous studies could be found on either the correlation between day 1 post-apheresis peripheral blood $CD34^+$ cells count and day 2 pre-apheresis peripheral blood $CD34^+$ cell count or the correlation between day 1 post-apheresis peripheral blood $CD34^+$ cells count and day 2 stem cell yield.

The collection of PBSC can be performed on various cell separator devices and in this study, the collection efficiency (CE) was evaluated on the Cobe Spectra apheresis machine using semiautomated MNC program. The median overall CE of $CD34^+$ cells was 63.7% with a range of (5.5% – 116.2%) and this value is comparable to the manufacturer's data which is in the range of 55-65%. The calculation of CE are dependent on the variables as in the following equation;

$$\frac{\text{Total } CD34^+ \text{ cells in Apheresis Product}}{\left[\frac{\text{Pre-apheresis } CD34^+ \text{ cells} + \text{Post-apheresis } CD34^+ \text{ cells}}{2}\right] \times \text{Total Blood Processed}} \times 100$$

In other words, CE is equal to stem cell yield divided by total quantity of cells processed (TQproc) through the channel during the procedure. The calculation for the TQproc is influenced by the % change in the $CD34^+$ count during the procedure. The post-apheresis peripheral blood $CD34^+$ count is usually lower than the pre-apheresis peripheral blood $CD34^+$ count, and the average fall may range from 0% to – 60% or more and in the present study, the median % change was 15% from pre-apheresis peripheral blood to post-apheresis peripheral blood count.

The % change in $CD34^+$ count from the pre-apheresis procedure value is primarily an indication of cell mobilization during the procedure. A 0% fall in the count suggests that a lot of cells have been mobilized; an 80% reduction in the post-apheresis count suggests few cells were mobilized. This influences the calculation of the quantity of cells that were processed through the channel, and thereby influences the calculations of CE. The CE for 0% in pre/post count yields a low CE while the CE for –80% fall in pre/post -apheresis counts yields a higher CE. The following variables have been observed to influence the percentage of CE;

i) CE is inversely related to the % change in the pre/post $CD34^+$ count. That is, the greater the drop in the $CD34^+$ count, post procedure, the higher the CE. This is an indirect reflection of cell mobilization during the procedure. The greater the mobilization, the less the fall in the count, and the lower the CE. The magnitude of mobilization is a biological factor over that could not be predicted. Inefficient device separation and collection such as poor interface positioning for collection and device

inefficiency, could result in a smaller % decease in the $CD34^+$ count

ii) CE is positively correlated with the $CD34^+$ yield, when the yield is expressed as some % of the starting quantity of $CD34^+$ available in the peripheral blood to be collected, a product of the pre-apheresis count x donor blood volume. Procedures may collect less than 100% of the initial CD34 quantity, but in well-performed procedures that process 2 and 3x the blood volume, the yield will be some value above 100% of the starting quantity

iii) The CE is also associated with the total blood volume (TBV) processed. TBV processed was calculated using the COBE Spectra software based on the sex, height and weight of the patient. In these patients, the median TBV processed was 8701 ml (range 4583 – 12918 ml). High TBV processed will result in low CE. For example if ≥ 3 x TBV processed, the CE falls unless the yield rises above 180% of the starting quantity.

Therefore, the lower or higher value of CE is very subjective in determining the efficiency of the apheresis machine as this value is also dependent on the mobilization process throughout the PBSC harvesting procedure. It is possible that the CE was low because of the post-apheresis count is high due to mobilization, which makes it look like the machine did not perform an efficient PBSC collection.

6.0 CONCLUSION

In summary, this study has shown a significant positive correlation between pre-apheresis peripheral blood $CD34^+$ cells and stem cell yield. A cut-off value of 27 cells/ul pre-apheresis peripheral blood $CD34^+$ cells count may be used to predict stem cell yield of $\geq 2 \times 10^6$ $CD34^+$ cells/kg body weight. By doing this, the number of apheresis with poor stem cell yield may be reduced and this may help to reduce the wastage of consumables, man power, costs and numbers of PBSC harvesting procedure.

Furthermore, the day 1 post-apheresis peripheral blood $CD34^+$ cell count with a value of ≥ 25 cells/ul may be used to predict the day 2 stem cell yield of $\geq 2 \times 10^6$ $CD34^+$ cells/kg body weight without performing a day 2 pre-apheresis peripheral blood $CD34^+$ cells count which may delay the PBSC harvesting procedure and thus the stem cell processing procedure.

Overall, the median CE of $CD34^+$ cells was 63.7% and this value is comparable to the manufacturer's data which is in the range of 55-65%. The value of CE is very subjective in determining the efficiency of the apheresis machine or a particular apheresis protocol as this value also dependent on the mobilization process of patients that is difficult to measure throughout the PBSC harvesting procedure.

In conclusion, the product yield and collection efficiency of peripheral blood stem cell harvesting can be estimated using peripheral blood $CD34^+$ cells quantification. The results obtained may serve as a guide to improve the PBSC harvesting procedure in UKM Medical Centre and shed new light on the the survival rate post-transplantation as well as the speed of engraftment.

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APPENDIX I

MATERIALS AND METHODOLOGY FOR PBSC HARVESTING

Instruments:

1. The COBE Spectra ™ Apheresis System
2. Tubing Heat Sealer
3. Hemostats
4. Tubing stripper

Consumables and Reagents:

1. AutoPBSC disposable set
2. One return line needle or catheter
3. Anti-coagulant (ACD-A)
4. 0.9% NaCl for injection (1000 ml)

Method:

1.0 SET UP

1.1 Equipment Set Up

1.1.1 Plug system into electrical outlet, as needed.
1.1.2 Turn power switch ON.

1.2 Setting up the Disposable Tubing Set

1.2.1 Install the standard dual-stage filler in the centrifuge, if necessary. Ensure that the filler in the machine is the standard dual-stage filler.

1.3 Installing Tubing on Front Panel

1.3.1 Install the collect flow path overlay on the front panel.
1.3.2 Remove the disposable package cover and record the lot number from the cover label onto the apheresis procedure record.
1.3.3 Remove the inlet coil, remove the tapes, and hang on left side of machine.
1.3.4 Place the access saline line over top of machine.
1.3.5 Remove the return coil, remove the tape, and hang on the left side of the machine.
1.3.6 Place the return saline spike over the top of the machine.
1.3.7 Remove the bags and place them in the correct position on the IV pole.
1.3.8 Remove the return pump cartridge and snap it into the cartridge clamp between the plasma and collect/replace pumps.
1.3.9 Remove the access pump cartridge and snap it into the cartridge clamp between the AC and the inlet pumps.

1.3.10 Place the AC line over the top of the machine.
1.3.11 Press the CONTINUE key to load the tubing into the pump housings. Verify that all four pumps are loaded.
1.3.12 Place the lines in the collect/replace and plasma valves.
1.3.13 Place the return pressure sensor in the return pressure sensor housing.
1.3.14 Install the cuvette into the collect concentration monitor housing. Take care not to touch the cuvette with your fingers. The cuvette will move freely on the collect line. Push downward and turn the CCM clockwise to lock into place.
1.3.15 Place the RBC line into the RBC valve. Ensure that the line is completely inserted into the RBC detector.
1.3.16 Position the return and inlet air chambers in the air detectors with the air chamber filters below the air detector housings. The RBC Reservoir sits freely on the front panel of the Spectra system.
1.3.17 Place the waste lines into the waste valve assembly.
1.3.18 Place the line in the centrifuge pressure sensor using a "flossing" action.
1.3.19 Place the access pressure sensor in the access pressure sensor housing.
1.3.20 Position the return line in the return valve so that the line runs horizontally through the center of the valve.

1.4 Installing Channel In Centrifuge

1.4.1 Press the UNLOCK COVER key.
1.4.2 Open the centrifuge by sliding the centrifuge cover back and lowering the door.
1.4.3 Gently extend the tubing leading to the centrifuge loop to full length to ensure that the tubing is not twisted.
1.4.4 Fold the centrifuge channel in half and load by inserting the channel through the loading port and pulling through from the top.
1.4.5 Position the channel in the correct orientation above the filler slots before placing the centrifuge collar into the collar holder.
1.4.6 Load the centrifuge collar into the centrifuge collar holder, closing the cover over the collar.
1.4.7 Lower the filler latch into the locked position.
1.4.8 Press the channel into position.
1.4.9 Press the tubes into the appropriate slots in the filler.
1.4.10 Place the lower bearing in the lower bearing holder, making sure that the hard plastic bearing is securely seated in the bearing holder.
1.4.11 Place the upper bearing in the upper bearing holder, making sure that the hard plastic bearing is securely seated in the bearing holder.
1.4.12 Place the upper collar into the upper collar holder.
1.4.13 Place the multi-lumen tubing in the exit slot on the right side of the system using a "flossing" action.
1.4.14 Rotate the centrifuge clockwise several times to ensure that the tubing does not twist and stays in place.
1.4.15 Close the centrifuge door and cover.

1.5 Priming the Disposable Tubing Set

1.5.1 Check the needle luer connections to make sure that they are secure.

1.5.2 Press the "3" key to select WBC when prompted to select set type. Press ENTER.

1.5.3 Close the white pinch clamps on the access and return lines. Close the roller clamps on both saline lines. The spike leading to the AC container is orange.

1.5.4 Press the CONTINUE key.

1.5.5 Connect the AC line with the orange spike to the AC container and place in the AC level detector.

1.5.6 Connect the access and return saline lines to the saline container. Press the CONTINUE key.

1.5.7 Open the roller clamps on the two saline lines. Press the CONTINUE key to prime the disposable set.

1.5.8 When prompted, prime the access and return patient connections by opening the white pinch clamps near the needle or luer connections.

1.5.9 Close the access saline line, clamp the access line and press the CONTINUE key to test the ration.

2.0. ALARM TEST

2.1 Press the CONTINUE key to clear the warning from the screen.

2.2 Press the YES key to perform the alarm tests and the NO key to bypass the alarm tests and the NO key to bypass the alarm tests. If the NO key is selected, you may proceed to Enter Donor/Patient Data.

2.3 Ensure that the access saline is closed and the access and return lines are clamped. Press the CONTINUE key. The COBE Spectra System will test the access pressure sensor low alarm and the air in return chamber alarm.

2.4 At the prompt, open the roller clamp on the access saline lines.

2.5 Press the CONTINUE key. The Cobe Spectra System will test the return pressure high alarm and the air in return chamber alarm.

2.6 At the prompt, verify that the return line valve is closed. Press the CONTINUE key to go on to the next test.

2.7 Press the UNLOCK COVER key and open the centrifuge door to perform the fluid leak detector alarm test.

2.8 Touch the fluid leak detector with your fingers to initiate the alarm. Verify that the COBE Spectra System indicates that fluid is detected in the centrifuge.

2.9 Close the centrifuge door and cover. Press the continue key.

3.0 ENTER DONOR/PATIENT DATA

3.1 Enter the donor/patient sex. Press ENTER.

3.2 Enter the donor/patient height. Press ENTER.

3.3 Enter the donor/patient weight. Press ENTER.

3.4 Respond to the Total Blood Volume display. Pressing the Yes key accepts the data and the NO key redisplays the data for collection.
3.5 Enter the hematocrit as a whole number. Press the ENTER key.
3.6 Enter the pre-procedure WBC concentration. Press ENTER. A WBC count and MNC differential from the day of collection are preferred. The system will not accept the zero default. You must enter a number here.
3.7 Enter the MNC percentage. Press ENTER. The system will not accept the zero default if no plasma is desired.
3.8 Pressing the Yes key approves the values. Pressing the NO key prompts the operator to change a value.

4.0 CONNECT DONOR/PATIENT

4.1 Perform the venipunctures for the access and return needle sites or connect a catheter. If performing a venipuncture, the return needle selected by the facility should be connected to the luer on the return site to maintain extended storage capability if closed system is desired.
4.2 Open the white pinch clamps on the return line to keep the return needle from clotting.
4.3 Leave a saline drip on the return line to keep the return needle from clotting.
4.4 Close the roller clamp on the access saline line.

5.0 RUN MODE

5.1 Press the CONTINUE key to initiate the run Mode.
5.2 When prompted, close the return saline roller clamp.
5.3 Press the CLEAR key to remove the message about closing the return saline from the screen. The system will first establish the interface, then proceed with the run.
5.4 When prompted, press the "1" key to initiate Rinseback.
5.5 If an alarm message is received during the procedure, the operator should consult the Troubleshooting section of the Operator's Manual and follow the instructions for that alarm.

6.0 RINSEBACK

6.1 Close the white pinch clamp on the access line and open the access saline line.
6.2 Press the CONTINUE key to begin Rinseback.
6.3 Remove/disconnect the access needle and place in an appropriate biohazard container.
6.4 Clamp and disconnect the collection bags when prompted. Press the CLEAR key. Both the product and the plasma bags should be permanently sealed.

7.0 DISCONNECT DONOR OR PATIENT/ UNLOAD PUMPS

7.1 When the Rinseback mode is completed, close the white pinch clamp on the return line. Remove/ disconnect the return needle and place in an appropriate biohazard container, The donor / patient must be disconnected before unloading the pumps.
7.2 Close the roller clamps on the access and the return saline lines.
7.3 Press the continue key. Record final values.
7.4 Press the CONTINUE key to unload pumps. The inlet pump will run to verify that the lines are closed.

8.0 REMOVING THE DISPOSABLE SET

8.1 Place the access and return connections in an appropriate biohazard disposable container.
8.2 Press the UNLOCK COVER key and open the centrifuge.
8.3 Remove the multi-lumen tubing from the exit slot on the right side of the system.
8.4 Remove the upper collar from the upper collar holder.
8.5 Remove the upper bearing from the upper bearing holder.
8.6 Remove the lower bearing from the lower bearing holder.
push the filler latching pin toward the center of the centrifuge and raise the filler latch.
8.7 Remove the tubes from the slots in the filler.
8.8 Remove the channel from the filler.
8.9 Remove the centrifuge collar.
8.10 Raise the channel above the filler.
8.11 Fold the channel in half and lower through the loading port. Remove form centrifuge compartment.
8.12 Close the centrifuge door and cover.
8.13 Remove the lines from the front panel, including: collect and plasma valves, return pressure sensor, waste divert valve, RBC line valve, CCM, return and inlet air detectors, centrifuge
pressure sensor, access pressure sensor, return line valve, AC level detector.
8.14 Remove the pump cartridges from the cartridges clamps.
8.15 Remove the fluid containers and waste bag from the pole and dispose of the disposable set in a biohazard bin.

APPENDIX II

MATERIALS AND METHODOLOGY FOR $CD34^+$ CELLS ENUMERATION

Instruments:

1. Flow cytometer (FacsCalibur, Becton Dickinson)
2. Vortex

Disposable items:

1. Trucount tube (Becton Dickinson)
2. Pipettes
3. Pipette tips

Samples and Reagents:

1. BD combined antibody reagent CD34/CD45 (Becton Dickinson)
2. 7-AAD Solution (Becton Dickinson)
3. PharM Lyse reagent (Becton Dickinson)
4. BD Stem Cell Control Sample (Becton Dickinson)
5. Phosphate-buffered saline (PBS, Gibco)

Method:

1) Specimen Collection & Preparation

- All samples were kept at room temperature until staining.
- The correct anti-coagulant for different sample types were used for example PBSC / mobilized PBSC use EDTA tube.
- All samples were stain within 8 hours of collection.
- Stained sample were acquired within 2 hours of staining.
- Dilution was to be done with 1X PBS for WBC count is $\geq 50x10^3$/uL.
- Smearing of blood down the side of the tube have to be avoided because if blood remains on the side of the tube, it will not be stained but can be picked up by the lysing solution.

2) Staining Method

- The steps for performing the staining of $CD34^+$ cells as shown in Figure 10.

3) <u>Instrument Set Up</u>:

1. Machine calibration was performed by using BD Calibrite 3 beads with FACSComp software. All the parameters have to pass the calibration.

2. The calibration steps as follow;

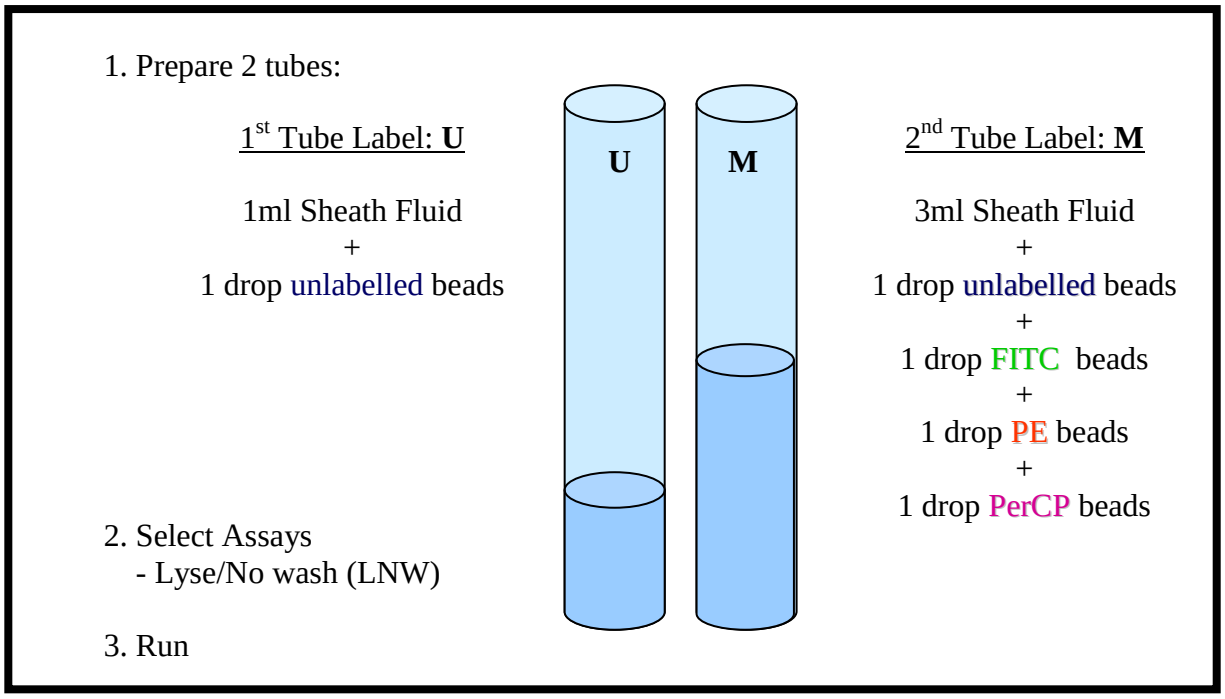
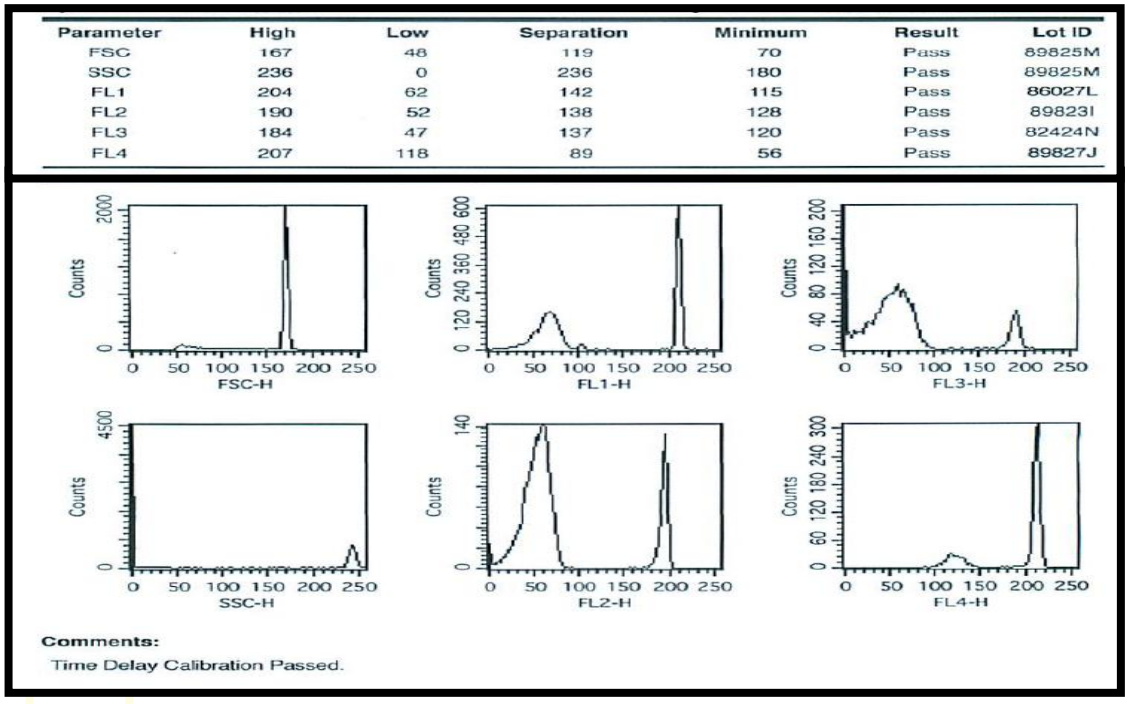


Figure 16: The Flow Cytometer Calibration Step

3. Assay Selection : **Lyse/No Wash** method.

4. Example of calibration result

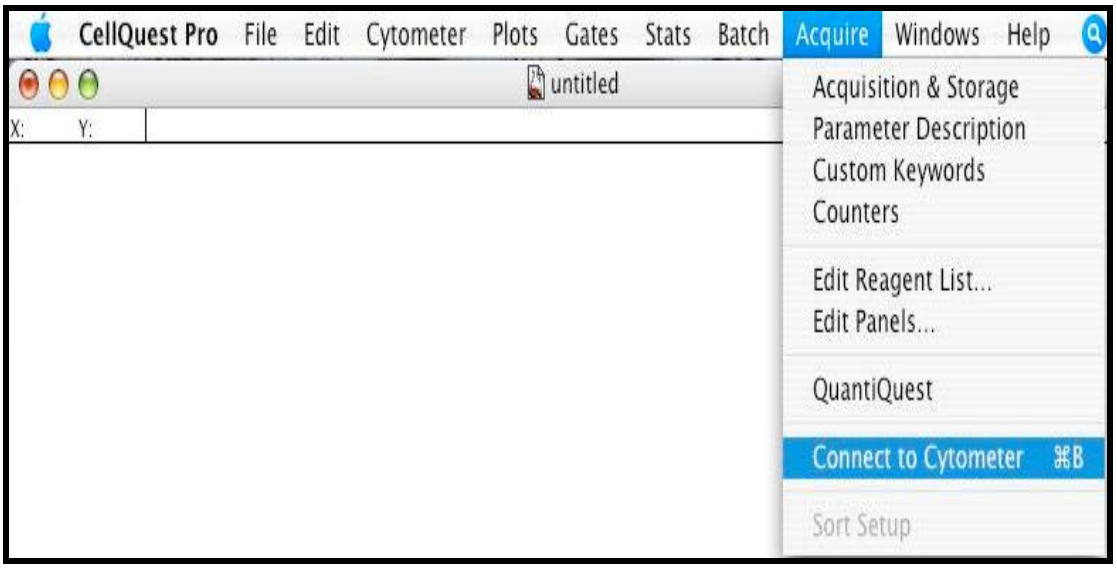


Parameter	High	Low	Separation	Minimum	Result	Lot ID
FSC	167	48	119	70	Pass	89825M
SSC	236	0	236	180	Pass	89825M
FL1	204	62	142	115	Pass	86027L
FL2	190	52	138	128	Pass	89823I
FL3	184	47	137	120	Pass	82424N
FL4	207	118	89	56	Pass	89827J

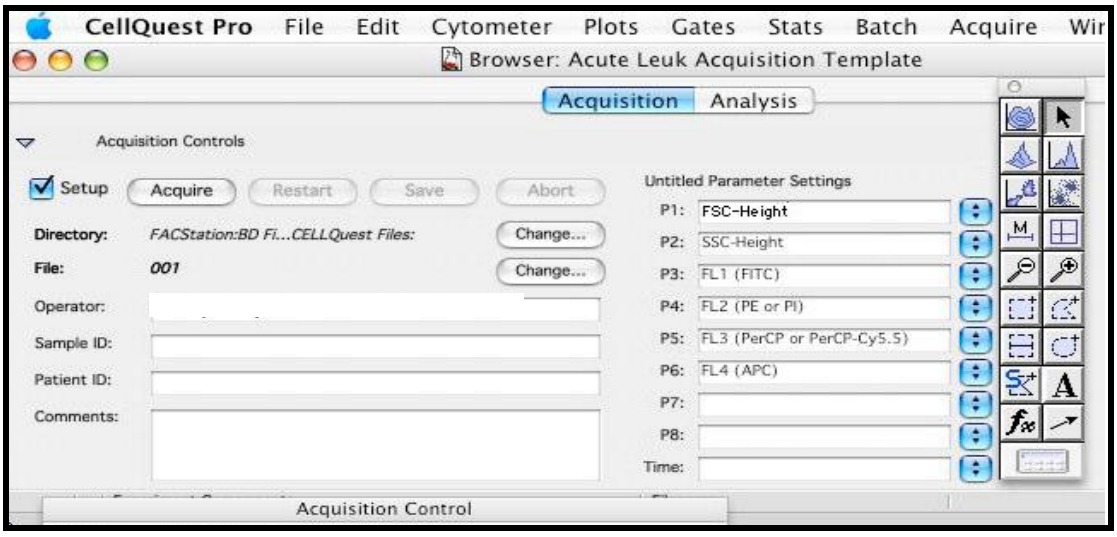
Figure 17: Example of Calibration Result

4). <u>Acquisition Set Up</u>:

- Launching CellQuest Pro software by double clicking the *"*CD34 ISHAGE template*"*.
- Choose <*Connect to Cytometer* > from the <*Acquire*> menu.



- The Acquisition view of the Browser appears:



- Identify the location for FCS file storage during acquisition:
- Key in the sample ID & patient ID.
- Apply the optimized instrument setting

5). Acquisition

- Place the sample in **Sample Injection Port** in flow cytometer and click <**run**> with <**low**> mode.
- Click to select the <Setup> box in <**Acquisition Control**> window. Select "Acquire".
- Check the subpopulations from the dot plots. Make sure all the subpopulations are in scale. Increase FL1 threshold to exclude unwanted debris.
- If satisfied with the setting, click "Pause" _ "Abort" _Deselect the "Setup". For actual acquisition, click "Acquire".
- Change the flow rate to "High".

The life-gate is used to collect ≥ 75,000 CD45 EVENTS.

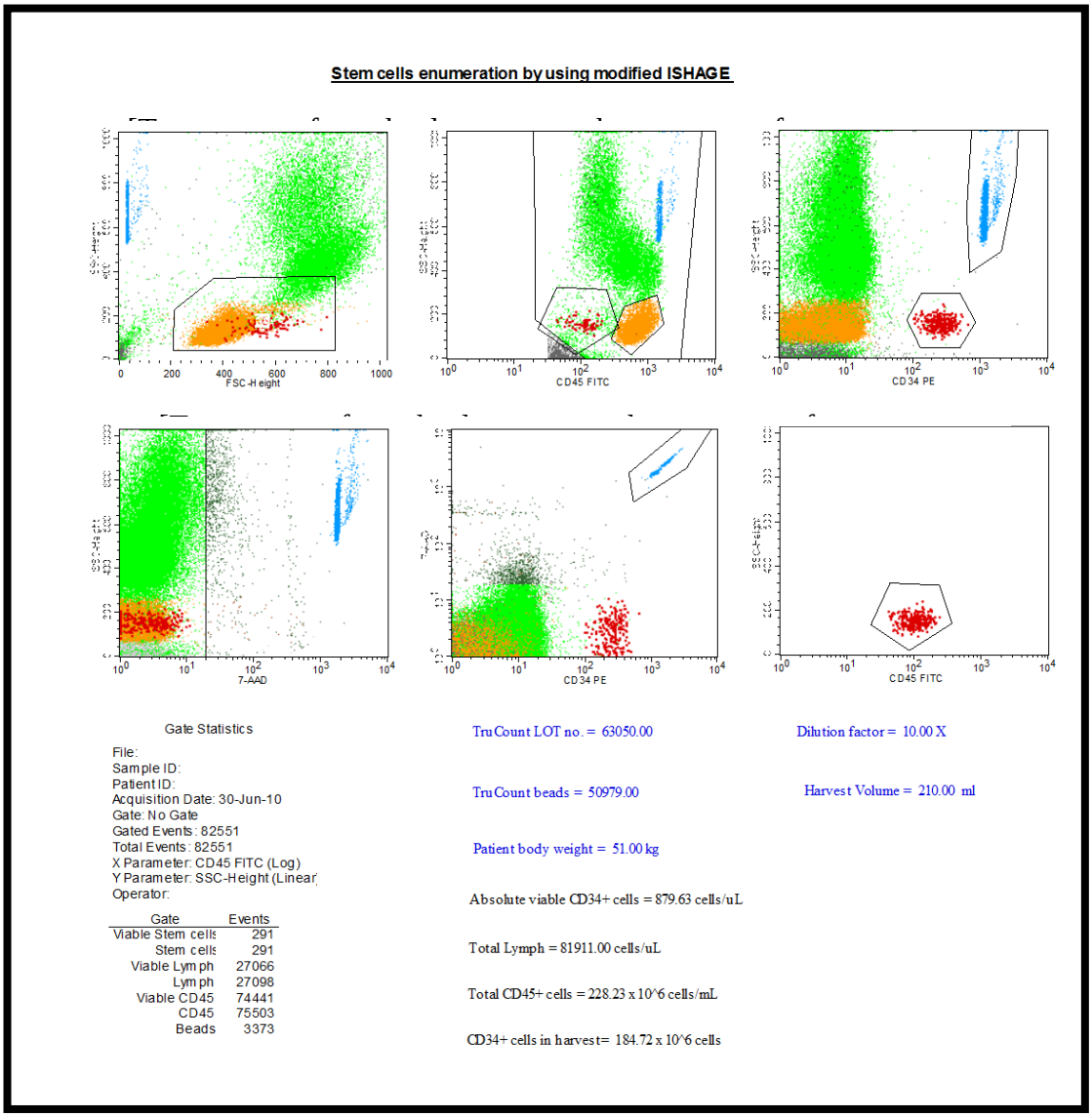


Figure 18: Example of Result After Acquisition

6) Data analysis

The CD34+ cell count is using this formula;

$$= \frac{\#\ CD34^{+}\ events}{\#\ Beads\ events} \times \frac{TruCount\ Beads}{Blood\ volume\ (50uL)} \times Dilution\ factor$$

APPENDIX III

Table 6: RAW DATA OF PATIENT

No	Patient's ID	Harvest Date	Age	Sex	Weight (kg)	Disease
1	N007209	01/03/07	28	F	45	Hodgkin's Lymphoma
2	N063986	01/09/07	45	F	52	Follicular Lymphoma
3	N098229	01/15/07	67	M	62	DLBCL
4	N102705	01/29/07	17	M	51	AML
5	N102713	03/05/07	45	M	44	AML
6	N098229	03/19/07	67	M	62	DLBCL
7	N107407	04/23/07	56	M	64	AML
8	N116876	05/07/07	58	M	73	DLBCL
9	N088027	06/11/07	39	F	48	AML
10	N104139	07/02/07	49	F	58	Multiple Myeloma
11	N128071	07/09/07	51	M	64	AML
12	N114544	08/03/07	63	M	63	DLBCL
13	N105997	08/09/07	17	F	34.6	Mediastinal NHL
14	N114544	08/25/07	63	M	66	DLBCL
15	M711767	09/04/07	46	F	54	AML
16	N136142	09/11/07	58	M	67	DLBCL
17	N140139	09/12/07	49	F	58	Multiple Myeloma
18	N113689	09/24/07	46	M	94	DLBCL
19	N140116	10/01/07	40	F	55	Multiple Myeloma
20	N049871	12/10/07	42	M	63	Multiple Myeloma
21	N167349	01/07/08	41	F	51	AML
22	M791130	01/21/08	54	M	61	Multiple Myeloma
23	N176296	01/28/08	50	M	68	DLBCL
24	N106564	02/11/08	31	M	79	DLBCL
25	N182038	10/3/208	26	F	45	Hodgkin's Lymphoma
26	N143357	03/12/08	14	F	47	AML
27	N032451	03/28/08	33	M	80	Waldenström's macroglobulinemia
28	N143357	04/15/08	14	F	47	AML
29	N168579	04/23/08	51	F	54	AML
30	N168579	06/11/08	51	F	54	AML
31	N184220	07/21/08	21	M	70	Burkitt's Lymphoma
32	N214557	10/28/08	45	M	48	DLBCL
33	N173722	10/29/08	26	M	78	NHL
34	N211185	11/03/08	57	M	60	DLBCL
35	N214081	12/29/08	48	F	44	AML
36	N172910	01/13/09	24	M	53	NHL
37	N218398	01/20/09	29	M	65	Hodgkin's Lymphoma
38	M001071	02/19/09	61	M	69	Richter's Transformation of CLL
39	N083268	03/09/09	28	F	49	DLBCL
40	N211483	04/20/09	59	M	101	Multiple Myeloma
41	M955006	04/29/09	27	F	38	Hodgkin's Lymphoma
42	M837505	05/04/09	42	F	58	Mantle Cell Lymphoma
43	M924781	05/26/09	22	M	72.5	Primary Lung Lymphoma
44	N246502	06/11/09	46	M	51	DLBCL
45	M237724	07/05/09	63	M	58	Multiple Myeloma
46	N248310	07/26/09	37	M	73	DLBCL
47	N239635	11/05/09	53	M	73	Multiple Myeloma
48	M727727	12/16/09	47	M	44	Follicular Lymphoma

No	Patient's ID	WBC count	Pre-apheresis Peripheral Blood Day 1	Total CD34+ Cells Day 1 (x106)	Stem Cell Yield Day 1 (x106/kg Body Weight)	Post-apheresis Peripheral Blood Day 1	Total Blood Volume Process	Volume (ml)	Lymph %	Mono %
1	N007209	29.4	65.9	184	4.09		7186	60	1.9	4.4
2	N063986	10.8	32.7	144	2.77		7139	91	4.2	10.1
3	N098229	19.6	30	124	2		9284	141	6	39.5
4	N102705	71.3	116.4	295	5.9		8667	171	2.1	17.4
5	N102713	31.5	14.9	4.4	0.1		8013	40	5.4	2.7
6	N098229	18.7	22	62	1		8859	141	6	6.8
7	N107407	56.2	30	135	2.11		9482	246	2.4	2.6
8	N116876	15.3	24.5	109.5	1.5	22	9425	153	9.2	17.3
9	N088027	11.7	13	48	1	7.5	6613	61	8.2	33
10	N104139	14.9	58	229.6	3.96	49	7264	181	5.2	22.9
11	N128071	22	40	115.2	1.8	28.5	9858	80	12.4	4.7
12	N114544	18.5	27	39.6	0.6	27.5	9073	81	5.9	19.7
13	N105997	10.9	23	38	1		6097	122	1.6	25.1
14	N114544	11.6	19	151.8	2.3	26	9953	169	10.3	11.9
15	M711767	7.7	24	124.2	2.3	26.5	7729	156	4.8	24.2
16	N136142	5.6	29.5	53.6	0.8	24	9150	50	33.1	13.9
17	N140139	6	37	145	2.5	53.5	9500	91	7.6	2.8
18	N113689	30.4	256.5	1101	12.7	156	11861	175	3.7	0.5
19	N140116	18.2	159	552.7	10		8100	159	5.8	1.3
20	N049871	37.2	371	1814.4	28.8	144	9801	221	8	2.2
21	N167349	11.9	51.5	112.2	2.2	38	8571	80	4.8	2
22	M791130	14.1	123.5	719.8	11.8	77	10033	183	4.9	3.9
23	N176296	2	58.5	319.6	4.7	83	9592	81	24.4	18.7
24	N106564	40.7	456.5	2567	32.5	236.5	11156	201	4.7	3.1
25	N182038	26.1	330	940.5	20.9	240.5	7052	80	3.3	4.4
26	N143357	7.1	32.8	38.2	0.8	23.7	7042	80	19	5.2
27	N032451	8.8	40.5	224	2.8	29.5	11015	172	1.9	7.1
28	N143357	6.7	9.5	19.12	0.4	15	6483	80	7.6	16
29	N168579	6.5	33.7	145.8	2.7	23.6	9588	235	13.4	22.2
30	N168579	15.6	28	156.8	2.9	34	7254	162	3	15.8
31	N184220	5.8	32	224	3.2	71.1	8991	161	11.7	6.6
32	N214557	11.5	75	432.22	9	75	8632	182	1	1.6
33	N173722	12.8	114	650.72	8.3	71.5	10010	177	16	26.4
34	N211185	31.3	194	856.37	14.2	97.1	10001	190	2.8	6.2
35	N214081	19.8	29.3	129.04	2.9	13.5	6507	159	3.5	1.4
36	N172910	11.6	29.8	140.3	2.6	18.2	10114	195	12.4	0.5
37	N218398	9.5	59	403.1	6.2	65.4	9908	182	1.7	5.5
38	M001071	10.1	28	161.4	2.3	23.2	10233	133	2	2.9
39	N083268	42.1	94	397	8.1	50.3	7303	159	4.4	29.2
40	N211483	8.3	132	1184	11.7	98.4	12918	215	8.1	3.4
41	M955006	14.7	73.4	286	7.5	48.3	7001	155	2.1	0.8
42	M837505	24.6	20.7	86.9	1.4	11.4	8512	168	9.2	7.8
43	M924781	5.1	42.1	32.8	0.5	64.9	11157	102	6.5	16.4
44	N246502	15.4	17.2	98.6	1.9	9.1	10847	204	7.5	15.2
45	M237724	3.1	65.5	568.4	9.8	81	10003	191	14.8	6.7
46	N248310	10.3	49	452	6.2	40.4	8701	157	8.2	2.3
47	N239635	35.3	16.9	88.3	1.2	6	8337	163	9.4	7.1
48	M727727	17.7	32	123.1	2.8	19.5	7502	161	3.7	1.3

No	Patient's ID	WBC count	Pre-apheresis Peripheral Blood Day 2	Total CD34+ Cells Day 2 (x106)	Stem Cell Yield Day 2 (x106/kg Body Weight)	Post-apheresis Peripheral Blood Day 2	Total Blood Volume Process	Volume (ml)	Lymph %	Mono %
1	N007209	52.6	54.10	130.5	2.9		7411	80	1.7	4.5
2	N063986	20.1	51.20	114.4	2.2		7352	100	2.6	29.3
3	N098229	28.2	15.50	62	1		9938	90	3.2	0.7
4	N102705									
5	N102713	54.2	10.50	22	0.5			144	3.2	20.2
6	N098229	24.7	10.90	24.8	0.4		9995	61	2.7	4.3
7	N107407	51.2	22.80	97.2	1.52		9263	156	1.9	2.5
8	N116876	31	22.00	62.7	0.88		9133	154	4	7.2
9	N088027	15.6	25.00	107.5	2.24	21.5	7032	72	9	12.3
10	N104139	24.5	76.50	150.8	2.6	49	7313	100	5.4	0.3
11	N128071	42	26.50	12.8	0.2	25	9158	70	2.3	2.1
12	N114544	23.7	11.60	13.2	0.2	5.4	9705	111	11.1	4.1
13	N105997	21.2	12.00	30.4	0.8	11.7	6000	120	1.5	8.9
14	N114544	31	29.00	126	2	13	7024	125	6	1
15	M711767	21.6	55.50	180.9	3.35	47	7206	163	2.2	2.3
16	N136142	12.6	40.00	167.5	2.5	29	10020	170	14.2	10.2
17	N140139	17.3	89.00	362.5	6.25	77	9518	150	3.1	1.9
18	N113689									
19	N140116									
20	N049871									
21	N167349	24.5	64.50	173.4	3.4	33.5	8553	102	2	31.8
22	M791130									
23	N176296									
24	N106564									
25	N182038									
26	N143357	10.7	46.00	38.2	0.8	14	6363	80	11.2	7.4
27	N032451	10	41.50	256	3.2	40	11052	100	2	2.5
28	N143357	19.2	17.00	76.48	1.6	20.5	6308	164	3.1	10.1
29	N168579	16.5	64.50	156.8	2.9	29.4	7243	167	5.1	7.6
30	N168579	15	48.00	221.4	4.1	37	7092	179	2.4	17.8
31	N184220	16.5	95.80	347.2	4.9	70	10898	168	3.9	11.6
32	N214557									
33	N173722									
34	N211185									
35	N214081	20.3	25.5	28.42	0.5	20.2	5736	62	4.5	7.4
36	N172910	12.9	28.50	162.9	3	11.6	9374	174	10.8	26.1
37	N218398									
38	M001071	19.7	51.00	179.4	2.6	31.2	10234	146	3	5.2
39	N083268	33	41.00	162	3.3	17.4	7309	151	2.2	25.4
40	N211483									
41	M955006	38.1	81.00	239	6.3	64.5	4583	104	1.9	0.9
42	M837505	26.4	21.30	37.8	0.65	15.3	9320	206	8.4	18.6
43	M924781	11.2	100.00	551	7.6	67.8	12015	186	2.2	5.4
44	N246502	25.7	14.00	43.2	0.8	3.58	11031	202	4.2	25.6
45	M237724	14.8	156.90	661	11.4	113.3	6982	136	3.2	1.7
46	N248310	19.4	74.00	379	5.2	54.4	11003	196	4.5	1.7
47	N239635	28.9	16.00	75.9	1	8.11	8581	166	9	5.3
48	M727727	21.5	34.00	194	4.4	24.9	7357	187	2.4	2

No	Patient's ID	WBC count	Pre-apheresis Peripheral Blood Day 3	Total CD34+ Cells Day 3 (x106)	Stem Cell Yield Day 3 (x106/kg Body Weight)	Post-apheresis Peripheral Blood Day 3	Total Blood Volume Process	Volume (ml)	Lymph %	Mono %
1	N007209									
2	N063986									
3	N098229									
4	N102705									
5	N102713									
6	N098229									
7	N107407	105.4	10.7	19.2	0.3	9.2	6359	70	1.8	2.2
8	N116876	41.2	20	51.1	0.7		7729	140	2.5	3.1
9	N088027	8.5	33.5	124.8	2.6	27.5	7015	90	18.7	20.3
10	N104139									
11	N128071	53.7	27	12.8	0.2	14.5	9158	61	2.9	1.9
12	N114544									
13	N105997									
14	N114544									
15	M711767									
16	N136142	27.9	34.5	120.6	1.8	27	10045	101	5.6	11.8
17	N140139									
18	N113689									
19	N140116									
20	N049871									
21	N167349									
22	M791130									
23	N176296									
24	N106564									
25	N182038									
26	N143357									
27	N032451									
28	N143357	24.9	29	112.8	2.4	26	5947	138	2.4	10.9
29	N168579	25.8	82.8	248.4	4.6	38.5	7244	165	16	26.4
30	N168579	19.9	91	297	5.5	41.5	7314	91	1	12.1
31	N184220									
32	N214557									
33	N173722									
34	N211185									
35	N214081	28.1	32	91.84	2	23.2	6903	157	2.5	0.3
36	N172910									
37	N218398									
38	M001071									
39	N083268									
40	N211483									
41	M955006									
42	M837505	29.6	15.4	37.8	0.7	13.5	8804	145	4.5	9.1
43	M924781									
44	N246502									
45	M237724									
46	N248310									
47	N239635	31.6	12.3	88.36	1.21	10.72	9308	183	5.4	1.6
48	M727727									

APPENDIX IV

Table 7 : COLLECTION EFFICIENCY

No	Patient's ID	Collection Efficiency Day 1	Collection Efficiency Day 2	Collection Efficiency Day 3
1	N007209			
2	N063986			
3	N098229			
4	N102705			
5	N102713			
6	N098229			
7	N107407			30.35%
8	N116876	50.0%		
9	N088027	70.8%	112.2%	58.33%
10	N104139	59.1%	52.1%	
11	N128071	34.1%	10.5%	6.74%
12	N114544	16.0%	23.1%	
13	N105997		79.2%	
14	N114544	67.8%	115.7%	
15	M711767	63.6%	85.3%	
16	N136142	21.9%	78.7%	39.04%
17	N140139	33.7%	80.0%	
18	N113689	45.0%		
19	N140116			
20	N049871	71.9%		
21	N167349	29.3%	59.7%	
22	M791130	71.6%		
23	N176296	47.1%		
24	N106564	66.4%		
25	N182038	46.8%		
26	N143357	19.2%	25.7%	
27	N032451	58.1%	103.6%	
28	N143357	24.1%	130.4%	68.97%
29	N168579	53.1%	64.2%	56.54%
30	N168579	69.7%	119.8%	61.29%
31	N184220	48.3%	63.3%	
32	N214557	66.8%		
33	N173722	70.1%		
34	N211185	58.8%		
35	N214081	92.7%	38.1%	48.20%
36	N172910	57.8%	110.3%	
37	N218398	65.4%		
38	M001071	61.6%	65.4%	
39	N083268	75.3%	100.1%	
40	N211483	79.6%		
41	M955006	67.1%	119.5%	
42	M837505	63.6%	37.0%	29.71%
43	M924781	5.5%	85.2%	
44	N246502	69.1%	52.9%	
45	M237724	77.6%	112.5%	
46	N248310	116.2%	87.0%	
47	N239635	92.5%	104.1%	82.48%
48	M727727	63.7%	137.3%	

APPENDIX V

CORRELATION RESULT

1) Pre-apheresis Peripheral Blood CD34$^+$ Cells and Product Yield

a) Overall pre-apheresis peripheral blood CD34$^+$ cells and stem cell yield for day 1, 2 and 3 PBSC harvesting

Table 8 (a): Correlation Result of Overall pre-apheresis peripheral blood CD34$^+$ cells and stem cell yield for day 1, 2 and 3 PBSC harvesting

		PRE_CD34_COUNT	POST_CD34_YIELD
PRE_CD34_COUNT	Pearson Correlation	1	.963(**)
	Sig. (2-tailed)		.000
	N	94	94
POST_CD34_YIELD	Pearson Correlation	.963(**)	1
	Sig. (2-tailed)	.000	
	N	94	94

** Correlation is significant at the 0.01 level (2-tailed).

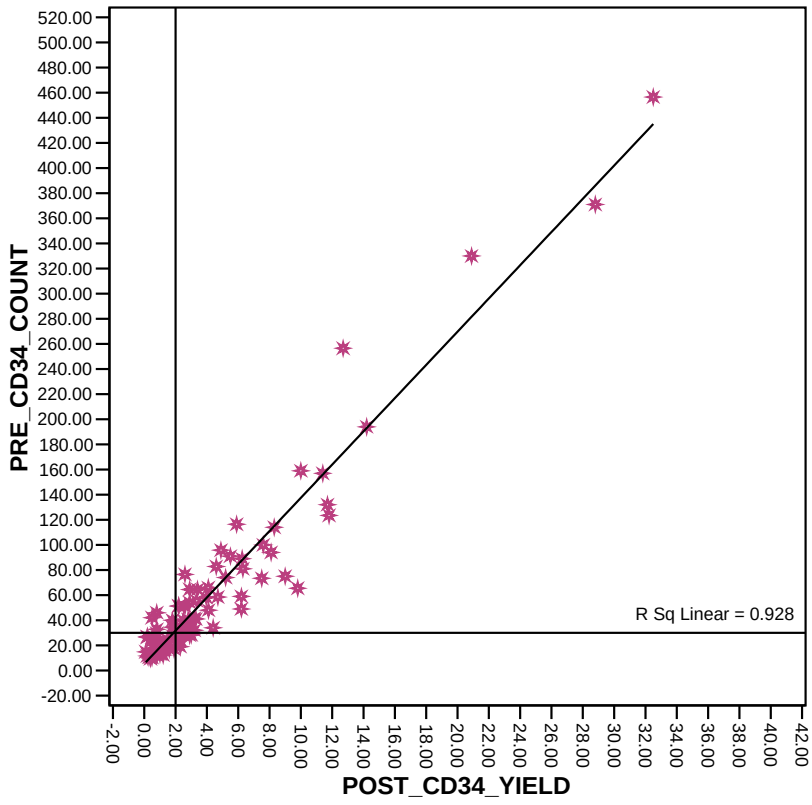


Figure 19(a): Correlation Analysis of Overall pre-apheresis peripheral blood CD34$^+$ cells and stem cell yield for day 1, 2 and 3 PBSC harvesting

b) Pre-apheresis peripheral blood $CD34^+$ cells and stem cell yield for day 1 PBSC harvesting

Table 8 (b): Correlation Result of Pre-apheresis peripheral blood $CD34^+$ cells and stem cell yield for day 1 PBSC harvesting

		PRECD34DAY1	POSTCD34YIELDDAY1
PRECD34DAY1	Pearson Correlation	1	.967(**)
	Sig. (2-tailed)		.000
	N	48	48
POSTCD34YIELDDAY1	Pearson Correlation	.967(**)	1
	Sig. (2-tailed)	.000	
	N	48	48

** Correlation is significant at the 0.01 level (2-tailed).

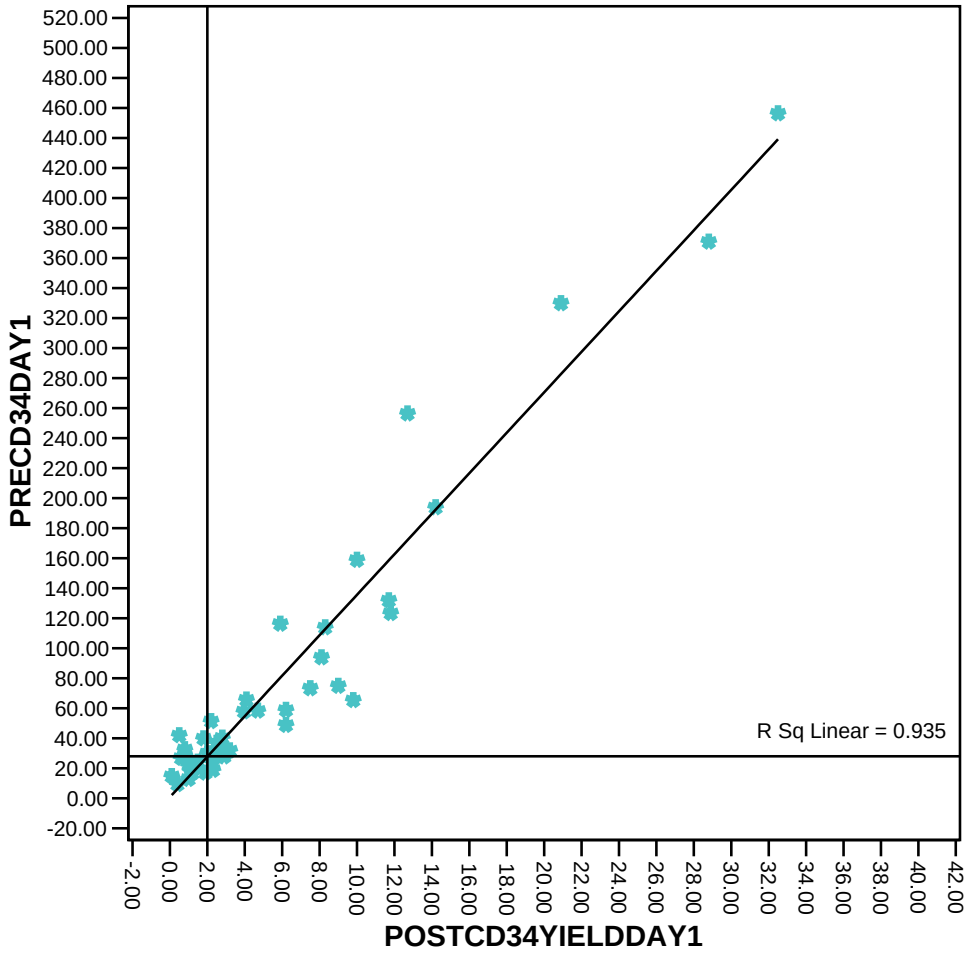


Figure 19(b): Correlation Analysis of Pre-apheresis peripheral blood $CD34^+$ cells and stem cell yield for day 1 PBSC harvesting

c) Pre-apheresis peripheral blood $CD34^+$ cells and stem cell yield for day 2 harvesting

Table 8 (c): Correlation Result Pre-apheresis peripheral blood $CD34^+$ cells and stem cell yield for day 2 harvesting

		PRECD34DAY2	POSTCD34YIELDDAY2
PRECD34DAY2	Pearson Correlation	1	.913(**)
	Sig. (2-tailed)		.000
	N	35	35
POSTCD34YIELDDAY2	Pearson Correlation	.913(**)	1
	Sig. (2-tailed)	.000	
	N	35	35

** Correlation is significant at the 0.01 level (2-tailed).

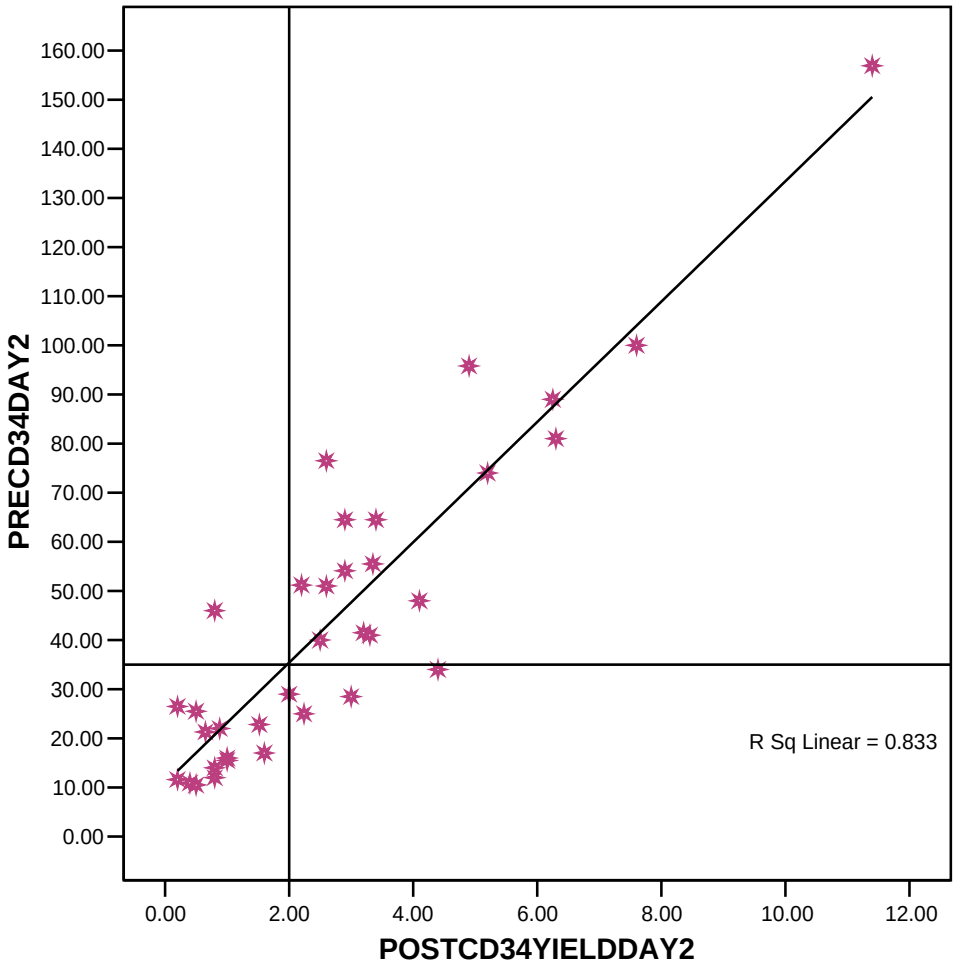


Figure 19(c): Correlation Analysis of Pre-apheresis peripheral blood $CD34^+$ cells and stem cell yield for day 2 harvesting

d) Pre-apheresis peripheral blood $CD34^+$ cells and stem cell yield for day 3 harvesting

Table 8 (d): Correlation Result of Pre-apheresis peripheral blood $CD34^+$ cells and stem cell yield for day 3 harvesting

		PRECD34DAY3	POSTCD34YIELDDAY3
PRECD34DAY3	Pearson Correlation	1	.939(**)
	Sig. (2-tailed)		.000
	N	11	11
POSTCD34YIELDDAY3	Pearson Correlation	.939(**)	1
	Sig. (2-tailed)	.000	
	N	11	11

** Correlation is significant at the 0.01 level (2-tailed).

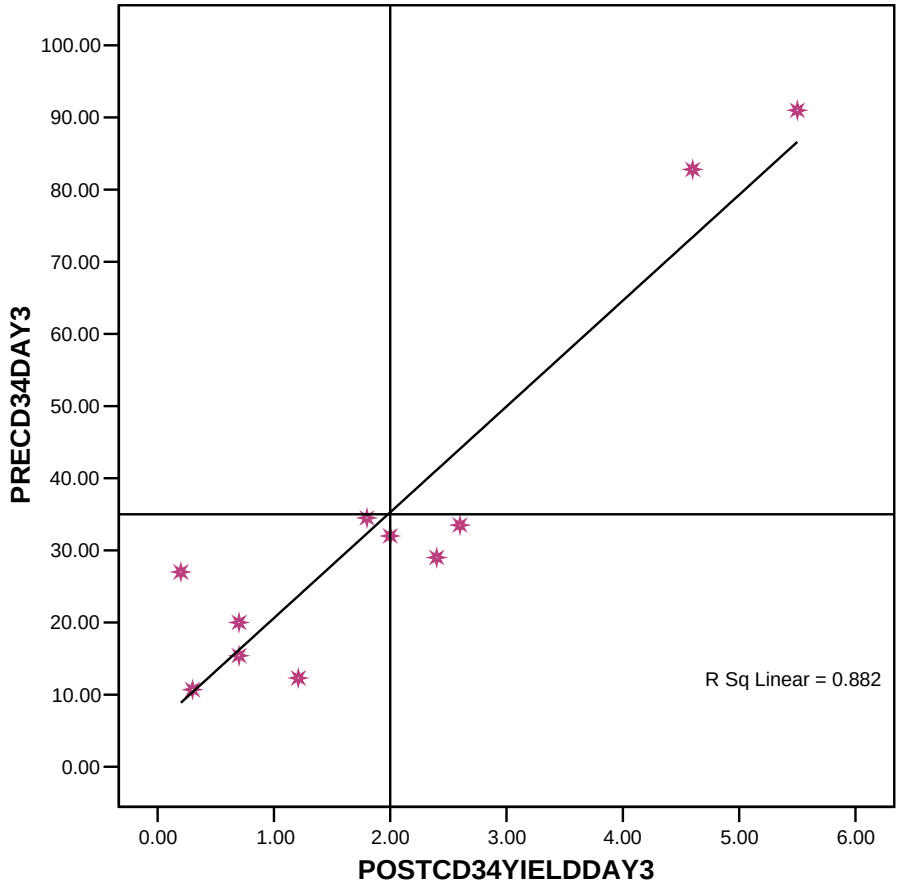


Figure 19(d): Correlation Analysis of Pre-apheresis peripheral blood $CD34^+$ cells and stem cell yield for day 3 harvesting

2) Post-apheresis Peripheral Blood CD34^{+} Cells Day 1 and Day 2 Pre-apheresis Peripheral Blood CD34^{+} Cells

Table 9: Correlation Result of Post-apheresis Peripheral Blood CD34^{+} Cells Day 1 and Day 2 Pre-apheresis Peripheral Blood CD34^{+} Cells

		POST_PB_CD34COUNT	PRE_CD34COUNT
POST_PB_CD34COUNT	Pearson Correlation	1	.895(**)
	Sig. (2-tailed)		.000
	N	28	28
PRE_CD34COUNT	Pearson Correlation	.895(**)	1
	Sig. (2-tailed)	.000	
	N	28	28

** Correlation is significant at the 0.01 level (2-tailed).

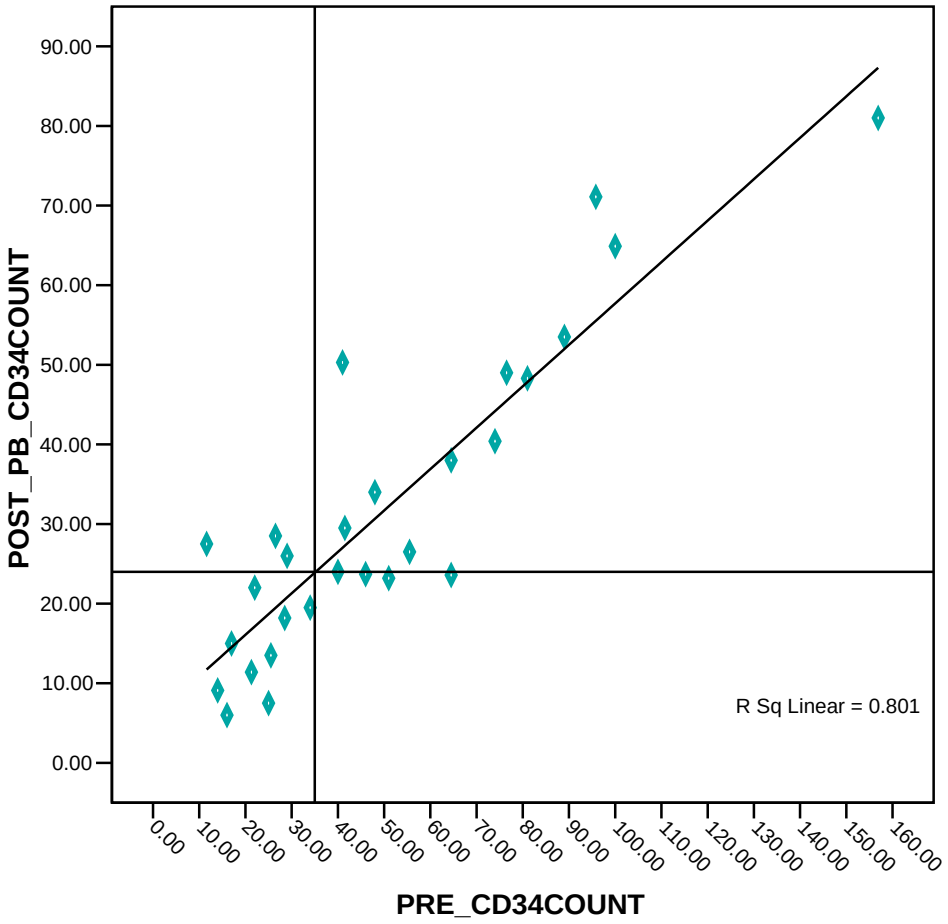


Figure 20: Correlation Analysis of Post-apheresis Peripheral Blood CD34^{+} Cells Day 1 and Day 2 Pre-apheresis Peripheral Blood CD34^{+} Cells

3) Post-apheresis Peripheral Blood CD34^{+} Cells Day 1 and Day 2 Stem Cell Yield

Table 10: Correlation Result Post-apheresis Peripheral Blood CD34^{+} Cells Day 1 and Day 2 Stem Cell Yield

		POST_PBDAY1	SC_YIELD_DAY2
POST_PBDAY1	Pearson Correlation	1	.811(**)
	Sig. (2-tailed)		.000
	N	28	28
SC_YIELD_DAY2	Pearson Correlation	.811(**)	1
	Sig. (2-tailed)	.000	
	N	28	28

** Correlation is significant at the 0.01 level (2-tailed).

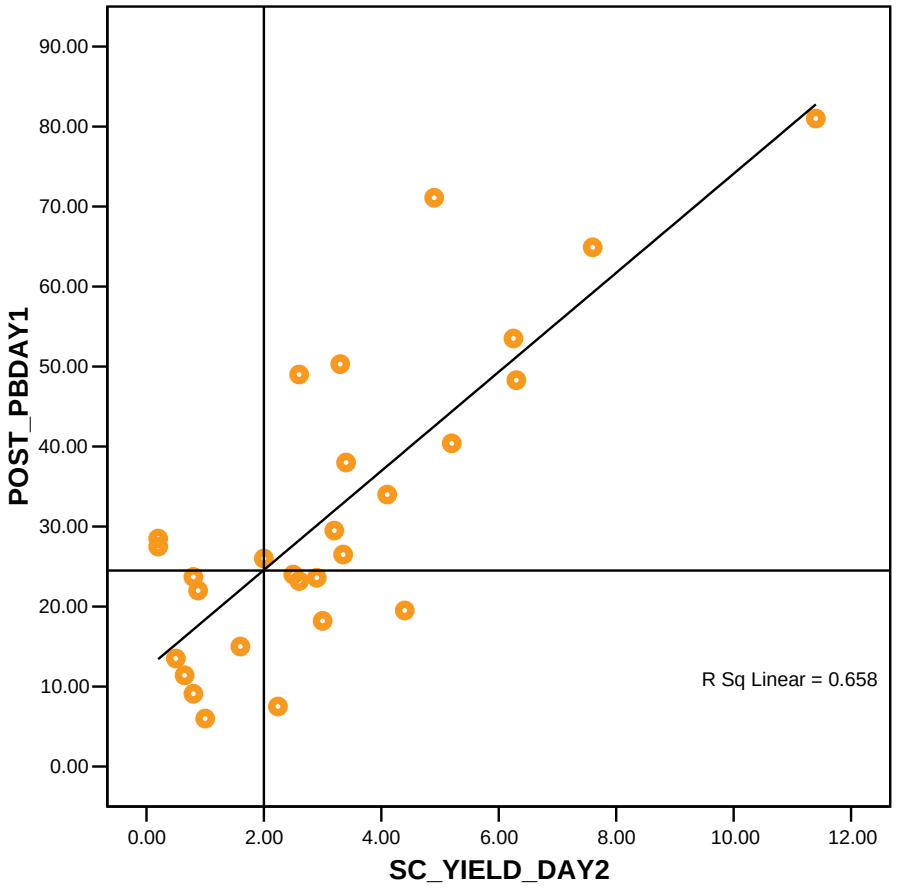


Figure 21: Correlation Result Post-apheresis Peripheral Blood CD34^{+} Cells Day 1 and Day 2 Stem Cell Yield

APPENDIX VI

RECEIVER OPERATING CHARACTERISTIC CURVE

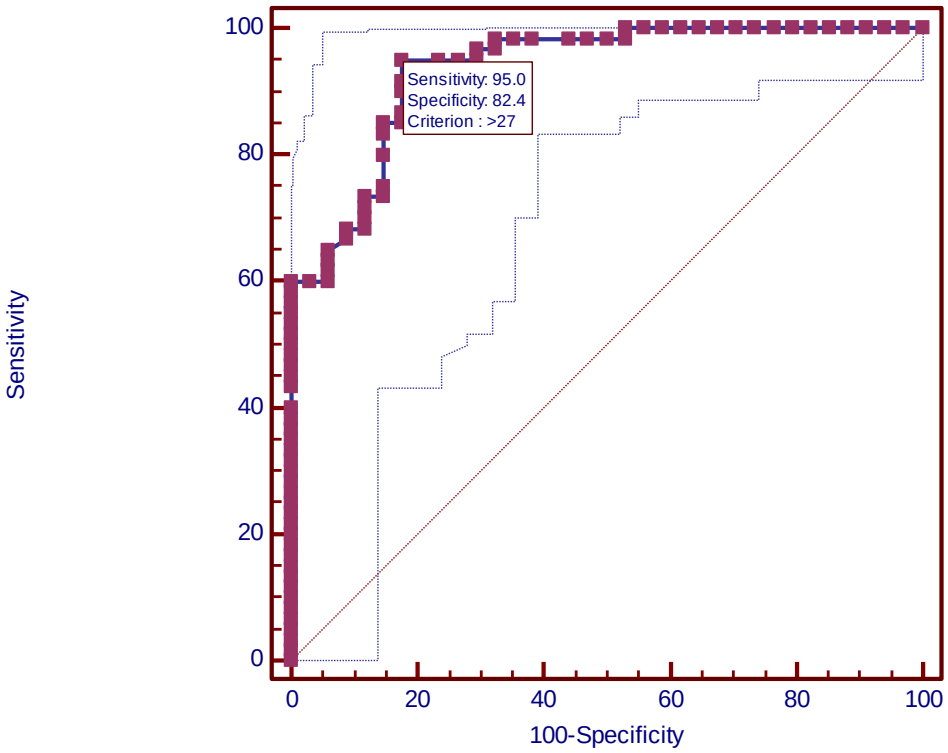


Figure 22(a): ROC Curve of Overall Pre-Apheresis Peripheral Blood $CD34^+$ Cells and Stem Cell Yield for Day 1, 2 and 3 PBSC Harvesting

Area under the ROC curve (AUC)	0.935
Standard Error [a]	0.0248
95% Confidence Interval [b]	0.864 to 0.975
z statistic	17.501
Significance level P (Area=0.5)	<0.0001

Table 11(a): Criterion Values and Coordinates of the ROC Curve for Day 1, 2 and 3 PBSC Harvesting

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>=9.5	100	94.0 - 100.0	0	0.0 - 10.3	1		63.8	
>9.5	100	94.0 - 100.0	2.94	0.07 - 15.3	1.03	0	64.5	100
>10.5	100	94.0 - 100.0	5.88	0.7 - 19.7	1.06	0	65.2	100
>10.7	100	94.0 - 100.0	8.82	1.9 - 23.7	1.1	0	65.9	100
>10.9	100	94.0 - 100.0	11.76	3.3 - 27.5	1.13	0	66.7	100
>11.6	100	94.0 - 100.0	14.71	5.0 - 31.1	1.17	0	67.4	100
>12	100	94.0 - 100.0	17.65	6.8 - 34.5	1.21	0	68.2	100
>12.3	100	94.0 - 100.0	20.59	8.7 - 37.9	1.26	0	69	100
>13	100	94.0 - 100.0	23.53	10.7 - 41.2	1.31	0	69.8	100
>14	100	94.0 - 100.0	26.47	12.9 - 44.4	1.36	0	70.6	100
>14.9	100	94.0 - 100.0	29.41	15.1 - 47.5	1.42	0	71.4	100
>15.4	100	94.0 - 100.0	32.35	17.4 - 50.5	1.48	0	72.3	100
>15.5	100	94.0 - 100.0	35.29	19.7 - 53.5	1.55	0	73.2	100
>16	100	94.0 - 100.0	38.24	22.2 - 56.4	1.62	0	74.1	100
>16.9	100	94.0 - 100.0	41.18	24.6 - 59.3	1.7	0	75	100
>17	100	94.0 - 100.0	44.12	27.2 - 62.1	1.79	0	75.9	100
>17.2	100	94.0 - 100.0	47.06	29.8 - 64.9	1.89	0	76.9	100
>19	98.33	91.1 - 100.0	47.06	29.8 - 64.9	1.86	0.035	76.6	94.1
>20	98.33	91.1 - 100.0	50	32.4 - 67.6	1.97	0.033	77.6	94.4
>20.7	98.33	91.1 - 100.0	52.94	35.1 - 70.2	2.09	0.031	78.7	94.7
>21.3	98.33	91.1 - 100.0	55.88	37.9 - 72.8	2.23	0.03	79.7	95
>22	98.33	91.1 - 100.0	61.76	43.6 - 77.8	2.57	0.027	81.9	95.5
>22.8	98.33	91.1 - 100.0	64.71	46.5 - 80.3	2.79	0.026	83.1	95.7
>23	98.33	91.1 - 100.0	67.65	49.5 - 82.6	3.04	0.025	84.3	95.8
>24	96.67	88.5 - 99.6	67.65	49.5 - 82.6	2.99	0.049	84.1	92

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>24.5	96.67	88.5 - 99.6	70.59	52.5 - 84.9	3.29	0.047	85.3	92.3
>25	95	86.1 - 99.0	70.59	52.5 - 84.9	3.23	0.071	85.1	88.9
>25.5	95	86.1 - 99.0	73.53	55.6 - 87.1	3.59	0.068	86.4	89.3
>26.5	95	86.1 - 99.0	76.47	58.8 - 89.3	4.04	0.065	87.7	89.7
>27 *	95	86.1 - 99.0	82.35	65.5 - 93.2	5.38	0.061	90.5	90.3
>28	91.67	81.6 - 97.2	82.35	65.5 - 93.2	5.19	0.1	90.2	84.8
>28.5	90	79.5 - 96.2	82.35	65.5 - 93.2	5.1	0.12	90	82.4
>29	86.67	75.4 - 94.1	82.35	65.5 - 93.2	4.91	0.16	89.7	77.8
>29.3	85	73.4 - 92.9	82.35	65.5 - 93.2	4.82	0.18	89.5	75.7
>29.5	85	73.4 - 92.9	85.29	68.9 - 95.0	5.78	0.18	91.1	76.3
>29.8	83.33	71.5 - 91.7	85.29	68.9 - 95.0	5.67	0.2	90.9	74.4
>30	80	67.7 - 89.2	85.29	68.9 - 95.0	5.44	0.23	90.6	70.7
>32	75	62.1 - 85.3	85.29	68.9 - 95.0	5.1	0.29	90	65.9
>32.7	73.33	60.3 - 83.9	85.29	68.9 - 95.0	4.99	0.31	89.8	64.4
>32.8	73.33	60.3 - 83.9	88.24	72.5 - 96.7	6.23	0.3	91.7	65.2
>33.5	71.67	58.6 - 82.5	88.24	72.5 - 96.7	6.09	0.32	91.5	63.8
>33.7	70	56.8 - 81.2	88.24	72.5 - 96.7	5.95	0.34	91.3	62.5
>34	68.33	55.0 - 79.7	88.24	72.5 - 96.7	5.81	0.36	91.1	61.2
>34.5	68.33	55.0 - 79.7	91.18	76.3 - 98.1	7.74	0.35	93.2	62
>37	66.67	53.3 - 78.3	91.18	76.3 - 98.1	7.56	0.37	93	60.8
>40	65	51.6 - 76.9	94.12	80.3 - 99.3	11.05	0.37	95.1	60.4
>40.5	63.33	49.9 - 75.4	94.12	80.3 - 99.3	10.77	0.39	95	59.3
>41	61.67	48.2 - 73.9	94.12	80.3 - 99.3	10.48	0.41	94.9	58.2
>41.5	60	46.5 - 72.4	94.12	80.3 - 99.3	10.2	0.43	94.7	57.1
>42.1	60	46.5 - 72.4	97.06	84.7 - 99.9	20.4	0.41	97.3	57.9
>46	60	46.5 - 72.4	100	89.7 - 100.0		0.4	100	58.6
>48	58.33	44.9 - 70.9	100	89.7 - 100.0		0.42	100	57.6

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>49	56.67	43.2 - 69.4	100	89.7 - 100.0		0.43	100	56.7
>51	55	41.6 - 67.9	100	89.7 - 100.0		0.45	100	55.7
>51.2	53.33	40.0 - 66.3	100	89.7 - 100.0		0.47	100	54.8
>51.5	51.67	38.4 - 64.8	100	89.7 - 100.0		0.48	100	54
>54.1	50	36.8 - 63.2	100	89.7 - 100.0		0.5	100	53.1
>55.5	48.33	35.2 - 61.6	100	89.7 - 100.0		0.52	100	52.3
>58	46.67	33.7 - 60.0	100	89.7 - 100.0		0.53	100	51.5
>58.5	45	32.1 - 58.4	100	89.7 - 100.0		0.55	100	50.7
>59	43.33	30.6 - 56.8	100	89.7 - 100.0		0.57	100	50
>64.5	40	27.6 - 53.5	100	89.7 - 100.0		0.6	100	48.6
>65.5	38.33	26.1 - 51.8	100	89.7 - 100.0		0.62	100	47.9
>65.9	36.67	24.6 - 50.1	100	89.7 - 100.0		0.63	100	47.2
>73.4	35	23.1 - 48.4	100	89.7 - 100.0		0.65	100	46.6
>74	33.33	21.7 - 46.7	100	89.7 - 100.0		0.67	100	45.9
>75	31.67	20.3 - 45.0	100	89.7 - 100.0		0.68	100	45.3
>76.5	30	18.8 - 43.2	100	89.7 - 100.0		0.7	100	44.7
>81	28.33	17.5 - 41.4	100	89.7 - 100.0		0.72	100	44.2
>82.8	26.67	16.1 - 39.7	100	89.7 - 100.0		0.73	100	43.6
>89	25	14.7 - 37.9	100	89.7 - 100.0		0.75	100	43
>91	23.33	13.4 - 36.0	100	89.7 - 100.0		0.77	100	42.5
>94	21.67	12.1 - 34.2	100	89.7 - 100.0		0.78	100	42
>95.8	20	10.8 - 32.3	100	89.7 - 100.0		0.8	100	41.5
>100	18.33	9.5 - 30.4	100	89.7 - 100.0		0.82	100	41
>114	16.67	8.3 - 28.5	100	89.7 - 100.0		0.83	100	40.5
>116.4	15	7.1 - 26.6	100	89.7 - 100.0		0.85	100	40
>123.5	13.33	5.9 - 24.6	100	89.7 - 100.0		0.87	100	39.5
>132	11.67	4.8 - 22.6	100	89.7 - 100.0		0.88	100	39.1

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>156.9	10	3.8 - 20.5	100	89.7 - 100.0		0.9	100	38.6
>159	8.33	2.8 - 18.4	100	89.7 - 100.0		0.92	100	38.2
>194	6.67	1.8 - 16.2	100	89.7 - 100.0		0.93	100	37.8
>256.5	5	1.0 - 13.9	100	89.7 - 100.0		0.95	100	37.4
>330	3.33	0.4 - 11.5	100	89.7 - 100.0		0.97	100	37
>371	1.67	0.04 - 8.9	100	89.7 - 100.0		0.98	100	36.6
>456.5	0	0.0 - 6.0	100	89.7 - 100.0		1		36.2

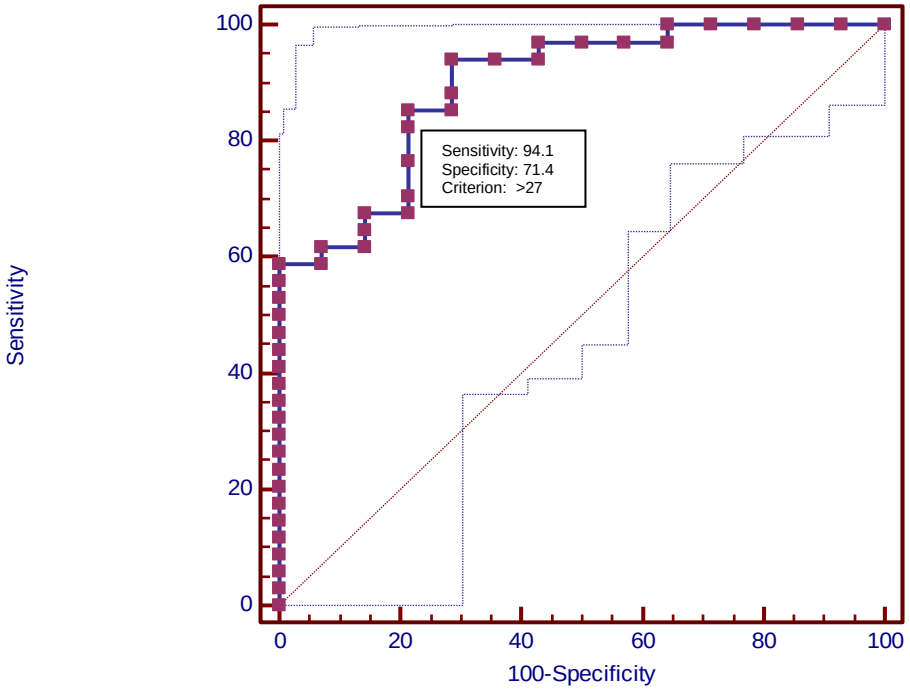


Figure 22(b): ROC Curve of Pre-apheresis Peripheral Blood $CD34^{+}$ Cells and Stem Cell Yield for Day 1 Harvesting

Area under the ROC curve (AUC)	0.895
Standard Error [a]	0.0451
95% Confidence Interval [b]	0.772 to 0.965
z statistic	8.767
Significance level P (Area=0.5)	<0.0001

[a] Hanley & McNeil, 1982
[b] Binomial exact

Table 11(b): Criterion Values and Coordinates of the ROC Curve for Day 1 PBSC Harvesting

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>=9.5	100	89.7 - 100.0	0	0.0 - 23.2	1		70.8	
>9.5	100	89.7 - 100.0	7.14	0.2 - 33.9	1.08	0	72.3	100
>13	100	89.7 - 100.0	14.29	1.8 - 42.8	1.17	0	73.9	100
>14.9	100	89.7 - 100.0	21.43	4.7 - 50.8	1.27	0	75.6	100
>16.9	100	89.7 - 100.0	28.57	8.4 - 58.1	1.4	0	77.3	100
>17.2	100	89.7 - 100.0	35.71	12.8 - 64.9	1.56	0	79.1	100
>19	97.06	84.7 - 99.9	35.71	12.8 - 64.9	1.51	0.082	78.6	83.3
>20.7	97.06	84.7 - 99.9	42.86	17.7 - 71.1	1.7	0.069	80.5	85.7
>22	97.06	84.7 - 99.9	50	23.0 - 77.0	1.94	0.059	82.5	87.5
>23	97.06	84.7 - 99.9	57.14	28.9 - 82.3	2.26	0.051	84.6	88.9
>24	94.12	80.3 - 99.3	57.14	28.9 - 82.3	2.2	0.1	84.2	80
>24.5	94.12	80.3 - 99.3	64.29	35.1 - 87.2	2.64	0.092	86.5	81.8
>27 *	94.12	80.3 - 99.3	71.43	41.9 - 91.6	3.29	0.082	88.9	83.3
>28	88.24	72.5 - 96.7	71.43	41.9 - 91.6	3.09	0.16	88.2	71.4
>29.3	85.29	68.9 - 95.0	71.43	41.9 - 91.6	2.99	0.21	87.9	66.7
>29.5	85.29	68.9 - 95.0	78.57	49.2 - 95.3	3.98	0.19	90.6	68.7
>29.8	82.35	65.5 - 93.2	78.57	49.2 - 95.3	3.84	0.22	90.3	64.7
>30	76.47	58.8 - 89.3	78.57	49.2 - 95.3	3.57	0.3	89.7	57.9
>32	70.59	52.5 - 84.9	78.57	49.2 - 95.3	3.29	0.37	88.9	52.4
>32.7	67.65	49.5 - 82.6	78.57	49.2 - 95.3	3.16	0.41	88.5	50
>32.8	67.65	49.5 - 82.6	85.71	57.2 - 98.2	4.74	0.38	92	52.2
>33.7	64.71	46.5 - 80.3	85.71	57.2 - 98.2	4.53	0.41	91.7	50
>37	61.76	43.6 - 77.8	85.71	57.2 - 98.2	4.32	0.45	91.3	48

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>40	61.76	43.6 - 77.8	92.86	66.1 - 99.8	8.65	0.41	95.5	50
>40.5	58.82	40.7 - 75.4	92.86	66.1 - 99.8	8.24	0.44	95.2	48.1
>42.1	58.82	40.7 - 75.4	100	76.8 - 100.0		0.41	100	50
>49	55.88	37.9 - 72.8	100	76.8 - 100.0		0.44	100	48.3
>51.5	52.94	35.1 - 70.2	100	76.8 - 100.0		0.47	100	46.7
>58	50	32.4 - 67.6	100	76.8 - 100.0		0.5	100	45.2
>58.5	47.06	29.8 - 64.9	100	76.8 - 100.0		0.53	100	43.7
>59	44.12	27.2 - 62.1	100	76.8 - 100.0		0.56	100	42.4
>65.5	41.18	24.6 - 59.3	100	76.8 - 100.0		0.59	100	41.2
>65.9	38.24	22.2 - 56.4	100	76.8 - 100.0		0.62	100	40
>73.4	35.29	19.7 - 53.5	100	76.8 - 100.0		0.65	100	38.9
>75	32.35	17.4 - 50.5	100	76.8 - 100.0		0.68	100	37.8
>94	29.41	15.1 - 47.5	100	76.8 - 100.0		0.71	100	36.8
>114	26.47	12.9 - 44.4	100	76.8 - 100.0		0.74	100	35.9
>116.4	23.53	10.7 - 41.2	100	76.8 - 100.0		0.76	100	35
>123.5	20.59	8.7 - 37.9	100	76.8 - 100.0		0.79	100	34.1
>132	17.65	6.8 - 34.5	100	76.8 - 100.0		0.82	100	33.3
>159	14.71	5.0 - 31.1	100	76.8 - 100.0		0.85	100	32.6
>194	11.76	3.3 - 27.5	100	76.8 - 100.0		0.88	100	31.8
>256.5	8.82	1.9 - 23.7	100	76.8 - 100.0		0.91	100	31.1
>330	5.88	0.7 - 19.7	100	76.8 - 100.0		0.94	100	30.4
>371	2.94	0.07 - 15.3	100	76.8 - 100.0		0.97	100	29.8
>456.5	0	0.0 - 10.3	100	76.8 - 100.0		1		29.2

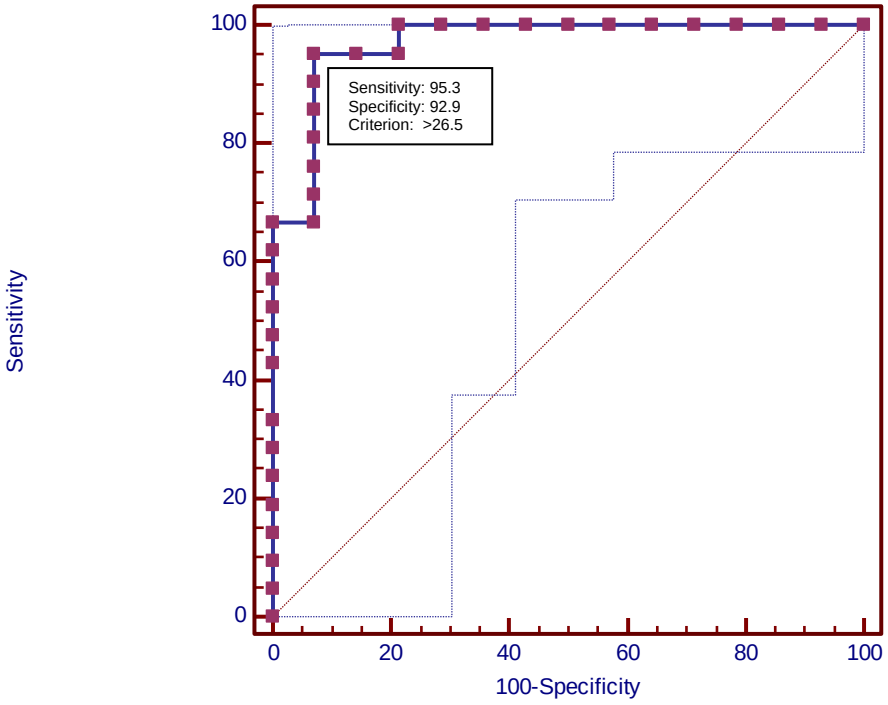


Figure 22(c): ROC Curve of Pre-apheresis Peripheral Blood $CD34^+$ Cells and Stem Cell Yield for Day 2 Harvesting

Area under the ROC curve (AUC)	0.969
Standard Error [a]	0.0284
95% Confidence Interval [b]	0.848 to 0.999
z statistic	16.525
Significance level P (Area=0.5)	<0.0001

[a] Hanley & McNeil, 1982

[b] Binomial exact

Table 11(c): Criterion Values and Coordinates of the ROC Curve for Day 2 PBSC Harvesting

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>=10.5	100	83.9 - 100.0	0	0.0 - 23.2	1		60	
>10.5	100	83.9 - 100.0	7.14	0.2 - 33.9	1.08	0	61.8	100
>10.9	100	83.9 - 100.0	14.29	1.8 - 42.8	1.17	0	63.6	100
>11.6	100	83.9 - 100.0	21.43	4.7 - 50.8	1.27	0	65.6	100
>12	100	83.9 - 100.0	28.57	8.4 - 58.1	1.4	0	67.7	100
>14	100	83.9 - 100.0	35.71	12.8 - 64.9	1.56	0	70	100
>15.5	100	83.9 - 100.0	42.86	17.7 - 71.1	1.75	0	72.4	100
>16	100	83.9 - 100.0	50	23.0 - 77.0	2	0	75	100
>17	100	83.9 - 100.0	57.14	28.9 - 82.3	2.33	0	77.8	100
>21.3	100	83.9 - 100.0	64.29	35.1 - 87.2	2.8	0	80.8	100
>22	100	83.9 - 100.0	71.43	41.9 - 91.6	3.5	0	84	100
>22.8	100	83.9 - 100.0	78.57	49.2 - 95.3	4.67	0	87.5	100
>25	95.24	76.2 - 99.9	78.57	49.2 - 95.3	4.44	0.061	87	91.7
>25.5	95.24	76.2 - 99.9	85.71	57.2 - 98.2	6.67	0.056	90.9	92.3
>26.5 *	95.24	76.2 - 99.9	92.86	66.1 - 99.8	13.33	0.051	95.2	92.9
>28.5	90.48	69.6 - 98.8	92.86	66.1 - 99.8	12.67	0.1	95	86.7
>29	85.71	63.7 - 97.0	92.86	66.1 - 99.8	12	0.15	94.7	81.2
>34	80.95	58.1 - 94.6	92.86	66.1 - 99.8	11.33	0.21	94.4	76.5
>40	76.19	52.8 - 91.8	92.86	66.1 - 99.8	10.67	0.26	94.1	72.2
>41	71.43	47.8 - 88.7	92.86	66.1 - 99.8	10	0.31	93.7	68.4
>41.5	66.67	43.0 - 85.4	92.86	66.1 - 99.8	9.33	0.36	93.3	65
>46	66.67	43.0 - 85.4	100	76.8 - 100.0		0.33	100	66.7
>48	61.9	38.4 - 81.9	100	76.8 - 100.0		0.38	100	63.6
>51	57.14	34.0 - 78.2	100	76.8 - 100.0		0.43	100	60.9

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>51.2	52.38	29.8 - 74.3	100	76.8 - 100.0		0.48	100	58.3
>54.1	47.62	25.7 - 70.2	100	76.8 - 100.0		0.52	100	56
>55.5	42.86	21.8 - 66.0	100	76.8 - 100.0		0.57	100	53.8
>64.5	33.33	14.6 - 57.0	100	76.8 - 100.0		0.67	100	50
>74	28.57	11.3 - 52.2	100	76.8 - 100.0		0.71	100	48.3
>76.5	23.81	8.2 - 47.2	100	76.8 - 100.0		0.76	100	46.7
>81	19.05	5.4 - 41.9	100	76.8 - 100.0		0.81	100	45.2
>89	14.29	3.0 - 36.3	100	76.8 - 100.0		0.86	100	43.7
>95.8	9.52	1.2 - 30.4	100	76.8 - 100.0		0.9	100	42.4
>100	4.76	0.1 - 23.8	100	76.8 - 100.0		0.95	100	41.2
>156.9	0	0.0 - 16.1	100	76.8 - 100.0		1		40

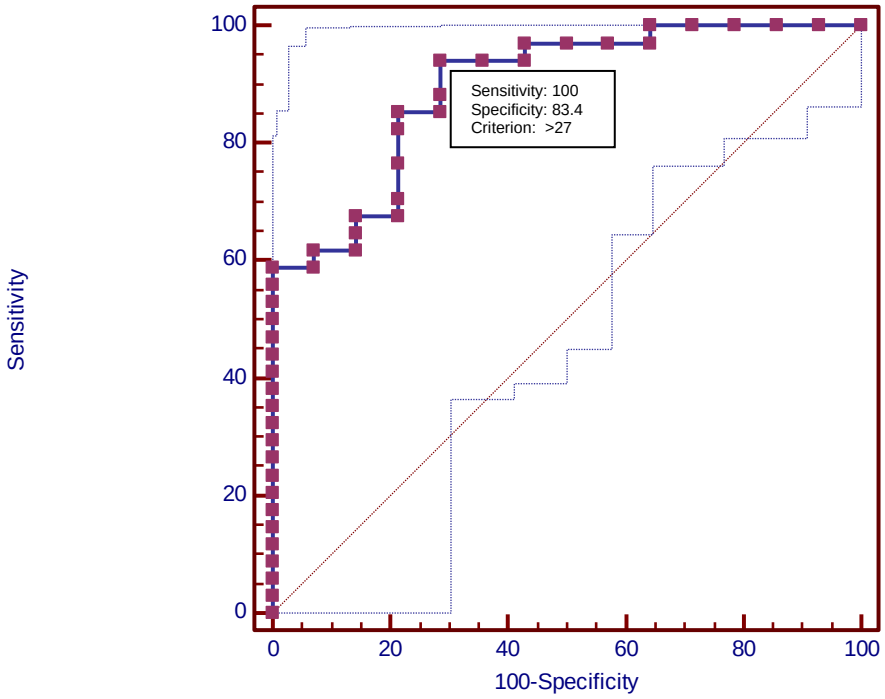


Figure 22(d): ROC Curve of Pre-apheresis Peripheral Blood $CD34^+$ Cells and Stem Cell Yield for Day 3 Harvesting

Area under the ROC curve (AUC)	0.9
Standard Error [a]	0.106
95% Confidence Interval [b]	0.576 to 0.997
z statistic	3.78
Significance level P (Area=0.5)	0.0002

[a] Hanley & McNeil, 1982

[b] Binomial exact

Table 11(d): Criterion Values and Coordinates of the ROC Curve for Day 3 PBSC Harvesting

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV
>=10.7	100	47.8 - 100.0	0	0.0 - 45.9	1		45.5	
>10.7	100	47.8 - 100.0	16.67	0.4 - 64.1	1.2	0	50	100
>12.3	100	47.8 - 100.0	33.33	4.3 - 77.7	1.5	0	55.6	100
>15.4	100	47.8 - 100.0	50	11.8 - 88.2	2	0	62.5	100
>20	100	47.8 - 100.0	66.67	22.3 - 95.7	3	0	71.4	100
>27 *	100	47.8 - 100.0	83.33	35.9 - 99.6	6	0	83.3	100
>29	80	28.4 - 99.5	83.33	35.9 - 99.6	4.8	0.24	80	83.3
>32	60	14.7 - 94.7	83.33	35.9 - 99.6	3.6	0.48	75	71.4
>33.5	40	5.3 - 85.3	83.33	35.9 - 99.6	2.4	0.72	66.7	62.5
>34.5	40	5.3 - 85.3	100	54.1 - 100.0		0.6	100	66.7
>82.8	20	0.5 - 71.6	100	54.1 - 100.0		0.8	100	60
>91	0	0.0 - 52.2	100	54.1 - 100.0		1		54.5