FACTORS INFLUENCING THE ENGRAFTMENT OF HAEMATOPOIETIC STEM CELL TRANSPLANTION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCY

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ABSTRACT

BACKGROUND

Haematopoietic stem cell transplantation remains the only curative option for many haematological malignancies. Currently, the majority of procedures for procurement of haematopoietic progenitor cells are performed by peripheral blood apheresis collection. The development of apheresis technology, the discovery of haematopoietic growth factors and small molecule CXCR4 antagonist for stem- cell mobilization and in vivo experimental transplantation studies that eventually led to clinical PBSCT.

The quality of PBSC graft be assessed by its speed of engraftment. The advantage of early engraftment of haematopoietic stem cell transplantation include, reduced incidence of post transplant neutropenia associated infections, mortality, morbidity and shorten the length of hospital stay that eventually reduce the overall cost.

Furthermore this valuable expensive procedure will be available for more number of patients who are waiting for their disease to be cured. This prospective observational study was undertaken to study (favorable and unfavorable) factors influencing the HSC engraftment.

AIM

To find out the factors that influence (favorable or unfavorable) engraftmentof haematopoietic stem cell transplantation.

METHODS

During the study period all patients who underwent HSCT procedure for hematological malignancies was included and the related factors were obtained. The relative speed of engraftment was analyzed depending on the median and range of values (neutrophil and platelet engraftment days) obtained under each individual factors.

RESULTS

In our study there was a definite correlation between CD34+ cell dose and speed of engraftment. CD 34 + cell dose of> 2.5×10^6 cells/kg, achieved faster neutrophil and platelet engraftment. CD 34+ cell dose of <$2.5x10^6$ cells/kg achieveddelayedneutrophil and platelet engraftment

Autologous PBSCT showed faster engraftment than allogenic PBSCT. The expected speed of engraftment could be achieved with higher dose of CD34+ cells even in patients with partial HLA match. Among various hematological malignancies multiple myeloma patients showed relatively rapid engraftment with autologous PBSC as a source. Total Body

irradiation and chemotherapeutic agent busalphan as a conditioning regimen showed relatively slower PBSCT engraftment.

CONCLUSION

Since early engraftment reduces length of hospital stay, morbidity, mortality and cost of this highly expensive treatment, it is imperative to utilize all available options to enhance the speed of engraftment. In a country like India where there are a few established haematopoietic stem cell transplantation centers available, there are many patients desperately waiting for their life to be saved by this specialized procedure. Hence, successful and faster PBSC graft engraftment is absolutely essential.

[Key words: Peripheral Blood Stem Cell Transplant (PBSCT), Peripheral Blood Stem Cell (PBSC), Haematopoietic Stem Cell (HSC), Human Leukocyte Antigen(HLA)]

LIST OF ABBREVIATIONS

AABB	-	American Association of Blood Bank
ANC	-	Absolute Neutophil Count
ALL	-	Acute Lymphocytic Leukemia
AML	-	Acute Myeloid Leukemia
BM	-	Bone Marrow
Bu Cy	-	Busalphan and Cyclophosphamide
BCNU	-	Carmustin
Bu Flu	-	Busalphan and Flucytocin
Bu Cy Flu	-	Busalphan, Cyclophosphamideand Flucytocin
CXCR4	-	Chemokine (C-X-C) Motif Receptor 4
CFU-GM	-	Granulocyte Macrophage Colony Forming Unit
cGY	-	Centigrey
CBV	-	Cyclophosphamide, BCNU(Carmustin), VP16(Etoposide)
Cy TBI	-	Cyclophosphamide and Total Body Irradiation
CLL	-	Chronic Lymphocytic Leukemia
CML	-	Chronic Myelogenous Leukemia
CMV	-	Cytomegalovirus
DMSO	-	Dimethyl Sulfoxide
EPO	-	Erythropoietin
Flt 3	-	FMS Like Tyrosine Kinase-3

G-CSF	-	Granulocyte- Colony Stimulating Factor
GM-CSF	-	Granulocyte Macrophage- Colony Stimulating Factor
GY	-	Grey
HSC	-	Haematopoietic Stem Cell
HSCT	-	Haematopoietic Stem Cell Transplant
HLA	-	Human Leukocyte Antigen
HPC(A)	-	Haematopoietic Progenitor Cell Apheresis
HPC(C)	-	Haematopoietic Progenitor Cell Cord
HPC(M)	-	Haematopoietic Progenitor Cell Marrow
HSV	-	Herpes Simplex virus
HIV	-	Human Immuno Deficiency Virus
HL	-	Hodgkin Lymphoma
ICM	-	Inner Cell Mass
iPSCs	-	Induced Pluripotent Stem Cells
IL3	-	Interleukin 3
ISHAG	-	International Society of Hematotherapy and Graft Engineering
LVL	-	Large Volume Leukapheresis
LACE	-	Lomustine, Adriamycine, Cyclophosphamide, Etoposide
MNCs	-	Mono Nuclear Cells
MMP-9	-	Matrix Metallo Protinase 9
MM	-	Multiple Myeloma

NVL	-	Normal Volume Leukapheresis
NHL	-	Non Hodgkin Lymphoma
PBSC	-	Peripheral Blood Stem Cell
PBSCT	-	Peripheral Blood Stem Cell Transplant
PCD	-	Plasma Cell Disorder
PCR	-	Polymerase Chain Reaction
SSCs	-	Somatic Stem Cells
SDF-1	-	Stromal cell Derived Factor
SCID	-	Severe Combined Immuno Deficiency
TPO	-	Thrombopoietin
VCAM	-	Vascular Cell Adhesion Molecule
VLA 4	-	Very Late Antigen 4 (Integrin Alpha 4 Beta 1)
VDRL	-	Venereal Disease Research Laboratory

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[Key words: Peripheral Blood Stem Cell Transplant (PBSCT), Peripheral Blood Stem Cell (PBSC), Haematopoietic Stem Cell (HSC), Human Leukocyte Antigen(HLA)]

INTRODUCTION

Haematopoietic stem cell (HSC) transplantation remains the only curative option for many haematopoietic malignancies. The pluripotent haematopoietic stem cells required for this procedure are usually obtained from the bone marrow or peripheral blood. Currently, the majority of procedures for procurement of haematopoietic progenitor cells are performed by peripheral blood apheresis collection.[1]

After all the disappointment of clinical stem cell transplants in the late 1950's and early 1960's there was general distrust about the field, nevertheless improvements in transfusion medicine and understanding of HLA typing encouraged the clinical application of stem cell transplantation.[2]

Since then, more patients received haematopoietic stem cell transplantation to treat life threatening malignant and nonmalignant diseases and observational outcome research studies were carried out worldwide to address the important issues in haematopoietic stem cells transplantation.[2]

The first successful transplantations of allogenic haematopoietic stem cells were performed in 1968 in three children with congenital immune deficiency diseases. In each instance, haematopoietic cells were collected from the bone marrow of sibling donors who were genotypically identical to

the recipient for human leukocyte antigens (HLAs). Since then, more than 800,000 patients have received haematopoietic cell transplantations (HSCTs) to treat life-threatening malignant and non-malignant diseases.[3]

Current estimates of annual number of HSCTs are 55,000–60,000, worldwide. Reasons for widespread use include proven and potential efficacy in many diseases, better understanding of the appropriate timing of harvest, greater ease of haematopoietic progenitor cell collection by apheresis, patient selection, greater availability of donors, improved transplantation strategies and supportive care, leading to less transplantation related morbidity and mortality and an increased ability to perform the procedure in older and sicker patients.[4]

AIM

To study the factors influencing the engraftment of haematopoietic stem cell transplantation in patients with hematological malignancy.

REVIEW OF LITERATURE

STEM CELLS [5]

Stem cells are unspecialised cells capable of renewing themselves through cell division, under certain physiologic or experimental conditions after long period of inactivity they can be induced to become tissue or organ specific cells with special functions.

1. Embryonic Stem Cells

Embryonic stem cells are totipotent or pluripotent in nature. Those derived from pre-implantation embryo (morula stage) which is derived from embryos before differentiation of trophoectoderm and inner cell mass, capable of giving rise to the entire organism and extra embryonic tissues are totipotent.

Those derived from the inner cell mass (ICM) are pluripotent, having ability to differentiate into derivatives of all three germ layers, (ectoderm, mesoderm and endoderm), except placenta.

Embryonic stem cells can also be generated by reprogramming of somatic cells, giving rise to induced pluripotent stem cells (iPSCs). Induced pluripotent stem cells (iPSCs), are pluripotent in nature, similar to the

embryonic stem cells they are capable of indefinite expansion and differentiation into ectodermal, mesodermal and endodermal cells.

These cells can be generated from somatic cells by a variety of genetic and epigenetic methods.

2. **Somatic Stem Cells (SSCs)**

Somatic stem cells (SSCs) are a resident, self-renewable population of cells which are present in virtually all organs and tissues of the body. They have limited differentiation capacity and may be multipotent or unipotent. They are essentially undifferentiated, resident in differentiated tissues and are committed to the lineage of that organ. They may, however, have limited plasticity.

Haematopoietic progenitor cells and haematopoietic stem cells are somatic stem cells (SSCs) used for haematopoietic reconstitution after myeloablative therapy.

Source of haematopoietic stem cells

1. **Bone Marrow[6]**

Healthy adult bone marrow contains 0.5-1% CD34+ cells. Bone marrow harvesting is a surgical procedure performed in an operating room under the general or regional anesthesia.

The marrow is aspirated 3to10 ml at a time from multiple punctures of both posterior iliac crests, using specially designed stainless steel beveled needles and syringes.

Optimal post transplantation haematopoietic recovery needs 2 to 5×10^6 CD 34 Cells/ kg or $2to5\times10^8$ total nucleated cells/kg recipient body weight. To achieve this optimal dose, require 1 to 1.5 litre marrow collection for an adult allogenic recipient. In case of allogeneic donors harvested for the Be The Match Registry maximum of 20 mL/kg donor weight marrow will be collected. [36aabb]

2. Umbilical or Placental Cord Blood Collection[7]

Cord blood collection can either be performed by the obstetrician with the placenta in situ, or immediately after delivery by a trained team. The usual volume collected is less than 170 ml with anticoagulant, usually ACD formula A (ACD-A) or citrate-phosphate-dextrose- adenine (CPDA-1)

3. Haematopoietic Stem Cell Collection by Apheresis[8]

The peripheral blood of healthy adults contain less than 0.1% HSCs, this number increases during recovery from cytotoxic therapy and even more when mobilising factors such as G-CSF are administered.

The donor stimulated with haematopoietic growth factors, or chemotherapy and growth factors, a sufficient number of circulating stem cells for marrow rescue collected in one to three apheresis procedures. Peak counts usually obtained at the end of 5 days after stimulation with G-CSF (10 to 20 μg/kg/day) or 10 to 14 days after chemotherapy and G-CSF. Leukapheresis started when the peripheral CD34 count reaches or exceeds 10 CD34+cells/ μL.

The apheresis device uses a centrifuge to separate and collect MNCs, including peripheral blood HSCs, from the blood. In order to achieve a target cell dose of 2 to 5 $\times 10^6$ CD34+ cells/kg it is necessary to process 12 to 25 liters of blood or 2.5 to 6.0 times the patient's calculated blood volume.

Instrument settings such as inlet flow rate, centrifuge speed, collect pump flow rate and anticoagulant: whole blood ratio vary depending on the target cell type to be collected.

Comparison of Peripheral Blood and Marrow as Haematopoietic Sources

Collection of PBPCs rather than marrow eliminated the need for general anesthesia, an operating room and the repeated insertion of marrow aspiration needles into the posterior iliac crest and free of tumor cells in a

patient with marrow metastases and opportunity to receive potentially curative marrow ablative therapy.[9]

Advantages of Peripheral Blood Stem Cell Collection (PBPCs) by Apheresis

Various studies suggest PBPCs as the preferred source of haematopoietic cells for both autologous and allogeneic transplantation. PBPCs are advantageous for the recipient, because the recovery (engraftment) of platelet and white cell counts following either autologous or allogenic PBPC transplantation is more rapid compared with recovery following transplantation of marrow cells.[10-13]

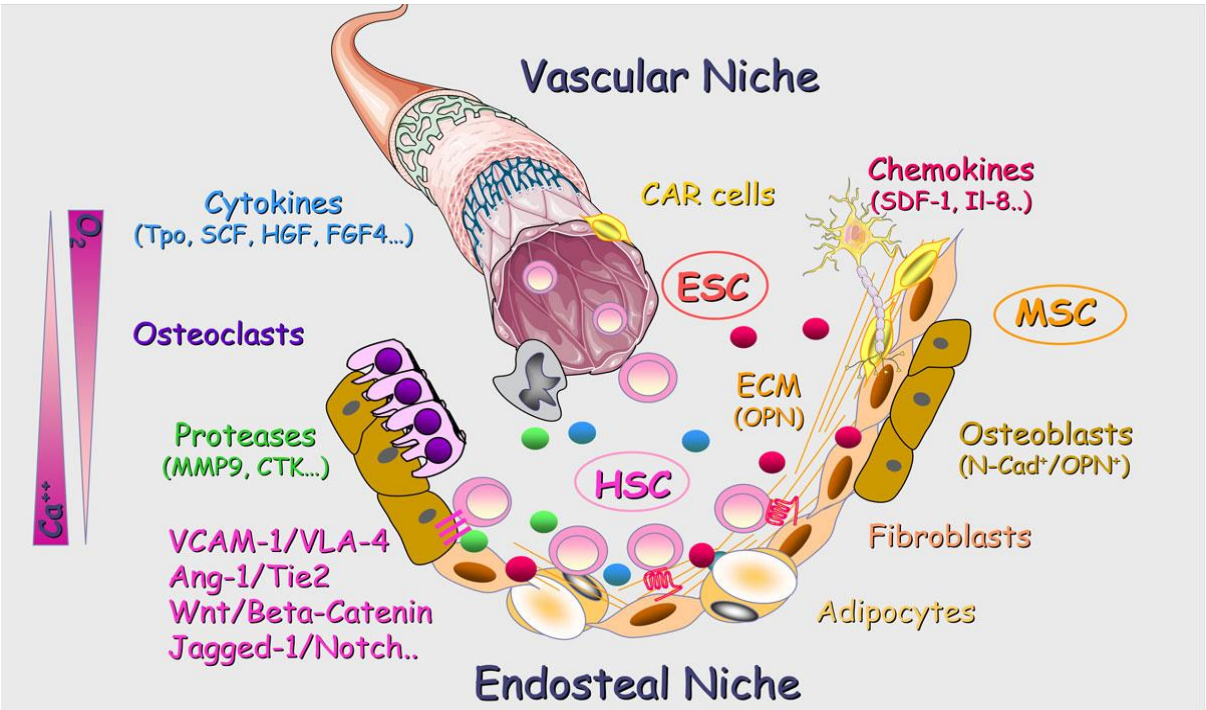
The stem cell mobilization techniques can increase the number of circulating progenitor cells and allow adequate collection of stem cells with minimum number of leukapheresis. PBPCs offer the potential advantage of containing fewer contaminating tumour cells than marrow. Presence of tumor cells in the collected marrow is an exclusion criterion for autologous marrow transplantation.[14-17]

Mobilization of Peripheral Blood Progenitor or Stem Cells

Biology of Haematopoietic Progenitor or Stem Cell Mobilization

Cottler-Fox et al in their study stated that haematopoietic stem cells are cells capable of self-renewal and differentiation into all blood cell lineages. In sufficient numbers, they give rise to complete sustained haematopoietic engraftment. In contrast, haematopoietic progenitor cells are committed to a blood cell lineage. They do not have the capacity for sustained self-renewal or the ability to differentiate into all haematopoietic lineages.[18]

Papayannopoulou T reported that the HPC adherence to the haematopoietic niche is mediated by a variety of adhesion molecule interactions, including the binding of a receptor, CXCR4 on HPCs, to stromal- cell-derived factor 1 (SDF1) the binding of beta-1 integrin adhesion molecules (VLA4 on HPCs) to vascular cell adhesion molecule 1 (VCAM1) on stromal cells and to fibronectin and the binding of HPC CD44 to hyaluronic acid, among others.[19] (Fig.1)



(Fig.1) Haematopoietic "Niche"

Yin T et al and Papayannopoulou T showed that the HPC mobilization stimulated by myelosuppressive chemotherapy or by administration of haematopoietic growth factors leads to temporary increase in the dissociation rate of HPCs from the niche through the disruption of those adhesive interactions and the migration of HPCs into the marrow and then into the peripheral blood circulation. [19,20]

The mobilising regimens are associated with the release of metalloproteases like MMP9, elastase, and cathepsin G which cleave one or more receptor-ligand pairs like SDF1 and release HPC from their stroma.[18,19]

In contrast, agents or antibodies those directly disrupt or block HPCs or stromal adhesive receptors such as VLA4 antibodies, or oligopeptides that block CXCR4, are associated high mobilisation of circulating HPCs in a matter of minutes to hours.[18]

Factors reported to affect HPC mobilization include the patient's age and gender, the presence of marrow disease, the extent and the type of prior myelotoxic therapy like cancer chemotherapy, radiotherapy or treatment of hepatitis C virus with interferon-γ and the dose, schedule, type of mobilizing regimen used and the patient's underlying disease, patient's genetic polymorphisms.[18,21-23]

Mobilization[198]

Approximately 0.03% to 0.05% of the white blood cells (WBCs) in the peripheral blood of normal healthy individuals express CD34 for a total of 0 to 5 CD34+ cells per μL of blood. In the marrow, 3% to 5% of cells express CD34 positivity.

The rationale for mobilization is to minimize the number of apheresis procedures needed to obtain enough stem and progenitor cells for successful post transplant engraftment of the absolute neutrophil count (ANC) to 500/μL and of the platelet count to 20,000/μL without platelet support.

Yin T et al in their study found that in the absence of mobilization, 8 to 14 apheresis procedures on separate days were required to obtain a yield of stem and progenitor cells adequate for transplantation (a median of 8.4×10^8 mononuclear cells (MNCs)/kg. With current mobilization methods, up to three apheresis procedures are needed to obtain a yield of stem and progenitor cells for adequate for engraftment.[24]

PBPC Collection during Steady-State Hematopoiesis[199]

Autologous PBPCs provided reliable, sustained haematopoietic recovery when transplanted, but the paucity of such cells in the circulation necessitated six to eight or more apheresis procedures to collect an acceptable graft product. While this large number of collections from a single patient is feasible, it is also cumbersome and time-consuming and discouraged PBPC transplantation except for very specific indications.

However, when the number of progenitors and stem cells in the circulation are deliberately increased, the number of collections is reduced, making the use of PBPCs more acceptable.

Haematopoietic Cytokines Used in Mobilisation and Its Effect on Engraftment

Chao N in their study concluded that the time to reach an ANC of 500/μL and a platelet count of 20,000/μL is significantly shorter with cytokine-mobilized PBPCs than with immobilised PBPCs.[25]

Nemunaitis J et al showed stem and progenitor cells mobilized by exogenous haematopoietic growth factors, currently, G-CSF (filgrastim) and GM-CSF (sargramostim) are the cytokines most commonly used for mobilization, which were used initially to accelerate recovery of the ANC after chemotherapy.[26]

1. G-CSF

Granulocyte Colony-Stimulating Factor (G-CSF) is a 177-amino acid protein of approximately25 kDa. Lane TA et al found G-CSF is more potent as a mobilizing agent and is far more widely used than GM-CSF, which is typically used in combination regimens. G-CSF administration to normal or autologous donor's results in dose-dependent increase in leukocyte, lymphocyte, and HPC counts.[27,28]

Weaver CH et al stated that G-CSF is typically administered once daily subcutaneously in doses ranging from 5 to 20 μg/kg/day, although higher doses may be used in poor mobilizers.[29] Kroger N et al suggest that

divided doses of G-CSF permit higher doses, result in improved mobilization with fewer side effects, or both, but those findings are not universal.[30]

Watts MJ in their study showed that, when used alone, G CSF is ideally administered at least 4 hours before scheduled HPC collection because of a transient decrease in HPC levels that occurs shortly after G-CSF administration.[31]

A single dose of long-acting, pegylated G-CSF has been reported to result in satisfactory allogeneic or autologous donor HPC mobilization, but this drug is not currently approved for HPC mobilization.[32]

Sato N, Sawada K, Takahashi A, et al in their study showed that in healthy individuals, G-CSF at 2 μg/kg/day induced maximal CFU-GM approximately 24 to 30 hours after five daily injections, with the peak level maintained for approximately 24 hours.[33] Grigg AP et al found that G-CSF at 10 μg/kg/day induced a median increase of 157-fold in circulating CFU-GM(range = 52-3940) and a 22-fold increase in circulating CD34+ cells (range = 8-105).[34]

Similarly Korbling M et al found that a 16-fold increase in circulatingCD34+ cells,[35] and Tjonnfjord GE et al found that a peak peripheral bloodCD34+ cell count on day 4 or 5 of 20 to 100CD34+ cells/μL

peripheral blood,[36] with wide inter individual variation after G-CSF mobilization.

At present, a widely used and effective mobilization protocol for allogeneic donors is 5 days of G-CSF (5-12 μg/kg/day subcutaneously) followed by 2 days of leukapheresis for collection of stem and progenitor cells[10]

Anderlini P, Korbling M, Dale D et al in their study found that when used in normal donors, G-CSF doses >10 μg/kg/day are of uncertain benefit and are associated with increased side effects. Most centers avoid leukocyte counts >70,000/μL by dose reduction if desirable before apheresis, especially for normal allogeneic donors.[37]

2. GM-CSF

Haas R, Ho AD, Bredthauer U et al in their study showed heavily pre-treated patients who were ineligible for marrow transplantation, continuous IV GM-CSF infusion induced a median 8.5-fold increase in circulating CFU-GM that allowed collection of adequate numbers of PBPCs for transplantation.[38,39]

With subcutaneous injection, there is a dose-dependent increase in CFU-GM, with a maximal effect at 10 μg/kg/day. Recommended doses of GM-CSF range from 3.0 to 10.0 μg/kg/day.[40]

3. G-CSF versus GM-CSF

Bolwell B, Goormastic M, Yanssens T et al studied on 44 patients with lymphoma received both marrow and PBPCs included random assignment to G-CSF or GM-CSF mobilization. The patients who received G-CSF achieved an ANC of 500/ μL and a platelet count of 20,000/μL after mean intervals of 9 and 13 days, respectively and for the patients who received GM-CSF, the intervals were 14 and 18 days, respectively.[9]

Capillary leak syndrome, including pericarditis and fluid retention, is a dose-limiting toxic effect of GM-CSF. Rare side effects of G-CSF include splenic rupture, iritis, cardiac ischemia, capillary leak syndrome, and gouty arthritis. [41]

Takaue Yet al and Lane T A et al found that improved mobilization of PBPCs can be achieved with combinations of haematopoietic cytokines. G-CSF and GM-CSF together stimulate more proliferation of immature cells than either cytokine alone both in vitro and in vivo.[42,43]

4. Adhesion-blocking agents and cytokines(Plerixafor /AMD3100)

DiPersio JF et al and DiPersio JF et al showed that Mozobil (AMD3100) was recently approved for use in combination with G-CSF for the mobilization of CD34+ cells in autologous transplant patients with multiple myeloma.[44]

Mozobil is a synthetic SDF1-like molecule that blocks the adhesive interaction between SDF1 on marrow stromal cells and CXCR4 on CD34+ cells. It is unique among clinical CD34+ cell mobilizing agents in that it stimulates the release of CD34+ cells within several hours after administration. Mozobil additive effects increase circulating CD34+ cells and T cells when it is administered in combination with G-CSF or myelosuppressive agents.[10]

DiPersio JF et al stated that the FDA approved protocol for HPC mobilization using Mozobil and G-CSF combined, calls for 10 μg/kg G-CSF to be administered each morning and for 0.24 mg/kg Mozobil to be administered subcutaneously (in patients with normal renal function) on day 4 of G-CSF mobilization, approximately 11 hours before leukapheresis.[10]

DiPersio JF et al and DiPersio JF et al showed that the combined use of G-CSF and Mozobil has been reported to increase the number of HPCs collected per apheresis procedure, to decrease the number of apheresis procedures required to collect a target dose of HPCs, and to improve the mobilization of HPCs in poor mobilizers.[44,10]

5. Flt3 Ligand[11]

Rusten LS et al showed that the cytokine Flt3 ligand, which binds to a tyrosine kinase receptor on the surface of stem and progenitor cells mobilizes them effectively.

Flt3 ligand is synergistic with SCF, G-CSF, GM-CSF, and IL-3 in vitro, producing 20-fold, 1.6-fold, 3.4-fold, and3.8-fold enhancements of CFU-GM, respectively.

6. Stem Cell Factor (SCF)

Nemunaitis J in his study on recombinant SCF revealed a mast-cell-related dose-limiting effect. Patients must therefore be pre medicated with antihistamines, albuterol, and/or pseudoephedrine before SCF is administered.[12]

There was a significant reduction ($p < 0.05$) in the number of collections required to reach target yields for patients receiving SCF plus G-CSF compared with patients receiving G-CSF alone; however, PBPCs collected with G-CSF alone engrafted equally rapidly.[13]

7. IL-3

The group receiving simultaneousIL-3 and G-CSF required four procedures and those patients receiving only IL-3required six procedures.[45]

Several additional agents that support HPC mobilization have been described, such as dextran, human growth factor, interleukin-8, erythropoietin (EPO) and thrombopoietin (TPO).

Additional agents that induce haematopoietic stem cell mobilization by interfering with their adhesive properties to the niche are currently under investigation.

Mobilization Using Chemotherapy

Richman CM et al showed in the setting of autologous haematopoietic stem cell transplantation, mobilizing chemotherapy perform various roles, including 1) Treating the patient's malignancy 2) Testing the sensitivity of the tumor to cytotoxic therapy 3) Mobilizing stem cells.[46]

Testa U et al showed that the mechanism of action chemotherapeutic agents mobilize stem cells is by endogenous production of haematopoietic cytokines in response to their action on dividing cells. Serum concentrations of interleukin-3 (IL-3), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, IL-8, thrombopoietin, and Fms-like tyrosine kinase 3 (Flt3) ligand, all increase 2 to 6 days after chemotherapy.[47]

A possible advantage of some disease specific chemotherapy-based regimens is the potential for “in-vivo purging” of tumor cells that might otherwise contaminate the HPC (A) product and/or the ability to keep rapidly progressive disease in check during the time required to mobilize and collect HPC and to prepare the patient for the high-dose anti neoplastictherapy.[48]

Mobilization Using Both Chemotherapy and Growth Factors

The combination of chemotherapy and cytokines is the most effective and commonly employed mobilization protocol in the autologous setting. Circulating progenitors can be increased by 100- to 160-fold, which is similar to the effect of cytokines alone in healthy allogeneic donors.[49]

Haas R, Hohaus S, Ehrhardt R et al have found that PBPCs mobilized by chemotherapy plus either G-CSF or GM-CSF gave faster engraftment than PBPCs induced by chemotherapy alone. The most common regimens include chemotherapy followed by daily G-CSF throughout the PBPC collection period.[14]

Oliver Rick et al in their study found that chemotherapy (paclitaxel,ifosfamide) + G-CCF mobilised CD34+ cells showed increased mobilisation and single 500 mg amifostine before chemotherapy also

increase the CD34+ cell counts on days 10 and 11without improving the overall PBPC collection result.[50]

Haas R, Hohaus S, Egerer G et al in their study reported that as with chemotherapy-alone regimens, combined modality regimens have the potential to result in chemotherapy-induced cytopenia, the frequency of which varies depending on the intensity of the chemotherapy regimen and on the patient's marrow reserve. Also, the administration of growth factor in combination with chemotherapy may diminish the duration of neutropenia.[15]

One widely used combined modality regimen (cyclophosphamide plus G-CSF) results in predictable mobilization with few side effects. The combination of Mozobil after chemotherapy with or without G-CSF has also been reported to further enhance HPC mobilization.[16]

Poor Mobilizers

Anderlini P et alin their study found that poor HPC mobilization (2% to 20% of mobilization attempts) is defined as the failure to achieve a minimum level of 5 to 20 CD34+ cells/μL in peripheral blood after completion of the mobilization regimen, or as the inability to collect at least 1 to 2 × 10^6 CD34+ cells/kg during a single apheresis procedure, or as the failure to collect a total of 5 × 10^6 CD34+ cells/ kg in all collections.[17,51]

Ewing JC et al indicate that the management of patients who fail to mobilize will remains a challenging problem and is a common reason that otherwise eligible patients do not receive an autologous transplant[42]. Pavone V et al found this is because patients transplanted with low doses of HPCs have prolonged times to engraftment, higher rates of graft failure and post transplant complications and poorer outcomes.[52]

Cottler-Fox M, Lapidot T indicate that factors such as the premobilization peripheral blood CD34+ cell count, flt3 ligand level or platelet count, have been reported to correlate with and to predict postmobilization CD34+ cell counts.[53]

Micallef IN et al and Calandra G et al indicate that either adding mozobil on day 4 for myeloma / lymphoma patients who are poorly mobilizing with G-CSF or remobilizing with growth factor, Mozobil are effective in most myeloma and non-Hodgkin lymphoma patients who mobilize poorly.[54,55-59]

Gazitt Y et al reported that the immediate administration of 32 μg/kg/day of G-CSF for 4 days led to acceptable mobilization in 80% of a heterogeneous group of poorly mobilizing autologous patients. [56]

Cassens U et al found that Large-volume leukapheresis, in which four to six patient blood volumes are processed, may also improve overall HPC collection in those patients.[57]

Lemoli RM et al found that the transplantation of autologous HPC(M) collected after marrow harvesting in poor HPC(A) mobilizers was also reported to enable marrow transplantation, even though with variable rates of engraftment speed.[58]

Factors Reported to Affect the Mobilization of HPC,

Mobilization method
Chemotherapy (Degree of transient myelosuppression)
Growth factors (Type, schedule, dose)
Combined chemotherapy and growth factors
Extent and type of prior chemotherapy, radiotherapy
Drugs (interferon-γ ,lenalidomide)
Patient or donor age
Patient or donor diagnosis
Patient or donor gender
Presence of marrow disease or metastases

COLLECTION OF PERIPHERAL BLOOD STEM CELLS

Collection of Stem Cells

Collection of PBPCs is performed by apheresis instruments. The blood cells sediments into distinct layers according to their molecular weight

when anticoagulated whole blood is subjected to centrifugal force. The apheresis instrument harvests the mono nuclear cells (MNCs) in the zone between the granulocyte layer and the platelet layer by a leukapheresis procedure.

Collection Goal

The HPC (A) collection goal range for allogeneic transplantation is similar to that for autologous transplantation (2 to 5 × 10^6 CD34+ cells/kg).[60]

Instrument-Related Variations inCollection and Engraftment

Dzieczkowski JS, McGonigal M, Cook J et al in their study found that all apheresis instruments that can collect WBCs can also collect stem cells. None provides "better" stem cells in terms of faster haematopoietic reconstitution.[61]

There are differences in the instruments and the products they collect. These include differences in extracorporeal volume, in the incidence of adverse effects, especially citrate reactions, in the degree of thrombocytopenia, and in flexibility to use anticoagulants other than citrate (such as heparin) in special circumstances such as for LVL or pediatric procedures.[61]

Ford DC, Pace N, Lehman C in their study found that the yield of CD34+ cells obtained from a collection depends, in part, on the efficiency of their collection, but other factors also have an influence. High WBC counts, haematocrit, and/or albumin concentrations can decrease collection efficiency.[62]

Ford CD, Greenwood J, Strupp A, et al in their study found that a rapid decrease in blood CD34+ levels, not due to hemodilution, has been reported during the first 30 to 70 minutes of a procedure; this is followed by a relatively stable level for the remainder of the procedure.[63]

Knudsen et al in their study showed that CD34+ cells and MNCs are recruited to the blood during PBPC collection, whereas granulocytes and platelets are not. Because recruitment is limited to the cell fraction being removed, a feedback mechanism may be responsible. CD34+ cells are most likely recruited from marrow or a marginal pool.There was no exhaustion of progenitor cell release and CD34+ cell recruitment was shown with several mobilization regimens. [64]

The clinical relevance of these findings is that the longer collection times will keep increasing the yield and will decrease the number of procedures needed to achieve the goal.

Ford CD et al measured CD34+ cell collection efficiency in 163 consecutive donations with blood CD34+ cell levels >5/μL and found it to be significantly higher with the Baxter CS3000.[65]

Snyder EL et al in a clinical study evaluating collection efficiency, infusion toxicity, and engraftment characteristics, the Amicus was shown to be safe and effective for PBPC collection.[66]

Timing of PBPC Collection

Once it is decided to perform stem cell transplantation, it remains to be determined when during mobilization to begin apheresis and when to stop. Donor response to mobilization vary differently to based on age, amount of prior chemotherapy and/or radiotherapy, type of malignancy and degree of marrow involvement.

The goal is to collect adequate PBPCs to give rapid, successful engraftment from the fewest apheresis procedures therefore scheduling the patients for apheresis in the autologous setting is more important.

When to Start PBPC Collections

Various criteria have been used to predict the optimal time to initiate collection of PBPCs. These include the absolute WBC count, the

kinetics of WBC recovery, the platelet count, and the blood level of CD34+ cells.[67]

Haas R et al has been recommended that leukapheresis begin when the WBC count reaches 1000/μL. They also found that the blood CD34+ cell count is predictive of the total yield of progenitors and can also be used to determine when to initiate apheresis.[68]

J.A. Perz-Simon et al in their study found that a minimum of 5 CD34+ cells per μl in peripheral blood is enough to initiate leukapheresis.[69]

Grigg AP, Roberts AW, Raunow H, et al,in their study have shown that for a 7-L collection using the Baxter CS3000 (Baxter Biotech, Deerfield, IL), a blood CD34+ cell count of 40 cells/μL predicted that the apheresis product would contain approximately 60×10^6 CFU-GM.[70]

Similarly Fruehauf S et al have shown that the CD34+ cell content in the peripheral blood before mobilization correlates with the number of collections needed to obtain an adequate number of CD34+ cells.[71]

Abba C. Zubair et al in their study showed that platelet (PLT) count before growth factor administration significantly correlated with total CD34+ cell yield in plasma cell disease patients who received prior chemotherapy. In addition, daily platelet count during PBPC harvest correlated with CD34+ cell yield for that day.

They suggest baseline platelet count is a sensitive indicator of autologous PBPC mobilization in PCD patients who received prior chemotherapy to determine the optimal period to mobilize treated PCD patients and to predict if enough cells can be collected for one or two transplants.[72]

Cheolwon Suh et al in their study found that initiation of peripheral blood progenitor cell harvest based on peripheral blood count of 5 CD34 cells per mm3 after chemotherapy plus lenograstim mobilisation in the autologous setting showed that the median time for neutrophil engraftment was 10 days (95% confidence interval 9-11 days; range, 9-20 days) and the median time for platelet engraftment was 12 days (95% confidence interval 10-14 days; range, 7-27 days) and there were no cases of engraftment failure.[73]

L.Pierelli et al in their study found that accurate prediction of apheresis yield can be accurately calculated by formula with pre apheresis peripheral blood CD34 cell count and peripheral blood volume to be processed.

CD34+cells per kg body weight collected = CD34+cells per ml of PB×(0.4)× (ml of peripheral blood processed per kg body weight).[74]

When to Stop Apheresis Collections

Programs that lack such readily available cytometry services, especially after normal hours, an alternative method to assess apheresis yields is to measure the CD34+ cell content of the cells collected by apheresis approximately midway through the apheresis procedure and to extrapolate the final yield.

When carefully used, that method has been reported to permit accurate decisions regarding whether to continue apheresis for another day or to discontinue it, without waiting for the final HPC (Apheresis derived) CD34+ cell count to be available. That approach may avoid unnecessary growth factor injections and apheresis procedures.[75]

Leukapheresis for Second Autograft during Haematopoietic Engraftment of First Autograft

N.Schwalla et al in their study concluded that (1) PBPC harvesting is feasible and well tolerated in the autologous setting. (2) In appropriate patients with efficient PBPC mobilization after conventional-dose chemotherapy, a further PBPC autograft can be collected during recovery of hematopoiesis after autologous blood progenitor cell transplantation, serving as a rescue for a second course of high dose chemotherapy.[76]

Harvest Schedules

1. Standard Collections

To obtain a predefined target yield, 1to 4 PBPC collections are needed. In a standard collection, 10 to 12 L of blood is usually processed in 3 to 4 hours. Haas R, Mohle R, Fruhauf S et al in their study found that patients with poor-prognosis lymphoma were mobilized with chemotherapy plus G-CSF and collected when the WBC count exceeded 1000/μL. [68]

Haas R et al also stated that the target yield of 2.5×10^6 CD34+ cells/kg was collected in a single 10-L leukapheresis in 34 of 61 patients. However 15 patients still had $<2.5 \times 10^6$ CD34+ cells/kg after a median of six apheresis procedures. In case of poorly mobilized patients, 8 to 10 procedures required to obtain an adequate yield of PBPCs. [68]

2. Large Volume Leukapheresis Increase the Stem Cell Mobilisation

To minimize the number of collections, large volume leukapheresis (LVL) procedures were performed, in which 15 to 40 L of blood is processed over 6 to 8 hours, provides an adequate yield for many well mobilized patients. It is also be desirable for poorly mobilized patients to achieve the collection goal.

All patients experience a significant drop in platelet count and prolongation of partial thromboplastin time when 15 to 35 L of blood is processed.[77]

Hillyer CD, Lackey DA III, Hart KK et al in their study found that more CFU-GM were harvested from the final blood volume collected than from any of the first three blood volumes during recruitment of HPCs throughout the course of LVL.[78]

Hillyer C, Tiegerman K, Berkman E et al also found that a 56% increase in the volume of blood processed (from 11.8 L to 18.5 L) lead to 142% increase in the CFU-GM content of the apheresis product. Additional evidence supporting recruitment is that no difference was noted during LVL between the number of CD34+ cells/kg/liter in the first hour and the last 2 hours.[79]

Cassens U, Momkvist PH, Zuehlsdorf M et al in their study on 24 patients undergoing LVL were compared with a control group of patients who were treated with G-CSF after mobilizing chemotherapy but did not undergo apheresis.

The LVL group experienced an increase in the relative yield during the second of six blood volumes processed but gradual decline of yield

thereafter. The timing of collection correlate well with peak blood CD34+ cell levels after G-CSF administration in the control patients.[80]

Lack of recruitment is also supported by studies showing that the number of PBPCs steadily decreases during apheresis. Bojko et al found that the mean peripheral blood CD34+ cell count decreases from116/μL at the start of the procedure to 57/μL after four blood volumes are processed.[81]

Prior Chemotherapy and Radiotherapy

Aurlien E, HolteH, Pharo A, et al in their study on 141 heavily pretreated, relapsed lymphoma patients found that radiation, conventional chemotherapy and high-dose chemotherapy all have a measurable negative impact on yields that may also influence engraftment kinetics.[82]

In addition, even 6 months of treatment with alkylating agents significantly delayed engraftment and in extensively pretreated patients, the CD34+ cell yield required for rapid platelet engraftment increased from 2.0 × 106/kg to 5.0 × 106/kg.

However above study explained negative impact of prior therapy. The extensive prior therapy need not prevent successful PBPC collection in all cases. The authors attributed their success to the use of a particular mobilizing regimen (mitguazon, ifosfamide, methotrexate, and etoposide) and higher doses of G-CSF.[82]

Toward that goal, a host of chemotherapy regimens that are tailored to the patient's malignant disease, most of which also use G-CSF or GM-CSF to augment HPC mobilization, have been reported to have adverse effect on yield.[83,84]

Impact of Disease State

Has et al in their study found that no significant difference in PBPC yields between the relapsed and the patients in complete remission. [68]

Passos-Coelho J, Braine HG, Davis J et al in their study PBPC collection by single LVL found that neither complete nor partial remission had a significant impact on CD34+ cell yield.[85]

Impact of Tumor Contamination[86]

Relapse remains the primary cause of death in patients who receive autologous stem cell support after myeloablative chemotherapy. The question remains, however, whether tumor cells reinfused with PBPCs cause relapses or whether the disease was not eradicated by myeloablative therapy. Disease recurrence is typically found in previous disease sites, suggesting that chemotherapy was not curative.

Rill DR et al in their gene-marking studies have demonstrated that the tumor cells reinfused with the PBPCs cause the relapse and these cells localize to sites where the original tumor is recurring.

The probability of tumor contamination in PBPCs correlates with both disease stage and the occurrence of relapse. It therefore seems prudent to collect PBPCs during remission, when the total body tumor burden will be minimized. Similarly, autologous marrow transplantation is best performed when there is minimal tumor detectable in the marrow.

Incidence of Tumor Contamination

Tumor contamination of PBPC collections has been clearly demonstrated for both hematologic malignancies and solid tumors.[87]Using immunoglobulin gene rearrangement polymerase chain reaction (PCR), Stewart et al found a contamination rate of 82% in 47 myeloma patients.[88]

McCann et al, also using PCR, detected tumor contamination in 82% of the PBPC collections from patients with relapsed non-Hodgkin's lymphoma.[89]

The Impact of Relapse and Remission on Tumor Contamination

Study by Dreyfus et al suggests that PBPCs from patients with relapsed refractory myeloma have a higher rate of tumor contamination. Tumor contamination was not found in one patient in remission, but was found in 31% of 16 patients in partial remission and 75% of four patients with refractory disease.[90]

Jacquy C et al in their study found that complete clinical remission does not eliminate the potential for mobilizing tumor. As many as 50% of patients in complete remission from diffuse large cell lymphoma have mobilized tumor cells.[91]

The Effect of Disease Stage and Mobilization on Tumor Contamination

Ross et al, who found that patients with localized disease did not have tumor cells in PBPCs while patients with widespread metastatic disease had tumor contamination rates ranging from 17% to 100%.

With the exception of bone, there was no correlation between the site of metastasis and the probability of finding tumor cells in PBPCs. On the other hand, marrow involvement was observed in six of the nine patients who had tumor cells in their PBPCs.[92]

Ladetto et al also found that all patients with advanced myeloma and marrow tumor also had tumor cells in PBPCs, suggesting that involved marrow is an important source of mobilized tumor cells. The authors concluded that both prevalence and concentration of tumor cells were low in blood before mobilization and that mobilization did not increase contamination. [93]

Thus, it remains controversial whether or in what circumstances mobilization of stem cells also mobilizes tumor cells.

Effect of Mobilization Regimen on Tumor Contamination

Different mobilization protocols have been studied for their effect on tumor contamination. Demirkazik et al found that patients who were mobilized with cytokines alone tended to have higher rates of tumor contamination in PBPCs than an unusual control group of nonmobilized patients. [94]

Knudsen LM et al in their study, cytokine mobilization was used for one collection while chemotherapy plus cytokines was used for the other, in random order. With each patient serving as his or her own control, tumor contamination was lower in PBPCs mobilized by chemotherapy plus cytokines than in PBPCs mobilized by cytokines alone.[95]

Mobilization kinetics of Tumor Cells can be Predictable

In daily collections from myeloma patients, Gazitt and Tian E et al found that the collections richest in stem cells were obtained on days 1 to 3, whereas 75% of tumor cells were collected on days 5 and 6.[96]

Gazitt Y et al in their study found that, only two of the 12 patients had >2.5% tumor cells on days 1 and 2, whereas 11 of 12 had >2.5% tumor cells by day 6. The same group also looked at low-grade (follicular) lymphoma to test the theory of differential mobilization of tumor. In this study, "poor mobilizers" (defined in part as those requiring ≥4 days of apheresis to yield 2 × 106 CD34+/kg) had lymphoma cells in 42% of PBPC collections as compared with 17% in "good mobilizers" who reached the same goal with 2 collections. [97]

Using TaqMan PCR and flow cytometry to detect tumor cells, Knudsen et al found that peak myeloma cell concentration in blood correlated with peak CD34+ cell concentration, regardless of mobilization regimen.[95]

McCann JC et al found that, the differential mobilization of tumor cells apparent in some studies after administration of chemotherapy may be due to tumor cells mobilizing from two different sources with different kinetics.[89]

Early mobilization may result from chemotherapy induced tumor necrosis, which causes haematogenous seeding as the primary tumor breaks down. Later mobilization may be due to release of metastatic tumor cells from marrow via the same mechanisms responsible for release of PBPCs.[89]

Dreger P et al suggest that patients with marrow metastases or diseases intrinsic to marrow should undergo apheresis early after mobilization. This approach, however, would be limited by the extent of mobilization because many patients need to achieve a relatively high WBC count before adequate numbers of PBPCs can be collected.[98]

In the subset of patients who mobilize well, a minimum number of LVL performed early in haematopoietic recovery may be preferable.

PERIPHERAL BLOOD PROGENITOR CELL GRAFT MANIPULATION/GRAFT ENGINEERING AND ITS INFLUENCE ON ENGRAFTMENT

The term “graft/cell engineering” is used to describe all varieties of stem cell graft manipulation, including removal of unwanted red cells or plasma from the stem cell graft to aid in stem cell cryopreservation or to reduce product volume before infusion for rapid engraftment.

Separation Methods[99]

1. Physical Methods

Early physical separation methods were performed with routine laboratory equipment such as floor-mounted centrifuges to separate a mononuclear cell fraction containing the stem cells from the graft containing red cells.

Later improvements utilized more sophisticated blood bank equipment such as the COBE 2991 Cell Washer (Gambro BCT, Lakewood, CO) with sterile disposable plastic ware to separate and concentrate mononuclear cells.

2. Immunologic Methods

The development of modern cell engineering techniques has been facilitated by improved phenotypic analysis with monoclonal antibodies and flow cytometry. The identification and characterization of the 115-kDa cell surface membrane molecule called CD34 on stem and committed progenitor cells allow specific haematopoietic progenitor cell isolation based on the presence or absence of CD34 and related antigenic markers.

The concept of positive or negative selection of cells based on cell surface phenotype identification upgrade the modern stem cell graft preparation. Coupling antibodies to magnetic beads, immunotoxins or microparticles offered new treatment options which significantly improve the outcome of stem cell transplantation.

Flow cytometry based high-speed cell sorting is an alternative method for selecting haematopoietic progenitors and its subpopulations.

a. Positive Selection

Positive selection techniques are designed to separate the cells bearing an antigenic marker of interest in a graft. Such techniques can isolate highly purified cell subsets, and this can affect the outcome of stem cell transplants.

The positive selection has been successfully employed to enrich stem or progenitor cell populations bearing the CD34 antigen this also accomplish some degree of separation of stem cells from contaminating precursors, mature immunocytes, and tumor cells.

b. Negative Selection

Negative selection techniques are designed to remove the cells bearing an antigenic marker of interest from a stem cell graft. The negative

selection is similar to cytotoxic depletion but unlike cytotoxic depletion not only confined to a malignant population it includes interfering cells such as T cells or subsets.

3. Tumour Cell Purging by Cytotoxic Depletion Techniques

Early immunologic methods depend on incubating harvested marrow with tumour antibodies in the presence of complement to achieve selective tumour cell killing. Now cytotoxic drugs and tumour antibodies have been used to purge occult tumour cells from autologous stem cell grafts.

Cytotoxic drugs such as mafosphamide or 4-hydroperoxycyclophosphamide have been used to kill a broad range of tumour cells while sparing stem cells. Stem cells have high levels of aldehyde dehydrogenase, which degrade these pharmacologic agents and escape from these agents, but tumour cells lack aldehyde dehydrogenase enzyme.

Application of Cell Engineering Techniques

Much effort has been focused on methods to manipulate PBPC grafts to improve clinical outcomes due to shift in use from marrow to mobilised PBPCs. Both enrichment (Positive selection) and depletion

(Negative selection) techniques have been employed in PBPC engineering procedure.

1. Tumor Purging and Engraftment

The techniques that are designed to eliminate unwanted tumor cells from an autologous stem cell graft called “tumor purging”. The rationale for tumor purging comes from clinical studies indicating that “contaminating” tumor cells in such grafts may contribute to disease recurrence following transplantation. MA Diaz et al in their study found that tumour cell purging delays platelet engraftment[100]

2. Enrichment Methods for Stem/Progenitor Cells

The earliest prototypes for cell selection methodology were antibody-coated tissue culture flasks that bound CD34+ cells to their surfaces. Subsequently, a more user-friendly cell selection system was developed that used monoclonal anti-CD34 and avidin-biotin immunoabsorption chromatography to purify CD34+ haematopoietic progenitor cells.[99]

The newer devices uses various platforms such as immuno magnetic or super paramagnetic iron dextran particles with monoclonal anti-CD34 conjugated for selection offers a closed system with sterile, disposable

plastic ware and clinical grade reagents and provides recovery of CD34+ cells with high purity.[99]

In various trials conducted in patients with non-Hodgkin's lymphoma, the selection procedure results in loss of about 50% of the CD34+ cells.

3. T-Cell Depletion and Engraftment

MA Dı´az1 et al reported that reduction in the number of T cells by manipulating allogeneic grafts before transplantation has been shown to reduce the incidence of graft versus host disease (GVHD).[99]

Koh MB et al and Solomon SR et al found that positive selection for CD34+ cells can bring about up to a 5-log reduction in the T-cell content of a graft, [62-64] and the engraftment of CD34+ cells selected from allogeneic PBPCs is similar to unmanipulated PBPC grafts.[101,102]

Mitsuyasu RT, Champlin RE, Gale RP et al and Noga SJ in their study found that T-cell-depleted grafts did not improve overall survival in patients compared with patients receiving unmanipulated stem cell grafts. Further ancillary cells in the stem cell graft that are mediators of GVHD are also important participants in stem cell engraftment and exhibit antileukemic activity.[103,104]

4. Cell Expansion and Engraftment

Brugger W et al and Henschler R, et al stated that, at present, mobilized PBPCs are the preferred stem cell source for transplantation and point out that myeloid and lymphoid stem/progenitor cells can be expanded for up to 2 weeks in the presence of cytokines.[105,106]

Boiron JM et al found that the empirical limit of engraftment speed with optimal CD34+ cell dose and growth factor administration is approximately 9 days. Preliminary clinical studies suggest that the time of 9 days may be shortened in the future by co administration with HPCs that have been expanded, activated ex vivo, or both.[107]

Tisdale JF et al and Haylock DN et al found that, adult CD34+ cells expanded ex vivo provide sufficient progeny to restore hematopoiesis in the short term, and expanded cells derived from PBPCs can correct neutropenia in transplant recipient's promptly.[108,109]

HAEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)[4]

The theoretical basis for stem cell transplantation came from the studies of Nowell and Ford, who demonstrated that bone marrow cells infused from one mouse in to a lethally irradiated mouse were capable of rescue by generating the entire repertoire of haematopoietic cells.

Based on this the first unsuccessful allogenic transplants was performed by Sir Donnel E Thomas in 1957and then first successful syngeneic transplant in 1959in leukemia patients following total body irradiation.

After understanding of the human histocompatibility, the first HLA matched sibling donor transplant was done by Dr Robert Good in an infant with immunodeficiency in 1968and by Sir Thomas in leukemia patient in 1969.

AUTOLOGOUS PERIPHERAL BLOOD PROGENITOR CELL TRANSPLANTATION

Early Studies

In the 1960s, animal studies detected stem cells in the circulation and, in the 1970s, haematopoietic stem and progenitor cells were identified in the human bloodstream. Subsequently transplantation of circulating

haematopoietic stem and progenitor cells was found to restore marrow function in lethally irradiated animals. [110]

However, to convert those observations in the clinical ground for therapeutic use, need development of techniques to collect the cells in large numbers. This was important because very few haematopoietic stem and progenitor cells circulate during steady-state haematopoiesis.[111]

The problem was solved when a report appeared in 1974 showing that large numbers of human stem and progenitor cells could be collected with an apheresis instrument.[103] Frequent apheresis procedures during steady state hematopoiesis and cryopreservation of the collected cells, allowed sufficient number of stem and progenitor cells for successful transplantation.[112]

Clinical Trials

McCarthy DM et al stated that, when the CML transformed to an accelerated phase, the patients received high-dose therapy and then their cryopreserved cells given to convert the disease to chronic phase. Forty seven of 50 patients recovered haematopoietic function with chronic phase disease but second chronic phase was short lived. [111]

Goldman JM et al found that since the blood of these patients contains high number of haematopoietic stem and progenitor cells harvesting of these cells is possible with 2 to 4 apheresis procedures. [113]

The outcomes of these transplants were more encouraging and the following year descriptions emerged of six successful autologous transplants from six different institutions throughout the world.[114]

ALLOGENEIC PERIPHERAL BLOOD PROGENITOR CELL TRANSPLANTATION

Allogeneic PBPC transplantation involves the transfer of cells from a normal donor to a recipient with either a malignant disease that was treated with high-dose therapy or an inherited marrow disorder that can be corrected by replacing the diseased marrow with normal cells. The patient and recipient must be matched closely for certain Histocompatibility antigens to avoid or minimize graft rejection and graft-versus-host disease (GVHD).[190]

Clinical Trials

The first clinical allogeneic PBPC transplant procedure was reported in 1989 and actually took place in late 1987, before cytokines were recognized as mobilizing agents. The clinical situation was unique because the matched sibling donor was unwilling to donate marrow but agreed to undergo multiple (10) apheresis procedures. [115]

Dreger P et al stated that, while the result of the 1987 transplant could be considered a qualified success, no further allogeneic PBPC transplantation attempts were reported until 1993.[116] Later that same year, a report of a successful allogeneic PBPC transplantation appeared.[117]

Bensinger WI et al stated that, in early 1995, three reports, together describing successful transplantation of allogeneic PBPCs in 25 patients, appeared simultaneously and from that time, use of peripheral blood rather than marrow for allogeneic transplantation became more common. [118]

Anderlini P et al stated that, in 2001, the International Bone Marrow Transplant Registry and the European Blood and Marrow Transplant Group reported that 1488 allogeneic PBPC transplants were reported between 1994 and 1998 by 152 teams, while in 1998, 26% of allogeneic transplants used PBPCs.[119]

Bensinger WI et al in their study found that randomized prospective studies have shown that haematopoietic recovery following transplantation of allogeneic PBPCs is more rapid than that of marrow.[120]

CHANGING SCENARIO

Present Scenario in the World [4]

Currently 55,000 to 60,000 Haematopoietic Stem Cell Transplants are performed worldwide. In 1970s, non-malignant diseases, like aplastic anemia (40%) and immune deficiencies (15%) were the major indications for HSCT. From 1985 to till date haematological malignancies (leukemia's 70%) are main indication for HSCT.

Aplastic anemia and immune deficiencies now account for 5% of allogenic transplants. Multiple myeloma is the most common (48%) indication for autologous transplants. Non-Hodgkin lymphoma (28%) and Hodgkin lymphoma (12%) is next common. Solid cancers account for 10% of all autologous stem cell transplants.

There are currently more than 1.5 million HLA-A,B and DR matched marrow donors registered in bone marrow donor registries worldwide,50% of the patients who require haematopoietic stem cell transplantation are still unable to find a suitable matched donor

Present Scenario in our Country

The data from six transplant centers in India were collected and it was found that a total of 1540 allogenic and autologous bone marrow

transplantations (BMT) have been performed in a country of over one billion population. Presently, more than 40,000 stem cell transplantations are being performed annually worldwide. In India, the transplantation procedure is being practiced in the larger transplant centers, located mostly in western, northern and southern India. [121]

In India, Haematopoietic Stem Cell Transplant Centre's are

- AIIMS, New Delhi
- Christian Medical College, Vellore
- Cancer Institute (WIA), Chennai
- Tata Memorial Hospital, Mumbai
- Apollo Cancer Hospital, Chennai
- SGPGI, Luknow
- Army Hospital R&R New Delhi
- Apollo Hospital, Hyderabad
- Rajiv Gandhi Cancer Institute New Delhi
- Command Hospital (SC), Pune
- Cancer Care Trust Hospital, Indore
- PGIMER,Chandigarh.
- Apollo Hospital, Hyderabad

Types of Haematopoietic Stem Cell Transplantation (HSCT)

Before the administration of the HPC product, the recipient is "conditioned." In patients with neoplastic diseases in both the allogeneic and autologous settings, the purpose of the conditioning regimen is to reduce the tumor burden. In the allogeneic setting, the conditioning regimen also creates "space" for the transplanted cells via myeloablation and suppresses the patient's immune system to allow for engraftment of the donor cells.[122]

Recently, conditioning regimens have been classified as[123]

1) Myeloablative,
2) Reduced intensity,
3) Nonmyeloablative.

Haematopoietic stem/progenitor cell transplants fall into one of three categories[4]

1. Autologous (Self)
2. Allogeneic (HLA-matched related or unrelated)
3. Syngeneic (Identical sibling)

Autologous

Intravenous infusion of patient own haematopoietic stem cells to rescue patient bone marrow due to severe bone marrow injury caused by high dose chemotherapy or radiotherapy as a part of treatment for the disease.

Allogenic

Infusion of haematopoietic stem cells from related or unrelated donor depending on the donor further classified as follows.

HLA Matched Related Sibling

Intravenous infusion of haematopoietic stem cells obtained from siblings. Every individual has a 33% chance of having a matched sibling donor.

HLA Matched Unrelated Donor

With the availability of bone marrow donor registries worldwide, it is now possible to find HLA Matched unrelated donors from these registries. It takes approximately 3 to 6 months with the availability of high resolution HLA match and better supportive care HLA Matched unrelated donors transplants now account for about half of all allogenic transplants worldwide.

Syngeneic

The transplant obtained from syngeneic twins which have the advantage of least complication due to complete HLA match.

Haploidentical Transplants

Donors share one haplotype with the recipient and mismatched for one or more antigens on the unshared haplotype with partial success. However this technique requires T-cell depleted grafts to reduce the possibility of life threatening GVHD.

Indications for Haematopoietic Transplantation[116]

Malignant diseases are the most common indication. Newer transplant trends include use in inborn disorders of metabolism, sickle cell disease, beta thalassemia major and autoimmune disorders. The success rate

of HPC transplantation depends on the disease of the patient being treated; the stage of the disease; the degree of prior treatment; the age and condition of the patient; and, in the case of allogeneic transplantation, the degree of HLA match between the donor and the patient.

Indications for Haematopoietic Stem Cell Transplantation

Malignant or Clonal Disease of the Marrow
Acute leukemia
Chronic myelogenous leukemia
Hodgkin & nonHodgkin lymphoma
Myelodysplatic syndromes
Myeloproliferative disorders
Multiple myeloma
Chronic lymphocytic leukemia

Hemoglobinopathies
Thalassemia
Sickle cell disease

Congenital Immune Deficiencies
SCID
Wiskott Aldrich syndrome
Marrow failure syndromes
Severe aplastic anemia
Fanconi anemia
Congenital hypoplastic anemia

Solid Tumours
Breast cancer
Ovarian cancer
Renal cell carcinoma
Testicular cancer

Childhood Solid Tumours
Wilm tumor
Neuroblastoma
Rhabdomyosarcoma
Ewing sarcoma
High-grade gliomas

Inborn Errors of Metabolism
Mucopolysaccharidoses
Leukodystrophies
Glycoprotein disorders
Lysosomal storage disorders
Osteopetrosis
Osteogenesis imperfect

PBSC ENGRAFTMENT

The speed of engraftment is measured by the number of days after infusion of the graftuntil a defined threshold of circulating neutrophil s (polymorphonuclear cells, or PMNs) or platelets is reached, typically the first of 3 days for PMNs >500/μL and platelets >20,000/μL, without transfusion.[188,124]

FACTORS REPORTED TO AFFECT THE RATE OF NEUTROPHIL ANDPLATELET ENGRAFTMENT

Infused dose of viable CD34+ cells/kg
Use of posttransplant growth factor
Type of pretransplant preparative regimen
Ease of mobilizing the donor
Extent and type of prior chemotherapy or radiotherapy
Patient or donor age

Graft Source and Engraftment

However, according to the results of large scale retrospective comparisons of unrelated donor marrow transplantation using HPC(A), HPC(M), or HPC(C), the potential advantages of HPC(A) or HPC(M) include more rapid engraftment, fewer graft failures, and an ability to obtain

additional cells if they are needed because of poor recipient engraftment or disease relapse.[125]

Visani G et al in their study found that, as a result of the administration of higher progenitor cell doses, autologous transplantation using HPC (A) grafts is associated with shorter times to neutrophil and platelet engraftment than HPC (M) grafts with fewer transfusions, fewer infections, shorter hospital stays and, in some studies, lower overall costs.[126]

Talmadge JE et al in their study found that immunologic recovery is also more rapid using HPC (A) when compared with HPC (M).[127]

Bensinger WI et al and Ringden O et al stated that related allogeneic donor transplantation in adults who received myeloablative conditioning also show that HPC (A) grafts are associated with shortened time to neutrophil and platelet engraftment with fewer transfusions, fewer infections, and shorter hospital stays when compared with HPC (M).[128,129]

Storek J et al concluded that recovery of immune cells and function are both more rapid after HPC (A) transplantation compared with HPC (M), both after related and matched unrelated allogeneic transplantation.[130]

Dey BR et al conducted a randomized clinical trial in adults to determine the advantages and disadvantages of both sources of HPC grafts for allogeneic transplantation in different diseases. Initial reports suggest that the advantages of rapid engraftment speed and disadvantages of chronic GVHD appear to apply as well to unrelated donor or nonmyeloablative conditioning using HPC (A) versus HPC (M) grafts according to observational studies.[131]

Following an evidence based review by executive committee, American Society for Blood and Marrow Transplantation, an expert panel recommended the preferential use of HPC (M) for matched related donor transplantation in paediatric patients who have AML and are in first remission, but no recommendation was made for unrelated- donor marrow transplantation.[132]

Richard K.Shaddu et al in their study found that in the autologous setting patients receiving apheresis derived PBSC transplants typically have faster neutrophil and platelet recoveries than bone marrow derived transplant and need fewer red cell and platelet transfusions. [133]

William I. Bensinger et al in their study reported that patients had rapid engraftment of both granulocytes and platelets after allogenic PBPC transplant and platelet engraftment was more rapid than bone marrow derived transplant.[134]

Martin Korbling et al in their study concluded that the use of PBPCs for transplantation represents a major advance. The temporary peripheralization of haematopoietic progenitor cells allows collection of large doses of progenitors and has a significant advantage for the donor including avoiding the need for general anesthesia and multiple BM aspiration. [135]

PBPC transplantation provides rapid hematologic recovery and in most studies has reduced hospitalization and costs. PBPCs are as effective as BM for autologous transplantation for the same indications as autologous BM transplantation has been proven efficacious. Allogeneic PBPC transplants are promising and do not appear to increase the risk of acute GVHD in preliminary studies.[135]

CD34+ Cell Dose and Engraftment

Attilio Oliveri et al in their study found that CD 34 cell dose is the only significant factor that affects neutrophil and platelet engraftment in haematological malignant patients who received G-CSF mobilised autologous pbsc after high dose chemotherapy.

In their study they concluded that optimal dose for rapid and complete engraftment ranges between 5 and 7.8×10^6 /kg $CD34^+$ cell dose and a graft size ranging from 2.5 to 4.9×10^6 /kg $CD34^+$ cell dose is safe for

complete stable and rapid neutrophil engraftment but not for rapid platelet engraftment and $<2.5\times10^6$ /kg $CD34^+$ cell dose is still optimal for neutrophil engraftment but not platelet engraftment and $>7.8\times10^6$ /kg $CD34^+$ cell dose is not advantageous.[136]

J.A.Perz-Simon et al in their study found that the time to engraftment significantly influenced by the number of CD34+ cell dose infused and they concluded that dose of 0.75×10^6 CD34+ cells per kg is sufficient for engraftment in all patients receiving autologous PBPC transplant.[69]

Similarly Bensinger et al also in their study found that the CD 34 cell dose is the only significant factor that affects neutrophil and platelet engraftment.[137,138]

Similarly H.E.Johnsen et al in their study found that the CD 34 cell dose is the only significant factor that affects time to neutrophil and platelet engraftment and they concluded that the quality assessment of autografts have fulfilled by enumerating CD34+ cell subset with flow cytometry.[139]

Similarly J.Reiffer et al also in their study found that the dose infused is the only significant factor that affects neutrophil and platelet engraftment.[140]

Similarly S Heimfeld et al also in their study found that the dose of CD34+ cells infused is the only significant factor that affects neutrophil and platelet engraftment.[141]

Bensinger W et al in their study found that CD34+ cell content was considered predictive of engraftment kinetics for neutrophil s and platelets. The minimum number of CD34+ cells required for rapid engraftment is also depend on the clinical setting, it may be twofold higher for heavily pre-treated patients.[87]

Civin CI, Strauss LC, Brovall C, et al found that in allogeneic transplantation, complete and sustained engraftment of neutrophil and platelets has been occurred after 2.2to2.5 × 10^6/kg CD34+ cell dose infusion.[142]

Rusten LS, Lyman SD, Veiby OP et al found that in case of autologous PBPC transplantation neutrophil and platelet engraftment occurred for all patients with advanced-stage or poor-prognosis malignant lymphoma who received >2.5 × 10^6 CD34+ cells/kg in patients and neutrophil engraftment took >2 weeks and platelet counts of 20,000/μL were not achieved until a median of 31 days in patients receiving fewer <2.5 × 10^6 CD34+ cells.[143]

Berenson RJ, Andrews RG, Bensinger WI, et al found that the influence of the CD34+ cell dose was so strong that the collection protocol was changed to specify a CD34+ cell dose of 5×10^6/kg rather than 5×10^8 MNC/kg.[144]

Bender JG et al and Tricot G et al stated that higher administered doses of CD34+ cells increase the speed of engraftment for both neutrophils and platelets, especially in heavily pretreated patients.[129, 130] Administration of $>5 \times 10^6$ viable CD34+ cells/kg is generally not advantageous, and administration of $<2\times10^6$ CD34+ cells/kg may compromise both the probability and speed of engraftment. [145-147]

Autologous PBSC Transplantation

Richman CM stated that significant increase in circulating progenitors 2 to 3 weeks after administration of myelosuppressive chemotherapy was reported in 1976.[148]

Juttner CA et al stated that these chemotherapy mobilized cells were collected, cryopreserved and reinfused following high-dose therapy. Hematopoiesis was restored in the patients, demonstrating that chemotherapy-mobilized stem and progenitor cells could provide a successful autograftproduct.[149]

Beyer J et al stated that from 1986 to 1991, autologous PBPC graft products were collected either in the steady state or after chemotherapy induced mobilization. Since chemotherapy induced mobilization increased the number of stem and progenitor cells in the blood, fewer apheresis procedures were required to collect a satisfactory graft product. In addition mobilized cells provide faster haematopoietic engraftment followingtransplantation.[150]

Allogeneic PBSC Transplantation

J.Szer et al in their study found that allogenic PBPC from HLA identical siblings speed the engraftment of neutrophil s and platelets without detrimental effects on GVHD or survival.[151]

Lee SH and Heimfeld.S et al stated that in allogeneic transplantation, CD34+ cell dose was also associated with decreased transplant-related mortality and relapse, as well as with improved overall patient survival.[152,153]

Pulsipher.MA et al and Heimfeld.S et al stated that however, because of the generally longer time to neutrophil engraftment and especially to platelet engraftment associated with allogeneic transplantation and with unrelated donors in comparison with autologous patients, a higher HPC(A) dose (at least 4×10^6 CD34+ cells/kg) is preferred.[152,154]

Administration of higher doses of CD34+ cells from related or unrelated, fully or partially matched donors that are given to adults or children is consistently associated with more rapid neutrophil and platelet engraftment. [155]

Heimfeld S et al and Zaucha JM et al stated that however, survival benefits are controversial because studies of adult or pediatric patients who received allogeneic HPC (A) transplants from sibling donors for ablative therapy show that very high CD34+ doses (>8× 10^6/kg) were associated with increased morbidity resulting from chronic GVHD, a common cause of severe morbidity and mortality after allogeneic transplantation and with no improvement in survival.[154, 156]

Pulsipher MA et al and Perez-Simon JA et al stated that however, graft selection and CD34+ cell dose should be individualized, because reports also indicate that increased CD34+ cell doses are associated with more rapid full-donor chimerism and may improve survival in high-risk leukemia patients who undergo nonmyeloablative regimens using HPC (A) from HLA-identical sibling donors or in standard to high risk patients after ablative or nonablative marrow transplantation using matched unrelated donors.[152,157]

Autologous Vs. Allogeneic

Thissiane et al in their study found that faster neutrophil and platelet engraftment was achieved in autologous PBSCT than allogeneic PBSCT.[158]

Disease Type

Thissaiane et al in their study on autologous and allogeneic PBSCT in 65 patients with hematological malignancies revealed multiple myeloma patients achieve rapid neutrophil and platelet engraftment than lymphoma and leukemia.[158]

M. J. Watts et al in their study on 20 patients who underwent autologous PBSCT found that multiple myeloma patients had earlier platelet engraftment than lymphoma patients.[159]

J. Reiffers et al in their study on 118 patients with hematological malignancies found AML patients achieved delayed engraftment than other malignancy.[161]

Shirong Wang et al in their study found that in each diagnostic subgroup, a stronger correlation between CD34+ cell dose and engraftment was seen in patients with MM and NHL. Patient sex was not a confounding factor in whole population analysis, except there were minor differences in

some diagnostic groups in the relationship between CD34+ cell dose and engraftment speed; that is, female AML patients engrafted much faster when they received a higher dose of CD34+ cells, while this was true for male patients with NHL. Patient age at transplantation did not confound the results. [162]

Conditioning Regimen

J.Reiffers et al in their study found that busalphan and total body irradiation conditioning regimens slower the engraftment.[160]

Mauricette Michellet et al in their study on pretransplantation and posttransplantaion factors and their impact on outcome found that the conditioning regimen had significant influence on engraftment.

Peter Dreger et al in their study on autologous progenitor cell transplantation found that BCNU and melphalan conditioning regimens had adversely affect PBCS graft performance engraftment[164]

Charles H. Weaver et al in their study on autologous HSCT found that patients receiving high dose cyclophosphamide, thiotepa and carboplatin regimens had more rapid platelet engraftment than patients receiving other regimens. Patients requiring two mobilization procedures to achieve $\geq 2.5\times10^{6}$ CD 34 + cells /kg experienced slower platelet engraftment.[191]

Bensinger WI et al and Dreger P et al stated that apart from CD34+ dose, additional factors that have been reported to affect engraftment after autologous transplantation include the type and extent of previous myelotoxic therapy (engraftment is slower after total body irradiation (TBI) or busulfan) and whether the patient was a poor HPC mobiliser [166,167]

Thissiane et al in their study, found that CBV, melphalan (M200) and Flu Cy conditioning regimens show faster engraftment than others and Cy TBI and Bu Cy achieve delayed engraftment than others.[158]

HLA Match

J. Szer et al in their study found that allogeneic PBPC from HLA-identical siblings speed the engraftment of neutrophil s and platelets without detrimental effects on GVHD or survival.[151]

Pulsipher M A et al in their study found that administration of higher doses of CD34+ cells from related or unrelated, fully or partially matched donors that are given to adults or children is consistently associated with more rapid neutrophil and platelet engraftment. [155,168]

Schulenburg A et al and Storek J et al stated that Immunologic recovery may be prolonged for up to a year, especially when the donor is unrelated, and prolonged immunologic recovery is associated with increased infections.[130,169]

Blood Group Match

G Stussi et al. in their study on 562 patients with allogeneic haematopoietic stem cell transplantation found that ABO incompatibility has no influence on neutrophil and platelet engraftment and that only RBC engraftment was delayed (particularly in major ABO incompatibility)they also suggest that ABO incompatibility does not seem to affect the outcome in most patients of stem cell transplant.[170]

Previous Radiotherapy and Chemotherapy

Peter Dreger et al in their study on autologous progenitor cell transplantation found that previous chemotherapy and radiotherapy had no influence on engraftment.[164]

Ross AA et al in their study found that the extent of prior chemotherapy may also have an effect on engraftment kinetics. This was seen in a large trial of double stem cell transplantation for multiple myeloma. In addition, even 6 months of treatment with alkylating agents like methotrexate, significantly delayed engraftment in extensively pretreated patients, the CD34+ cell yield required for rapid platelet engraftment increased from 2×10^6/kg to 5.0 × 106/kg.[171]

Age and Engraftment

Similarly Thissiane et al revealed that PBSC engraftment was more rapid in the 50-59 year age group and they found this finding could be due to the fact that associated favorable factors like autologous source of PBSC and received melphalan 200 and CBV conditioning regimen which were also associated with faster engraftment.[158]

Shaji K. Kumar et al in their study found that the proportion of patients attaining WBC engraftment (as indicated by an ANC >500 for three consecutive days) by day 15 was 94% for the elderly group (≥70years) compared to 78% for the control group(≤65) Similarly, the proportion of patients achieving a non-transfused platelet count of over 50,000 by day 30 was similar for both groups (81% and 80% respectively) and they concluded that chronologic age alone should not be used to decide on transplant eligibility.[172]

DMSO Depletion

Cigdem A. Akkok et al in their study concluded that simple single wash DMSO depletion causes significant CD34+ cell loss and delayed platelet engraftment and increased platelet transfusion requirement. They suggest this procedure should be recommended for those patients with increased risk of toxicity.[173]

Sex

Thissiane et al their study found that male gender achieved faster neutrophil and platelet engraftment than female gender.[158]

Number of Mobilization

Fraipont.V in their study found that autologous PBPC obtained from 2nd mobilisation with chemotherapy plus G-CSF after first failed mobilisation with chemotherapy plus G-CSF delays engraftment.[174]

Second Apheresis Yield (Graft) from Poor Mobilisers and Engraftment

M. J. Watts, al. in their study on previously treated patient's conclude that poor mobilizers on second attempt yield adequate cells to enable high dose therapy and prompt engraftment and remobilisation is worth among these patients.[159]

Large Volume Leukapheresis vs. Normal Volume Leukapheresis

J.F.Abrahamsen et al. in their study showed that LVL leukapheresis yields more CD34+ cells particularly in poor mobilizers and faster engraftment of platelets (10 days for LVL and 11 days for NVL) and neutrophil engraftment is 10 days for both LVL and NVL. [175]

Pettengal et al in their study showed that long term engraftment achieved from single LVL apheresis in an attempt to reduce number of apheresis procedure.[176]

Collection and Engraftment

Schwella et a1 in their study reported that when the number of circulating CD34+ cells is greater than 40 per µl, more than 2.5 x 10^6 CD34+ cells per kg can be collected, resulting in a rapid haematopoieticengraftment.[200,69]

Good and Poor Mobilisers and Engraftment

Liuyan Jiang et al in their study concluded that haematopoietic stem cell source from good and poor mobilisers show similar time to engraftment particularly neutrophil engraftment which is a most sensitive predictor for long term engraftment.[177]

Similarly Shirong Wang et al in their study concluded that poor mobilizers can still benefit from autologous haematopoietic stem cell transplantation and the efficacy of haematopoietic stem cell mobilization and collection, defined as number of days to reach a CD34+ cell dose of 2 × 10^6 per kg, and should not be used independently to estimate posttransplantaion engraftment.[162]

Density-Enriched Peripheral Blood CD34+ Cells

Thai M.Cao et al. in their study found that allogeneic transplantation with density enriched PBPC CD34+ graft resulted in rapid haematopoietic engraftment with no incidence of graft failure in patients with advanced haematological malignancy.

Neutrophil engraftment was rapid with median time to ANC greater than 0.5×10^9/L taking place at 10.5 days (range, 8–18 days). The median time to a platelet count greater than 20×10^9/L was 13 days. [178]

Positively CD34+ Cell Selection

Wichard Vogel et al in their study concluded that in both allogenic and autologous PBPC transplantation setting the time to engraftment is not different between positively selected CD34+ cells and unmanipulated CD34+ cells.[179]

Ex Vivo Expansion

Wichard Vogel et al in their study found that ex-vivo expansion shorter the PBSC engraftment time. [179]

T cell Depletion

William I. Bensinger et al in their study reported that the addition of T cell-depleted PBSC assisted the establishment of allogeneic engraftment.[168]

G-CSF Dose

Marc Andrew et al in their study concluded that the engraftment data of 5 and 10 µg per kg filgrastim mobilised groups (group A 5 µg per kg, group B 10 µg per kg) produce similar engraftment time(8 days for neutrophil engraftment and 9 days for platelet engraftment for both group A and B).[180]

Plerixafor

Nina Worel et al, in their study concluded that plerixafor in combination with G-CSF is an effective and well-tolerated mobilization regimen in patients with previous mobilization failure. Importantly, patients mobilized with plerixafor presenting with low PB CD34+ cell counts on the first day of apheresis show more efficient stem cell collections than patients mobilized without plerixafor. The median dose of PBSC graft infused was 2.93×10^6 CD34+ cells/kg recipient body weight. The median numbers of days to neutrophil and platelet engraftment were 12 and 15day, respectively.[181]

Freezing and Storage Procedure

Virginia Fisher et al in their study found that G-CSF mobilised PBSC that have been collected from unrelated donors can be stored, transported and cryopreserved without a significant loss of CD34+ cells. Nevertheless in marked difference, under the same conditions, there is a significant reduction in viable CD34+ cells cause delayed PBSC engraftment.[182,183]

Pegfilgrastim (G-CSF) Vs. Filgrastim (G-CSF)

Simone Cesaro et al in their study found that a single dose of 100 mg/kg pegfilgrastim can be used successfully for PBSC collection, and the resulting autografts have an overall morbidity and rate of neutrophil and platelet engraftment similar to that observed in autografts performed with filgrastim-stimulated PBSC.[184]

Preceding Chemotherapy, Tumour Load and Age Influence Engraftment in Multiple Myeloma Patients Mobilized With Granulocyte Colony-Stimulating Factor Alone.

K. R. Desikan et al in their study on multiple myeloma patients concluded that disease status, previous therapy with alkylating agents had minimal effect on engraftment and tumour load had significant impact on engraftment (delay the speed of engraftment). They also found the number

of CD34+ cells/kg infused remains an important determinant of rapidity of engraftment irrespective of mobilization regimens used.[185]

Cell Viability and Engraftment

S. Lee et al in their study concluded that quantification of post-thaw viable CD34+ cells better represents the actual composition of the graft and may be a more accurate predictor of haematopoietic engraftment than post-thaw total CD34+ cell counts, or prefreeze determinations, especially for platelet engraftment. It is necessary to develop good quality controls for freezing and thawing procedures to minimize variance in cell viability.[186]

Post Transplant G-CSF and GVHD Prophylaxis Use.

A Urbano-Ispizua et al in their study on 33 patients with allogenic PBSCT found that the speed of neutrophil engraftment strongly influenced by the use of rhG-CSF post transplant and marginally by the type of GVHD prophylaxis which contain MTX (Methotrexate). [187]

MATERIALS AND METHODS

The study was conducted in the Department of Transfusion Medicine, The Tamilnadu Dr.M.G.R. Medical University Guindy, in collaboration with the Cancer Institute Adyar (W.I.A), Chennai.

In our study between June 2013 and August 2014, the factors influencing the engraftment of haematopoietic stem cell transplantation for patients with haematological malignancies were analyzed based on the neutrophil and platelet engraftment criteria.

As per AABB[188], the speed of engraftment is measured by the number of days after infusion of the graft until a defined threshold of circulating neutrophils (polymorphonuclear cells or PMNs) or platelets is reached, typically the first of three days for PMNs >500/µL and platelets >20,000/µL, without transfusion.

Accordingly, the following factors were analyzed:

1. Patient Related Factors
    - Patient Age Group
    - Patient Sex
    - Diagnosis
2. Donor Related Factors
    - HLA match
    - PBSCT Type (Autologous/Allogeneic)
    - ABO Blood Group Match

3. Treatment Related Factors
    - Previous Chemotherapy
    - Previous Radiotherapy
    - Conditioning Regimen (Reduced intensity/Myeloablative)

4. Graft Related
    - CD 34+ cell Dose Infused (Per kg recipient body weight)
    - CD34+ cell Viability

The following inclusion and exclusion criteria were applied

Inclusion criteria

All patients with haematological malignancy undergoing haematopoietic stem cell transplantation with written consent.

Exclusion Criteria

Patients undergoing haematopoietic stem cell transplantation for disorders other than haematological malignancy.

<u>Details of PBSCT Procedure Followed in Our Study Centre</u>

1. **Mobilization**
2. **Collection and Cryopreservation**
3. **Enumeration of Haematopoietic Stem Cells**
4. **Pretransplant match for allogenic PBSCT**
5. **Patient preparation and PBSCT**
6. **Post Transplant Care**

1. Mobilization

i) Autologous Donor

ii) Allogenic Donor

Autologous Donor

After completing chemotherapy and referral from the treating physician along with informed consent, autologous donors (patients) were mobilised with either G-CSF alone or G-CSF with plerixafor (CXCR4 competitive inhibitor).

For donors with G-CSF alone, 10µg/kg body weight/day subcutaneous injection of G-CSF for four days was given and stem cell collections by apheresis were started on fifth day.

For donors with G-CSF with plerixafor, G-CSF 10µg/kg body weight was given for four days, followed by a single dose of plerixafor 0.24mg/kg body weight subcutaneously, stem cell collection by apheresis were started after approximately 11 hours of plerixafor injection.[10]

Allogeneic Donor

After obtaining fitness opinion from the physician along with informed consent from the allogeneic donors, the same mobilization regimen used for autologous donors were followed.

2. Collection and Cryopreservation

Apheresis were carried out using an automatic blood cell separator Haemonetics MCS +LN 9000-220 E intermittent type stem cell collection device, after establishing peripheral vein/central vein access line under aseptic precaution, with 80 mL/min draw speed, 50ml/min recirculation speed, 30ml/min collect speed with ACD anticoagulant in the ratio 1:9 to 1:12.

The final product collected in the stem cell bag were either stored at 4^0C for immediate use within 48 hours or for long term storage they were cryopreserved in DMSO (7.5 % Final concentration) with a controlled-rate freezing device and stored at -70^0C in the mechanical freezer.

3. Enumeration of HSC by ISHAG protocol[189]

Under aseptic precaution 3ml of stem cell product from the stem cell bags were collected in to the sterile disposable 5 ml syringe with the help of sterile connecting device and aliquoted in to 1ml each in to three test tubes after proper labeling, for the following tests to measure the graft size by using flowcytometry:

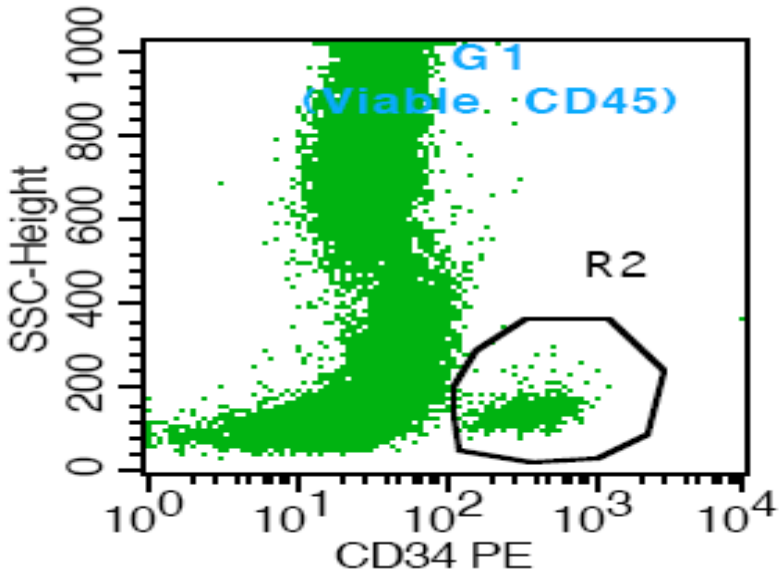
i. Total Nucleated Cell count
ii. Absolute Mono Nuclear Cell count/CD 34+ cell count
iii. CD34+ cell viability

The harvest size was calculated after each leukapheresis by total nucleated cell count, MNC count, CD34+ cell viability and cytofluorimetric count of CD34+ cells. MNC were identified by basophil channel H*1-Technicon coulter. The CD34+ cells were counted using a Becton-Dickinson FACScan after erythrocyte lysis (EDTA-ammonium- chloride solution) and direct incubation for 30 minutes with phycoerythrin (PhE) conjugated monoclonal anti-CD34+ antibody.

CD34+ cell counts were performed both in open gate, considering the whole cell population, and by choosing a proper lymphomonocyte population as follows: the entire population was gated using double staining with anti-CD45/14 PhEFITC conjugated antibodies; in this way only those cells with lymphomonocyte scatter characteristics were acquired, and the double staining with anti- CD34/14 PhE-FITC conjugated antibodies ruled out non-specific binding by the monocytes. (Fig.2)

A negative control for non specific fluorescence as used with unstained cells; the minimal number of events acquired for each determination was 20,000 and the entire procedure was performed at 4 °C. In all cases only the CD34+ cell count performed in the lymphomonocyte gate has been considered for data analysis.

(Fig.2) Viable CD34+ cell event in flowcytometry



Absolute CD34+ cell count which is expressed by number of cells per microliter is converted in to CD 34+ cell dose by following formula,

$$\text{CD 34+ cell dose} = \frac{\text{CD 34+ cells/µL} \times \text{Volume of the stem cell (ml)}}{\text{Recipient body weight (Kg)}}$$

4. Pretransplant match for allogenic PBSCT

In allogenic PBSCT setting donor and patient were evaluated for viral status (CMV IgG, CMV IgM, HSV IgG, HSV IgM, Hepatitis BsAg, Hepatitis BcAb, HCV, HIV, and VDRL. Patient and donor HLA match done by either high resolution HLA matching (10/10) or low resolution HLA matching (6/6), ABO blood group by serology.

5. Patient Preparation and Peripheral Blood Stem Cell Transplantation

Prophylactic drug regimens were started on day -7, depending on the diagnosis, all patients myeloablated by giving appropriate conditioning regimens started on day -3, after completion of conditioning regimen, PBSC were infused on day 0 with steroid cover.

Conditioning regimens included, fractioned total body irradiation (TBI 1200 cGy) with cyclophosphamide 60mg/kg, melphalan 200mg/m^2, busalphan 1mg/kg with cyclophosphamide 60mg/kg in, lomustine 200mg/m^2, ARA-C 2000mg/m^2 cyclophosphamide1800mg/m^2, etoposide1000mg/m^2 (LACE) in 6, Cyclophosphamide 750mg/ m^2 and carmustin 112.5mg/ m^2 (BCNU) with etoposide200mg/ m^2 (VP-16) in 1 patient.

6. Post Transplant Care

During the aplastic phase all patients were kept in a positive pressure HEPA filtered room and received antimicrobial prophylaxis with ciprofloxacin 1000mg/day and fluconazole 100 mg/day orally and acycloguanosine15 mg/kg/day intravenously.

All patients received G-CSF 5 µg/kg/day until WBCcount reached 0.5×10^9/µL starting after Peripheral Blood Stem Cell Transplantation,

irradiated blood products were given in order to maintain the hemoglobin and platelet levels over 8 g/dL and $20{,}000\times10^{9}$/μL, respectively.

The values (speed of engraftment) obtained for individual factors will be analyzed based on median and range.

RESULTS

A total of 36 patients undergoing PBSCT procedure were prospectively observed during the study period. Out of 36 patients 5 patients was not received peripheral blood stem cell transplantation due to inadequate yield of peripheral blood stem cells by apheresis, in the remaining 31 patient's one patient died before engraftment.

In the present study on factors influencing peripheral blood stem cell transplantation in 30 patients with hematological malignancies, the speed of engraftment was assessed by the first appearance and persistence of neutrophils of >500cells/µL and platelets of >20,000/µL respectively, without transfusion for three consecutive days.

The speed of PBSC engraftment based on neutrophil and Platelet appearance in 30 patients of hematological malignancies was analyzed based on the median values obtained for each of the factors studied.

In our study, the range observed was between 4 and 21 days for neutrophil engraftment. The range observed for platelet engraftment was between 10 and 46. In the remaining 1 case, which was diagnosed as CLL, the patient died due to multi-organ failure on the 11^{th} day post PBSC infusion, neutrophils did not appear till then.

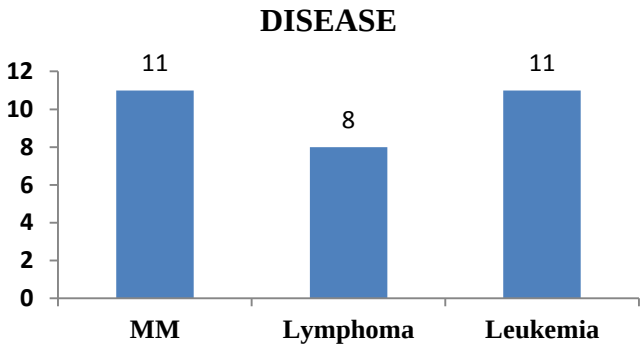
The data obtained for various factors analyzed for the speed of PBSC engraftment are as follows:

1. **DISEASE**

Frequency Distribution of Disease

Table.1

Diagnosis	Frequency	Percent
MM	11	36.7
Lymphoma	8	26.7
Leukemia	11	36.7
Total	30	100.0



Disease and PBSC Engraftment

Table.2

Diagnosis	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
Multiple Myeloma	10 (4-12)*	13 (11-19)
Lymphoma (HL,NHL)	11 (13-46)	16.5 (13-46)
Leukemia (ALL,AML,CML)	14 (12-21)	25 (10-30)

*Range

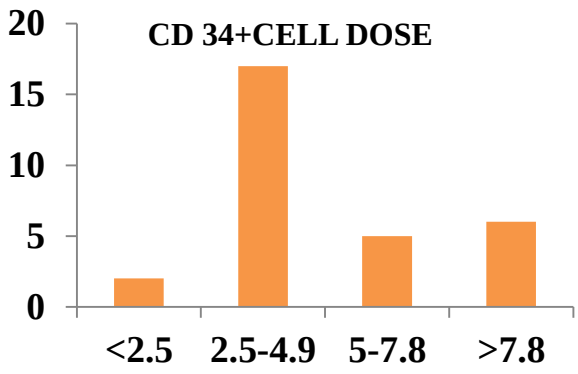
- In our study among hematological malignancies, MM, lymphoma and leukemia patients achieve neutrophil engraftment in median on day10, 11 and 14 respectively
- Platelet engraftment occurred in median on day13, 16.5 and 25 respectively. (Table.1,2)

2. CD 34+CELL DOSE

Frequency Distribution of CD34+ Cell Dose

Table.3

CD34+ Cell Dose× 10^6/Kg	Frequency	Percent
<2.5	2	7.0
2.5-4.9	17	56.0
5-7.8	5	17.0
>7.8	6	20.0
Total	30	100.0



CD34+ Cell Dose and PBSC Engraftment

Table.4

CD 34+cell dose/Kg Recipient Body Weight	Neutrophil Engraftment in Median on Day	Platelet Engraftment in Median on Day
< 2.5 × 10^6	18.5 (16&21)*	24.5 (19& 30)
2.5 – 4.9 × 10^6	11 (4-13)	15 (11-46)
5.00 – 7.8 × 10^6	11 (10-14)	13 (12-19)
> 7.8× 10^6	14.5 (12-15)	17 (10-23)

*Range

- In our study patients who received < 2.5 × 10^6 CD 34+cell dose/Kg recipient body weight were achieved neutrophil and platelet engraftment in median on day 18.5 and 24.5 respectively.

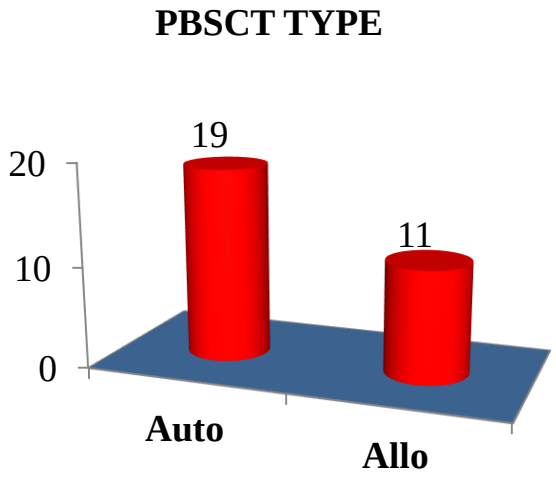
- Patients who received CD 34+cell dose between 2.5 and 4.9 × 10^6 cells/Kg were achieved neutrophil and platelet engraftment in median on day 11 and 15 respectively.
- Patients who received CD 34+cell dose between 5 and 7.8 × 10^6 cells/Kg were achieved neutrophil and platelet engraftment in median on day 11 and 13 respectively.
- Patients who received CD 34+cell dose >7.8 × 10^6 cells/Kg were achieved neutrophil and platelet engraftment in median on day 14.5 and 17 respectively. (Table3,4)

3. AUTOLOGOUS VS. ALLOGENEIC

Frequency Distribution of PBSCT Type

Table.5

PBSCT Type	Frequency	Percent
Autologous	19	63.3
Allogenic	11	36.7
Total	30	100.0



PBSCT Type and Engraftment

Table.6

PBSCT Type	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
Autologous	11 (4-13)*	15 (11-46)
Allogenic	14 (11-21)	18 (10-30)

*Range

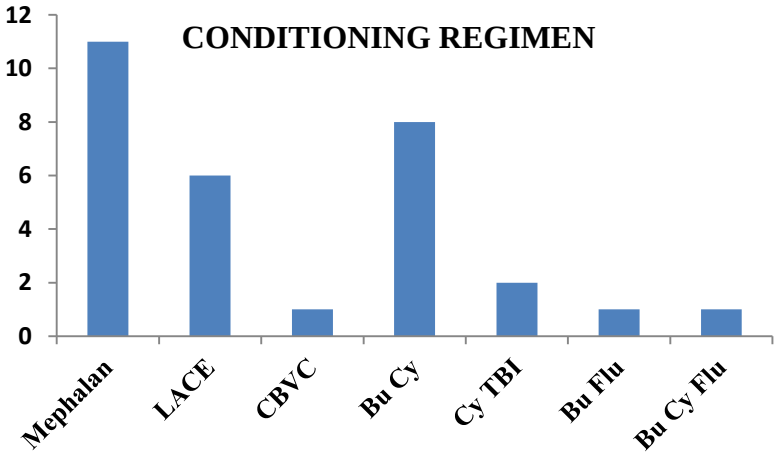
- In our study patients who received autologous PBSCT achieved neutrophil and platelet engraftment in median on day 11 and 15 respectively.
- Patients who received allogenic PBSCT were achieved neutrophil and platelet engraftment in median on day 14 and 18 respectively.(Table5,6)

4. CONDITIONING REGIMEN

Frequency Distribution of Conditioning Regimen

Table.7

Conditioning Regimen	Frequency	Percent
Melphalan	11	36.7
LACE	6	20.0
CBV	1	3.3
Bu Cy	8	26.7
Cy TBI	2	6.7
Bu Flu	1	3.3
Bu Cy Flu	1	3.3
Total	30	100.0



Conditioning Regimen and Engraftment

Table.8

Myeloablative Conditioning regimen	**Neutrophil Engraftment In Median on Day**	**Platelet Engraftment In Median on Day**
Melphalan (200 mg)	10 (4-12)*	13 (11-13)
LACE	10.5 (9-16)	17.5 (16-46)
CBV	11	13
Bu Cy	13.5 (11-16)	15 (13-23)
Cy-TBI	16.5 (12 & 21)	24 (18 & 30)
Reduced Intensity Conditioning regimen	**Neutrophil Engraftment In Median on Day**	**Platelet Engraftment In Median on Day**
Bu Flu	14 day	10 day
Bu Cy Flu	14 day	12 day

*Range

Myeloablative Conditioning regimen

- Patients who received melphalan (200mg) were achieved neutrophil and platelet engraftment in median on day 10 and 13 respectively.
- Patients who received LACE were achieved neutrophil and platelet engraftment in median on day 10.5 and 17.5 respectively, one patient with CBV were achieved on day 11 and 13, patients with Bu Cy achieved neutrophil and platelet engraftment in median on day 13.5 and 15 respectively.

- Patients who received Cy-TBI were achieved neutrophil and platelet engraftment in median on day 16.5 and 24 respectively. (Table.4)

Reduced intensity conditioning regimen

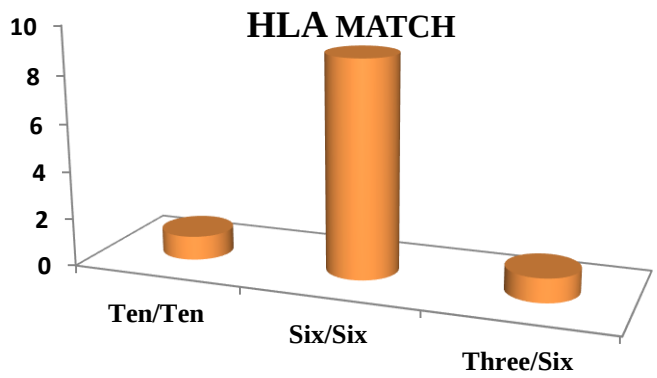
- Patients who received Bu Flu and Bu Cy Flu regimen were achieved neutrophil engraftment in median on day 14 and platelet engraftment in median on day 10 and 12 respectively. (Table.7,8)

5. HLA MATCH

Frequency Distribution of HLA Match

Table.9

HLA Match	Frequency	Percent
Ten/Ten	1	3.3
Six/Six	9	30.0
Three/Six	1	3.3
Total	30	100.0



HLA match and Engraftment

Table.10

HLA Match	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
10/10	21#	30#
6/6	14 (11-16)*	18 (10-23)
3/6	14#	12#

#Single patient with neutrophil and platelet engraftment day achieved *Range

Among patients who received allogeneic PBSCT (n=11), one patient with 10/10 HLA match achieved neutrophil engraftment on day 21 and platelet engraftment on day 30.

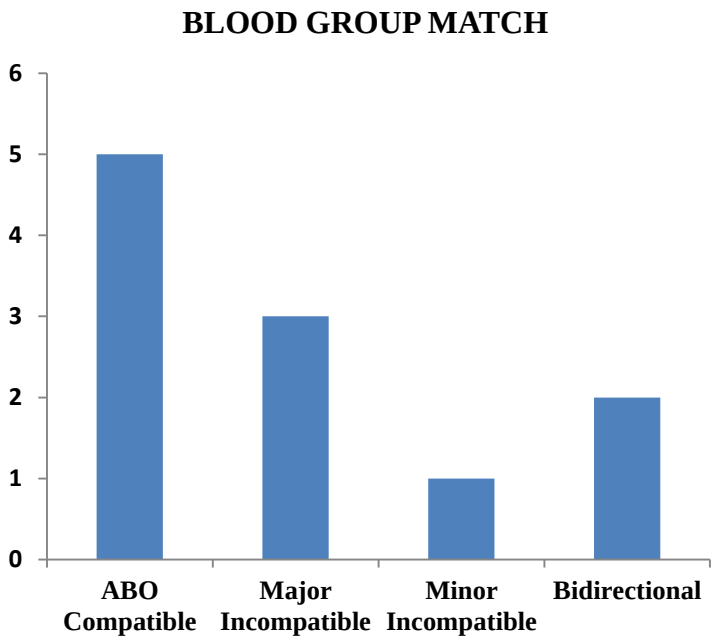
- Patients who received 6/6 HLA matched PBSCT achieved neutrophil and platelet engraftment in median on day 14 and 18 respectively.
- One patient who received 3/6 HLA matched PBSCT achieved neutrophil engraftment on day 14 and platelet engraftment on day 12.(Table.9,10)

6. BLOOD GROUP MATCH

Frequency Distribution of Blood Group Match

Table.11

Blood Group Match	Frequency	Percent
ABO Compatible	5	16.7
Major Incompatible	3	10.0
Minor Incompatible	1	3.3
Bidirectional	2	6.7
Total	30	100.0



Blood Group Match and Engraftment

Table.12

ABO Blood Group Match	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
ABO compatible	15 (12-21)*	19 (10-30)
Major Incompatible	14 (12-15)	18 (12-13)
Minor Incompatible	16	19
Bidirectional	13 (12& 14)	12.5 (12& 13)

*Range

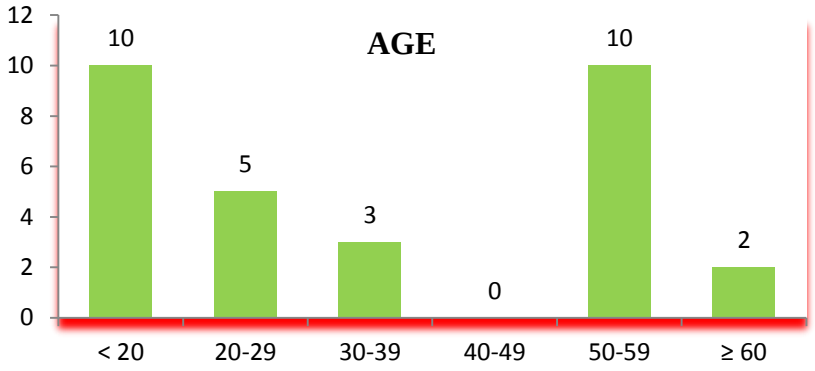
- Patients who received ABO compatible PBSCT achieved neutrophil and platelet engraftment in median on day 15 and 19 respectively.
- Patients who received major incompatible PBSCT achieved neutrophil and platelet engraftment in median on day 14 and 18 respectively.
- Patient who received minor incompatible PBSCT achieved neutrophil and platelet engraftment on day 16 and 19 respectively.
- Patients who received bi-directional PBSCT achieved neutrophil and platelet engraftment in median on day 13 and 12.5 respectively.(Table.11,12)

7. AGE

Frequency Distribution of Age Group

Table.13

Age Group (Years)	Frequency	Percent
< 20	10	33.3
20-29	5	16.7
30-39	3	10.0
40-49	0	.0
50-59	10	33.3
≥ 60	2	6.7
Total	30	100.0



Age and Engraftment

Table.14

Age in Years	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
<20	12 (10-16)*	15 (12-46)
20-29	13 (10-21)	17 (12-30)
30-39	14 (13-15)	15 (10-23)
40-49	-	-
50-59	10 (4-12)	13.5 (11-17)
>60	11	15.5 (12,19)

*Range

- In our study patients in the age group <20 years were achieved neutrophil and platelet engraftment in median on day 12 and 15 respectively.
- Patients in the age group 20-29 years were achieved neutrophil and platelet engraftment in median on day 13 and 17 respectively.

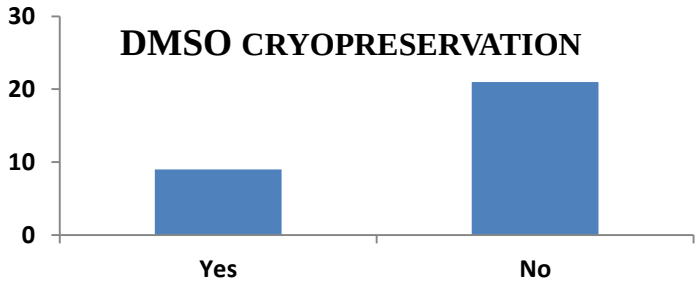
- Patients in the age group 30-39 years were achieved neutrophil and platelet engraftment in median on day 14 and 15 respectively.
- Patients in the age group 50-59 years were achieved neutrophil and platelet engraftment in median on day 10 and 13.5 respectively.
- Patients in the age group >60 years were achieved neutrophil and platelet engraftment in median on day 11 and 15.5 respectively.(Table.13,14)

8. DMSO CRYOPRESERVATION

Frequency Distribution of DMSO Cryopreservation

Table.15

DMSO CRYO PRESERVATION	Frequency	Percent
Yes	9	30.0
No	21	70.0
Total	30	100.0



DMSO Cryopreservation and Engraftment

Table.16

DMSO Cryo Preservation 7.5% Final Concentration	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
YES	11 (9-13)*	15 (11-46)
NO	12 (4-21)	15 (12-30)

*Range

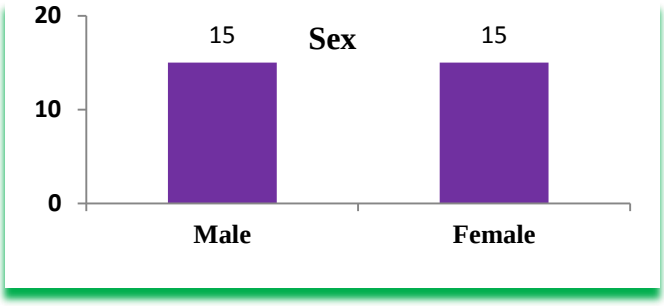
- Patients who received DMSO Cryo preserved and thawed PBSC graft infusion were achieved neutrophil and platelet engraftment in median on day 11 and 15 respectively.
- Patients who received fresh (<48 hrs) PBSC graft without DMSO Cryo preservation and thawing were achieved neutrophil and platelet engraftment in median on day 12 and 15 respectively.(Table.15,16)

9. SEX

Frequency Distribution of Sex

Table.17

SEX	Frequency	Percent
Male	15	50.0
Female	15	50.0
Total	30	100.0



Sex and Engraftment

Table.18

Sex	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
Male	12 (10-21)*	11 (4-16)
Female	27 (10-46)	15 (11-23)

*Range

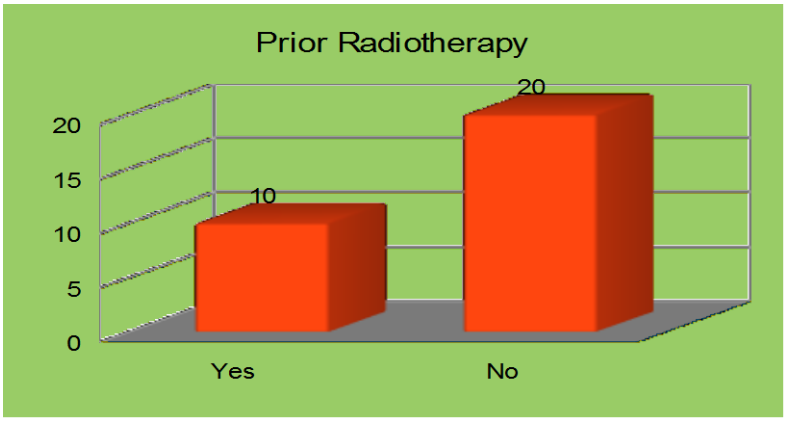
- In our study male gender achieved neutrophil and platelet engraftment in median on day 12 and 11 respectively.
- Female gender achieved neutrophil and platelet engraftment in median on day 27 and 15 respectively.(Table.17,18)

10.PREVIOUS RADIOTHERAPY

Frequency Distribution of Previous Radiotherapy

Table.19

Prior Radiotherapy	Frequency	Percent
Yes	10	33.0
No	20	67.0
Total	30	100.0



Previous Radiotherapy

Table.20

Prior Radiotherapy	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
Yes	10.5 (10-12)	13.5 (12-18)
No	12 (4-21)	15.5 (12-46)

*Range

- In our study 10 patients received prior radiotherapy dose ranging from 20-60 CGY was achieved neutrophil and platelet engraftment in median on day 10.5 and 13.5 respectively.

- 20 patients was achieved neutrophil and platelet engraftment in median on day 12 and 15.5 respectively without any prior radiotherapy. (Table.19,20)
- In our study due to large number of different drugs administered at different doses with various combinations we unable to group and find significant influence of chemotherapy regimen on PBSC engraftment.

DISCUSSION

1. DISEASE AND PBSC ENGRAFTMENT

In our study among hematological malignancies, MM patients achieve rapid neutrophil and platelet engraftment than lymphoma and leukemia.(Table.21)

Similarly Thissaiane et al in their study on autologous and allogeneic PBSCT in 65 patients with hematological malignancies revealed rapid engraftment of neutrophil s and platelets in MM patients than lymphoma and leukemia.[158] (Table.22)

Present study (Table.21)

Diagnosis	**Neutrophil Engraftment In Median on Day**	**Platelet Engraftment In Median on Day**
Multiple Myeloma	10 (4-12)*	13 (11-19)
Lymphoma (HL,NHL)	11 (13-46)	16.5 (13-46)
Leukemia (ALL,AML,CML)	14 (12-21)	25 (10-30)

*Range

Thissaiane et al [158] (Table.22)

Diagnosis	**Neutrophil Engraftment In Median on Day**	**Platelet Engraftment In Median on Day**
Multiple Myeloma	10	11
Lymphoma (HL,NHL)	11	13
Leukemia (ALL,AML,CML)	18	21

In concordance with our study, M. J. Watts et al in their study on 20 patients who underwent autologous PBSCT found that Multiple Myeloma patients had earlier platelet engraftment than Lymphoma patients. [159]

In our study among 30 cases of hematological malignancies, Leukemic patients achieved delayed neutrophil and platelet engraftment than MM and lymphoma.

J. Reiffers et al in their study on 118 patients with hematological malignancies found AML patients achieved delayed engraftment than other malignancy.[160]

Shirong Wang et al in their study found that in each diagnostic subgroup, a stronger correlation between CD34+ cell dose and engraftment was seen in patients with MM and NHL. [162]Similarly, in our study majority (8 out of 11) of MM patients received higher CD34+ cell dose ranging from 5.3 to 11.4 × 10^6, which could be the possible reason for faster PBSC engraftment.

In our study all MM patients had received autologous PBSCT. This could be another reason for faster engraftment in these group patients. This is similar to the study by Bensinger W et al on MM patients on whom autologous haematopoietic stem cell transplant yielded faster engraftment.[166]

2. DOSE AND PBSC ENGRAFTMENT

In our study all patients received 98-100% viable CD 34+ cells and those who received graft size ranging from 2.5 – 4.9 × 10^6 cells/kg achieved rapid neutrophil engraftment. However, with regard to the rapid platelet engraftment, the graft size infused was 5.0- 7.8× 10^6 cells/kg.

When the graft size was < 2.50 × 10^6 the neutrophil and platelet engraftment was delayed.(Table.23)

Present study (Table.23)

CD 34+cell dose/Kg Recipient body weight	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
< 2.5 × 10^6	18.5 (16&21)*	24.5 (19& 30)
2.5 – 4.9 × 10^6	11 (4-13)	15 (11-46)
5.00 – 7.8 × 10^6	11 (10-14)	13 (12-19)
> 7.8× 10^6	14.5 (12-15)	17 (10-23)

*Range

Table.24

Author/Reference	CD 34+cell dose	Neutrophil Engraftment (Median Day)	Platelet Engraftment (Median Day)
Attilio Oliveri et al[136]	< 2.5 × 10^6	11	17
	2.5 – 4.9 × 10^6	11	13
	5.00 – 7.8 × 10^6	11	12
	> 7.8× 10^6	10	11

Similarly Attilio Oliveri et al in their study on 80 patients with hematological malignancy undergoing autologous PBSCT revealed that a graft size ranging from 2.5 – 4.9 × 10^6 cells/kg is safe for complete stable and rapid neutrophil engraftment. In the same study, they also found out that CD 34+ cell number of 5.0 - 7.8× 10^6 is the optimal number for obtaining rapid platelet recovery. Further, they also found out that infusion of CD 34+ cell number exceeding the threshold of 7.8× 10^6 cells/kg is not advantageous. [136,145-147](Table 24)

However, Charles H. Weaver et al suggested that there is a correlation between CD 34+ cell dose of more than 5.0-7.50 × 10^6 and rapidity of engraftment of neutrophil s and platelets.[191] (Table.25)

Table.25

Author/Reference	**CD 34+cell dose**	**Neutrophil Engraftment (Median Day)**	**Platelet Engraftment (Median Day)**
Charles H.Weaver[191] (Autologous PBSCT)	0.5-2.5 × 10^6	11	12
	2.5-5.0 × 10^6	10	11
	5.0-7.50 × 10^6	10	11
	7.5-10 × 10^6	9	10
	10-12.5 × 10^6	9	10
	>12.5 × 10^6	8	8

Similarly, Nicolas Ketterer et al in their study on 168 patients with hematological malignancy undergoing autologous PBSCT, suggested that reinfusion of $\geq 15 \times 10^6$ cells further shortens haematopoieticengraftment.[192]

Table.26

Author/Reference	**CD 34+cell dose (Median)**	**Neutrophil Engraftment (Median Day)**	**Platelet Engraftment (Median Day)**
Nicolas Ketterer et al. [192] (Autologous PBSCT)	$\leq 2.5 \times 10^6$	12	14
	$2.5 - 15 \times 10^6$	11	10
	$\geq 15 \times 10^6$	10	8

Similarly, Bender JG et al and Tricot G et al in their studies stated that higher administered doses of CD34+ cells increase the speed of engraftment for both neutrophils and platelets, especially in heavily pretreated patients.[193,194]

Similarly, W Bensinger et al in their study on 243 patients with hematological and non hematological malignancy undergoing autologous PBSCT concluded that CD 34+ cell dose is an important predictor of engraftment kinetics after PBSC transplant regardless of disease or mobilization technique.[195]

Heimfeld S et al and Zaucha JM et al stated that however, survival benefits are controversial because studies of adult or pediatric patients who received allogeneic HPC (A) transplants from sibling donors for ablative

therapy show that very high CD34+ cell doses (>8× 10^6/kg) were associated with increased morbidity resulting from chronic GVHD, a common cause of severe morbidity and mortality after allogeneic transplantation and with no improvement in survival.[154, 156,165]

Similarly H.E.Johnsen et al in their study found that the CD 34+ cell dose is the only significant factor that affects the speed of neutrophil and platelet engraftment. They also concluded that the quality assessment of autografts have been fulfilled by enumerating CD34+ cell subset with flow cytometry.[196]

Similarly S Heimfeld et al also in their study found that the dose of CD34+ cells infused is the only significant factor that affects neutrophil and platelet engraftment.[141]

Rusten LS, Lyman SD, Veiby OP et al found that in case of autologous PBSC transplantation, successful neutrophil and platelet engraftment occurred for all patients with advanced stage or poor-prognosis malignant lymphoma who received >2.5 × 10^6 CD34+ cells/kg in patients. In their study, neutrophil engraftment took >2 weeks and platelet counts of 20,000/μL were not achieved until a median of 31 days in patients receiving fewer <2.5 × 10^6 CD34+ cells.[143]

3. AUTOLOGOUS VS. ALLOGENIC

In our study patients who underwent autologous PBSCT achieved rapid neutrophil and platelet engraftment in median on day 11 and 15 respectively than those who underwent allogeneic PBSCT in median on day14 and 18. (Table27)

These findings were similar to the study done by Thissiane et al who found faster neutrophil and platelet engraftment in autologous PBSCT than allogeneic PBSCT.[158] (Table28)

Present study (Table.27)

Source (AT/AL)	**Neutrophil Engraftment In Median on Day**	**Platelet Engraftment In Median on Day**
Autologous	11 (4-13)*	15 (11-46)
Allogeniec	14 (11-21)	18 (10-30)

*Range

Table.28

Author/Reference	**Source (AT/AL)**	**Neutrophil Engraftment In Median on Day**	**Platelet Engraftment In Median on Day**
Thissiane et al [158]	Autologous	10	11
	Allogeniec	19	21

4. CONDITIONING REGIMEN

In our study patients who received melphalan conditioning regimen achieved rapid neutrophil and platelet engraftment than others and those who received Bu Cy and Cy TBI achieved delayed engraftment.

In our study 2 patients who had received reduced intensity conditioning regimen viz., Bu Flu and Bu Cy Flu (Reduced intensity) achieved rapid neutrophil and platelet engraftment than Cy TBI (Myeloablative) (Table.29)

Present study (Table.29)

Myeloablative Conditioning regimen	**Neutrophil Engraftment In Median on Day**	**Platelet Engraftment In Median on Day**
Melphalan (200 mg)	10 (4-12)*	13 (11-13)
LACE	10.5 (9-16)	17.5 (16-46)
CBV	11	13
Bu Cy	13.5 (11-16)	15 (13-23)
Cy-TBI	16.5 (12 & 21)	24 (18 & 30)
Reduced Intensity Conditioning regimen	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
Bu Flu	14^{th} day	10^{th} day
Bu Cy Flu	14^{th} day	12^{th} day

*Range

Similarly, Thissiane et al in their study, found that CBV, Melphalan (M200) and Flu Cy shows faster neutrophil and platelet engraftment engraftment than others and Cy TBI and Bu Cy achieve delayed engraftment than others.[158] Table.30

Table.30

Author& Reference	**Conditioning Regimen**	**Neutrophil Engraftment**	**Platelet Engraftment**
Thissiane et al[158]	Melphalan(200mg)	11	11
	FLU,CY	12.50	12.50
	BU,CY(120mg)	19.00	19.00
	CY 200mg	19.00	19.00
	CY,TBI	20	20
	BU,CY(200)	23.5	23.5

In a study carried out on patients with hematological malignancies, J.Reiffers et al found that Bu TBI regimen slows the speed of neutrophil engraftment.160

Bensinger WI et al and Dreger P et al in their studies stated that apart from CD34+ dose, additional factors that have been reported to affect engraftment after autologous transplantation include the type and extent of

previous myelotoxic therapy. They found that engraftment is slower after total body irradiation (TBI) or busulfan. [182,162]

Bensinger W et al in their study stated that patients with MM achieve faster engraftment after autologous haematopoietic stem cell transplant with prior high dose chemotherapy. This has been widely accepted as a standard therapy because of its faster tempo of engraftment.[166]

Similarly in our study the finding of rapid engraftment in MM patients due to melphalan conditioning regimen could be the fact that all of them had received autologous PBSCT.

5. HLA MATCH

In our study one patient with 3/6 HLA match achieved rapid neutrophil and platelet engraftment and one patient with 10/10 HLA match delayed neutrophil and platelet engraftment than others (6/6 HLA matched patients) (Table.31)

Present study (Table.31)

HLA Match	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
10/10	21#	30#
6/6	14 (11-16)*	18 (10-23)
3/6	14#	12#

#Single patient with neutrophil and platelet engraftment day achieved *Range

J. Szer et al in their study found that allogeneic PBSC from HLA-identical siblings enhances the speed of neutrophil s and platelets engraftment without detrimental effects on GVHD or survival.[151]

Although allogeneic PBSC from HLA-identical siblings augments the speed of engraftment of neutrophils and platelets,[145] in our study the finding of delayed engraftment in 10/10 HLA matched PBSCT could be due to the fact that inadequate CD 34+ cell dose of 2.10×10^6 cells/kg was given.

The finding of rapid engraftment in 3/6 HLA matched PBSCT could be due to the fact that higher CD 34+ cell dose of 6.3×10^6 cells/kg was given.

This is similar to Pulsipher M A et al study, that is administration of higher doses of CD34+ cells from related or unrelated, fully or partially matched donors that are given to adults or children is consistently associated with more rapid neutrophil and platelet engraftment. [155,157]

Pulsipher.MA et al and Heimfeld.S et al in their study stated that however, because of the generally longer time to neutrophil engraftment and especially to platelet engraftment associated with allogeneic transplantation and with unrelated donors in comparison with autologous patients, a higher HPC (A) dose (at least 4×10^6 CD34+ cells/kg) is preferred.[152,154]

6. BLOOD GROUP MATCH

In our study, among allogeneic PBSCT (n=11), there is no significant difference in the speed of engraftment between ABO compatible and incompatible PBSC graft. (Table.32)

Present study (Table.32)

ABO Blood Group Match	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
ABO compatible	15 (12-21)*	19 (10-30)
Major Incompatible	14 (12-15)	18 (12-13)
Minor Incompatible	16	19
Bidirectional	13 (12& 14)	12.5 (12& 13)

*Range

Similarly G Stussi et al. in their study on 562 patients with allogeneic haematopoietic stem cell transplantation found that ABO incompatibility has no influence on neutrophil and platelet engraftment and that only RBC engraftment was delayed (particularly in major ABO incompatibility). They also suggested that ABO incompatibility does not seem to affect the outcome in most patients of stem cell Transplant. [170]

7. AGE AND ENGRAFTMENT

In our study patients in the age group between 50-59 years had achieved faster neutrophil and platelet engraftment than others. (Table.33)

Present study (Table.33)

Age in Years	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
<20	12 (10-16)*	15 (12-46)
20-29	13 (10-21)	17 (12-30)
30-39	14 (13-15)	15 (10-23)
40-49	-	-
50-59	10 (4-12)	13.5 (11-17)
>60	11	15.5 (12,19)

*Range

Similarly Thissiane et al revealed that PBSC engraftment was more rapid in the 50-59 year age group and they found that the reason could be due to the associated favorable factors like autologous source of PBSC and pretransplant conditioning regimen with melphalan 200 mg and CBV.[158]

Table.34

Author/Reference	Age in Years	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
Thissiane et al[158]	<20	19	21
	20-29	18.5	22
	30-39	17	15
	40-49	15	18.5
	50-59	11	13
	>60	11.5	15

Similar to Thissiane et al study we found, this could be due to the fact that all patients in this group except one patient received autologous PBSCT and all received melphalan 200 except two patient who received LACE and Bu Cy which were also associated with faster engraftment. [158](Table34)

Shaji K. Kumar, et al in their study found that the proportion of patients attaining WBC engraftment (as indicated by an ANC >500 for three consecutive days) by day 15 was 94% for the elderly group (≥70years) compared to 78% for the control group (≤65). Similarly, the proportion of patients achieving a non-transfused platelet count of over 50,000 by day 30 was similar for both groups (81% and 80% respectively) and they concluded that chronologic age alone should not be used to decide on transplant eligibility.[197]

8. DMSO CRYO PRESERVATION AND THAWING

In our study, the speed of neutrophil and platelet engraftment between two groups of patients, one group who were infused with non-manipulated PBSC graft and other group who were infused with DMSO Cryo preserved and thawed PBSCgraft remain almost same.

This finding is not similar to the study done by Cigdem A. Akkok,et al, who had observed delayed PBSC graft engraftment following DMSO cryopreservation (10% final concentration) and depletion procedure due to DMSO toxicity and cell loss.[173]

Present studyTable.35

DMSO Cryo Preservation and Thawing (7.5% Final Concentration)	Neutrophil Engraftment In Median on Day	Platelet Engraftment In Median on Day
YES	11 (9-13)*	15 (11-46)
NO	12 (4-21)	15 (12-30)

*Range

9. SEX

In our study male gender achieved faster neutrophil and platelet engraftment than female gender. This could be due to the fact that majority of male patients received autologous PBSCT compare to female patients.

Present Study Table.36

Sex	**Neutrophil Engraftment In Median on Day**	**Platelet Engraftment In Median on Day**
Male	12 (10-21)*	11 (4-16)
Female	27 (10-46)	15 (11-23)

*Range

Similarly Thissiane et al in their study also found that male gender achieved faster neutrophil and platelet engraftment than female gender.[158]

Table.37

Author/Reference	**Sex**	**Neutrophil Engraftment In Median on Day**	**Platelet Engraftment In Median on Day**
Thissiane et al[158]	Male	13	16
	Female	16	20

10.PREVIOUS RADIOTHERAPY

To find out the influence of previous radiotherapy treatment, we compared the speed of engraftment in patients with or without previous radiotherapy treatment, in which we had observed faster neutrophil and platelet engraftment in former than latter. This is unlike the observation made by Peter Dreger et al on autologous progenitor cell transplantation, who found that previous radiotherapy was associated with lower CD34+ cell yield but had no influence on engraftment.[164]

In our study out of 19 patients who had undergone autologous PBSCT, 10 had received radiotherapy. This could be the possible reason for relatively faster engraftment in patients who had received previous radiotherapy treatment. (Table.38)

Present study (Table.38)

Prior Radiotherapy	**Neutrophil Engraftment In Median on Day**	**Platelet Engraftment In Median on Day**
Yes	10.5 (10-12)	13.5 (12-18)
No	12 (4-21)	15.5 (12-46)

*Range

In our study due to large number of different drugs administered at different doses with various combinations we unable to group and find significant influence of chemotherapy regimen on PBSC engraftment.

- Similarly Peter Dreger et al[164] in their study stated that chemotherapy drugs administered at different doses with various combinations may give contradictory results furthermore confounding factors such as underlying disease radiation dose and conditioning regimen may mask the effect of chemotherapy.

SUMMARY

In our study on factors influencing the engraftment of peripheral blood stem cell transplantation on 30 cases of hematological malignancies the following features were observed based on AABB engraftment criteria. The relative speed of engraftment was analyzed depending on the median and range of values (Neutrophil and platelet engraftment days) obtained under each individual factors.

- 28out of 30 cases received CD 34 + cell dose of > 2.5×10^6 cells/kg, achieved faster neutrophil and platelet engraftment when compared to the remaining 2 cases who had received CD 34+ cell dose of $<2.5x10^6$ cells/kg.

- 11 out of 30 cases diagnosed as multiple myeloma achieved faster neutrophil and platelet engraftment than other hematological malignancies. All these 11 cases had received melphalan as their conditioning regimen.

- 19 out of 30 cases who had received autologous peripheral blood stem cell transplantation achieved faster neutrophil and platelet engraftment than allogenic PBSCT.

- 2 out of 30 cases who had received Cyclophosphamide+Total body irradiation TBI (12 CGy) conditioning regimen achieved delayed neutrophil and platelet engraftment.

- 1 out of 30 cases who had received partially HLA matched PBSC (3/6) graft achieved same speed of neutrophil and faster platelet engraftment when compared to fully HLA matched graft recipients. This is a case of AML who had received CD34+ cell dose of $6.30x10^6$/kg recipient body weight.

- 6 out of 11 allogenic cases who had received ABO incompatible allogeneic peripheral blood stem cell graft (3 major incompatible, 1 minor incompatible and 2 bidirectional) showed no significant influence on the speed of engraftment.

- With appropriate conditioning regimen and peripheral blood stem cell graft, patients in the age group of 50 to 59 years achieved faster neutrophil and platelet engraftment than patients at age group < 50 years and > 59 years.

- When compared to 11 female patients who had received autologous PBSCT 15 males who had also received autologous PBSCT achieved faster neutrophil and platelet engraftment.

- 9 out of 30 cases who had received DMSO cryopreserved (7.5 % final concentration) and thawed peripheral blood stem cell graft (without DMSO depletion) showed no significant variation on speed of neutrophil and platelet engraftment when compared the remaining cases who had received unmanipulated PBSC graft.

- 10 out of 30 patients, who had received previous radiotherapy showed no significant variation on speed of neutrophil and platelet engraftment when compared to other patients who had not received prior radiotherapy.

CONCLUSION

In our study there was a definite correlation between CD34+ cell dose and speed of engraftment. Autologous PBSCT showed faster PBSC graft engraftment than allogenic PBSCT. The expected speed of engraftment could be achieved with higher dose of CD34+ cells even in patients with partial HLA match. Among various hematological malignancies multiple myeloma patients showed relatively rapid PBSC graft engraftment with autologous PBSC as a source. Total body irradiation and chemotherapeutic agent busalphan as a conditioning regimen showed relatively slower PBSC engraftment.

Since early engraftment reduces length of hospital stay, morbidity, mortality and cost of this highly expensive treatment, it is imperative to utilize all available options to enhance the speed of engraftment.

In a country like India where there are a few established haematopoietic stem cell transplantation centers available, there are many patients desperately waiting for their life to be saved by this specialized procedure. Hence, successful and faster PBSC graft engraftment is absolutely essential.

However, analysis of factors influencing successful engraftment from larger number of PBSCT patients would provide some more relevant information in this regard.

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INSTITUTIONAL ETHICS COMMITTEE

Address of Ethics Committee: The Tamilnadu Dr MGR Medical University Chennai, India	
Principal Investigator: Dr A. Sivaramakrishnan, MBBS	
Proposal title: Factors influencing the engraftment of haematopoietic stem cell transplantation in patients with haematological malignancy (ECMGR0309025)	
Documents filed	✓
Protocol	✓
Informed consent documents	✓
Any other documents	

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DECISION

pinion of the institutional Ethics Committee-PLEASE CHECK ONE

✓ Approved

— Modification required prior to approval (please specify on the space below

— Disapproved

ate of review: 17/9/13

igned : _____ (please print name) DR. O SHANTHARAM

lease delete as appropriate, Chairperson, Secretary)

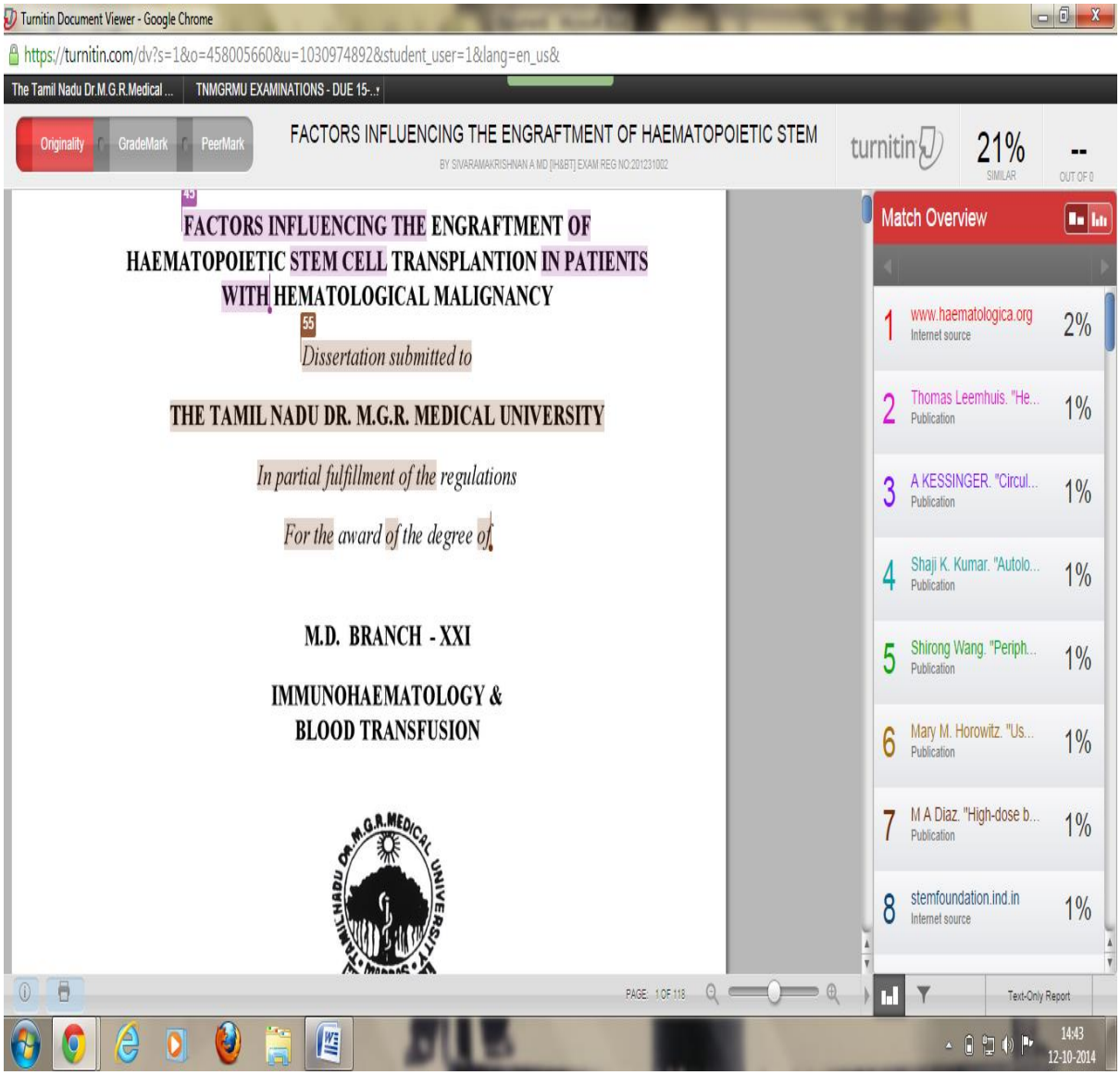
lodification needed
he Study should be done in the National Institute of Siddha instead of Chennai Corporation
hools.
he pilot study should be done in adults.

he research proponent is hereby informed that the Institutional Ethics Committee will require
e following:

1) All adverse drug reaction (ADRs) that are both serious and unexpected to be reported promptly to the IEC within 7 working days.

2) The progress report to be submitted to the IEC at least annually.

3) Upon completion of the study, a final study status report to submitted to the IEC.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In partial fulfillment of the regulations
For the award of the degree of
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The Department of Transfusion Medicine,

The Tamilnadu Dr. M.G.R. Medical University

No. 69, Anna salai, Chennai – 32

Title of dissertation: Factors influencing the Engraftment of haematopoietic stem cell transplantation in patients with haematological malignancy

PROFORMA

Donor or Patient ID: _____

Patient/Donor Name: _____IP NO: _____

Age: _____ Sex: _____ Weight:_____

Address:_____

_____

Phone no: _____

Blood group:

1. O / A / B / AB / Oh
2. Rh: Positive/Negative

Diagnosis: _____

Associated Medical Illness: _____

Previous medical illness and treatment history: _____

_____

Donor status:

1. Identical twin (syngeneic)_____
2. Sibling_____
3. Unrelated donor(allogenic)_____
4. patient's own (autologous)_____

Peripheral mobilising growth factor:

1. Type
2. Schedule
3. Dose

Stem cell collection (CD34+ cells) per apheresis: _____

Type of Chemotherapy regimen /Total body irradiation:

1. Reduced intensity
2. Myeloablative

Degree of HLA Match with patient: _____

Type of peripheral blood stem cell transplantation:

1. Allogenic
2. Autologous

Dose of CD34+ cells at the time of Transplantation_____

Hematopoietic recovery after stem cell Transplantation

- Absolute neutrophil count _____
- Platelet count_____
- Total WBC count_____
- RBC count_____
- Note:

PATIENT& DONOR INFORMATION SHEET

Factors Influencing the Engaftment of Haematopoietic Stem Cell Transplantation in Patients with Haematological Malignancy

Haematopoietic stem cell (HPC) transplantation remains the only curative option for many haematopoietic malignancies. The haematopoietic stem cells required for this procedure are usually obtained from the bone marrow or peripheral blood. Currently, the majority of procedures for procurement of haematopoietic progenitor cells are performed by peripheral blood apheresis collection.

The ultimate aim of this observational study is to find out the favorable and unfavorable factors influencing the outcome of the hematopoietic stem cell transplantation in patients with haematological malignancy. Based upon the observation in our study in comparison to other studies, suggestions to overcome the unfavorable factors for successful hematopoietic stem cell transplantation would be given.

நோயாளி / கொடையாளி தகவல் படிவம்

புற்று நோய் சிகிச்சையின் பலன்களை பாதிக்கும் காரணிகள்

குருதி மற்றும் அதனைச் சார்ந்த இரத்தப் புற்று நோயிலிருந்து குணமாக குருதியில் உள்ள தாய் அணுக்களே தற்போது உள்ள சிகிச்சை முறையில் நிரந்தர தீர்வாக உள்ளது. இந்த தாய் அணுக்கள் குருதியில் இருந்து பிரித்து எடுக்கப்பட்டு (ஏ பிரசிஸ் முறைமூலம்) பின்பு நோயாளிக்கு செலுத்தப்படுகிறது.

இந்த மருத்துவ ஆய்வின் முக்கிய குறிக்கோள் மேற்கண்ட ஆய்வுகளின் முடிவுளை கொண்டு தற்போது உள்ள சிகிச்சையினை மேம்படுத்த மருத்துவ ரீதியான குறிப்புகளை வழங்குவதே முக்கிய நோக்கமாகும்.

Consent Form

The Department of Transfusion Medicine,

The Tamilnadu Dr. M.G.R. Medical University

No. 69, Anna salai, Chennai – 32

Title of dissertation: Factors influencing the Engraftment of haematopoietic stem cell transplantation in patients with haematological malignancy.

I confirm that I read and understood the information about the above research study dated _____ and I had chance to ask the questions.

My participation in this study is voluntary and I know that I am free to withdraw from the study at any time, without giving any reason and without affecting my legal rights.

I agree to this access. I know that my identification or any details will not be revealed to third persons or published.

I agree not to restrict or interfere with any data or results that are obtained from this study.

I agree to participate in this research study for the above listed purpose.

Donor's name :

Signature : Date :

Signature of the person

Who obtains consent : Date :

Donor ID Number :

DIAGNOSIS	AT/AL	HLA MATCH	BLOOD GROUP MATCH	RBC DEPLETION	CO MORBIDITY	CONDITIONING REGIMEN	DISEASE STATUS	AGE	SEX	WEIGHT	RELAPSE	CD34 CELL VIABILITY	CD34 CELL DOSE($\times 10^6$/Kg)	NEUTROPHIL ENGRAFTMENT DAY	PLATELET ENGRAFTMENT DAY
ALL	AL	ten/ten	ABO compatible	No	Bil Femur AVN	CY T BI	CR	26	M	91	Nil	99.00	2.10	21	30
HL (IIB)(NS)	AL	six/six	Minor incompatible	Yes	Pericardial effusion	LACE	CR	21	F	78	Nil	100.00	2.20	16	19
HL (NS)	AT	NA	NA	No	Hard of Hearing	BU-CY	CR	30	M	56	Nil	99.00	2.50	13	15
HL (III)	AT	NA	NA	No	Nil	LACE	CR	22	M	71	Nil	100.00	2.58	10	18
NLPHL	AT	NA	NA	No	Nil	LACE	CR	12	M	43	Nil	99.50	2.59	11	46
PCL	AT	NA	NA	No	IHD,DM,HT,CCF,HyT	Mel	CR	51	F	60	Nil	99.00	2.60	11	13
ALL	AL	six/six	Major incompatible	Yes	Nil	Cy-TBI	CR	17	F	71	1	100.00	2.90	12	18
AML	AL	six/six	ABO compatible	No	Nil	CY,BU	CR	13	F	39	Nil	98.00	3.10	11	15
MM (IIIA)	AT	NA	NA	No	Type 2DM	Mel	CR	53	M	71	Nil	100.00	3.14	10	14
NHL (III)	AT	NA	NA	No	Nil	LACE	CR	14	F	38	Nil	100.00	3.18	10	15
HL (IIA)	AT	NA	NA	No	Nil	LACE	CR	28	M	67	Nil	100.00	3.50	12	12
MM (IIIA)	AT	NA	NA	No	DM Type 2,HT	Mel	CR	51	M	85	Nil	100.00	3.59	12	14
MM (IIIA)	AT	NA	NA	No	Nil	Mel	PR	25	F	46	Nil	100.00	3.60	10	13
HL (III A)	AT	NA	NA	No	Nil	CBVC	CR	13	F	74	Nil	99.00	3.84	11	13
MM (IIIA)	AT	NA	NA	No	Nil	Mel	CR	52	F	61	Nil	100.00	4.11	10	11
MM (IIIA)	AT	NA	NA	No	Nil	Mel	CR	52	F	55	Nil	100.00	4.60	10	13
MM (IIIA)	AT	NA	NA	No	Nil	Mel	CR	62	M	85	Nil	100.00	4.70	11	12
MM (IIIA)	AT	NA	NA	No	DM,ATT	Mel	CR	57	F	54	Nil	100.00	4.82	4	17
MCL (IV)	AT	NA	NA	No	Nil	LACE	CR	53	F	53	Nil	100.00	4.88	9	17
CLL	AL	six/six	ABO compatible	No	Nil	*	CR	51	M	67	Nil	98.00	5.00	*	*
MM (IIIA)	AT	NA	NA	No	Nil	Mel	CR	55	M	66	Nil	100.00	5.20	11	16
CML	AL	six/six	Bidirectional	Yes	Nil	BU-CY	CR	53	M	61	Nil	100.00	5.30	12	13
MM (IIIA)	AT	NA	NA	No	Nil	Mel	CR	58	M	72	Nil	100.00	5.50	10	12
MM (IIIA)	AT	NA	NA	No	IHD,DM,HT,CAD,COPD	Mel	CR	62	M	76	Nil	98.50	5.59	11	19
AML	AL	three/six	Major incompatible	Yes	Nil	BUCYFLU	CR	18	F	71	2	100.00	6.30	14	12
AML	AL	six/six	ABO compatible	No	Nil	BU-CY	CR	15	F	54	Nil	98.00	8.40	15	23
APML	AT	NA	NA	No	HBSAg Positive	CY,BU	CR	12	M	45	Nil	99.00	8.80	12	15
CML	AL	six/six	ABO compatible	No	HbsAg+	BU-FLU	CR	31	M	55	Nil	100.00	9.00	14	10
AML	AL	six/six	ABO compatible	No	HCV+	BU-CY	PR	10	F	46	Nil	99.20	9.20	16	19
AML	AL	six/six	Bidirectional	Yes	Nil	BU-CY	CR	20	F	66	Nil	100.00	10.00	14	12
AML	AL	six/six	Major incompatible	Yes	Nil	BU-CY	CR	32	M	50	3	100.00	11.14	15	23
AT=Autologous						CR=Complete Remission									
AL=alloganeic							PR=Partial Remission								