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Allogeneic Haematopoietic Stem Cell Transplantation : Army Hospital Experience

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ABSTRACT

This article outlines our experiences with **allo**geneic **h**aematopoietic **s**tem **c**ell **t**ransplantation (allo-HSCT) at Army Hospital (Research & Referral) with effect from February 1998 to March 2010. A total of 132 patients underwent 137 transplants, five of them undergoing second transplant due to the failure of first. The initial 18 allogeneic HSCTs were done in single, air-conditioned, side rooms of the general hematology ward. In February 2002, a three-bed, **h**igh **e**fficiency **p**articulate **a**ir (HEPA) filter equipped unit was established, where 119 allo-HSCTs have been carried out. The details of 114 patients who underwent 119 allo-HSCTs in the HEPA filter unit are being discussed in this article. Indications for allo-HSCT included various genetic disorders and haematological malignancies.

One hundred and ninteen transplants were performed in 114 patients for various indications of which 79 were males and 35 were females with a median age of 17 (2-60) years. Peripheral blood stem cells were used in 75 (63%) cases, bone marrow in 43 (36%) and in one patient, bone marrow plus cord blood was used. **G**raft **v**ersus **h**ost **d**isease (GVHD) was noted in forty-two (36%) patients including acute GVHD in seventeen (14%) patients and chronic GVHD in twenty-five (22%) patients. Grade-III/IV acute GVHD was noted in 11 (10%) patients and so was extensive chronic GVHD noted in 11 (10%) patients. Seventy-four (64.92%) patients are surviving on a median follow-up of 34 (3-95) months. The overall mortality was 35.08% (40/114) and the main causes of death were GVHD, infections and regimen related toxicity.

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These results are comparable and in some instances superior to those reported from India and the West.

Keywords : Allogenic haematopoietic stem cell transplantation (allo HSCT), haematological malignancies, graft versns host disease (GVHD)

Introduction

HSCT has revolutionized the treatment of numerous hematological disorders, which were considered incurable in the past (1, 2). The term bone marrow transplantation (BMT) is used synonymously with HSCT. Technically HSCT is the correct nomenclature as stem cells can be sourced from the bone marrow for BMT, from peripheral blood for peripheral blood stem cell transplantation (PBSCT) and umbilical cord blood for cord blood transplant (CBT).

In the developed countries there are adequate numbers of transplant centers, HSCT being an established form of treatment. However, in the developing world there are very few centers, the main limiting factors being, the availability of trained personnel; the prohibitive cost and the high level of multidisciplinary support required for such a center (3).

In India the first HSCT was done in TMH, Mumbai in 1983. This was followed by transplants in CMCH, Vellore; Army Hospital (Research & Referral), Delhi Cantt; AIIMS, New Delhi; Apollo Hospital, Chennai and Sahayadri Hospital, Pune. There are several other centers which have started a transplant program. To date, centers doing more than ten allo-HSCTs yearly are numbered and a total of 1757 allo-HSCTs have been performed till December 2008 in India. Hence, there is a significant supply demand imbalance, necessitating more centers to meet the requirement for a population of over a billion.

Army Hospital (Research & Referral) is the premier referral center for over a hundred Armed Forces hospitals spread all over the country. Presently, we run a successful and effective HSCT program for the Armed Forces of India carrying out approx 35 HSCTs yearly (4).

Healthcare is provided totally free of cost to all entitled patients in the Armed Forces. All soldiers and their dependents (spouse, children, and parents) are entitled for enrolling in the HSCT program and all patients who had a 6/6 HLA antigen match from a related donor were considered for HSCT.

HSCT in Indian setting differs from that in the West in the following aspects.

1. There is no national health program supporting HSCT unlike the NHS (National Health Scheme) in UK. In the public sector, most patients are self supported, whereas, some state and central government agencies and private cooperative enterprises financially support HSCT. Insurance based healthcare has been started but yet is to be set up in a comprehensive manner.
2. There is no functional donor registry for HSCT in India, while this is well established in Europe, North America and Japan. These registries improve the chances of finding a fully matched HLA (unrelated) donor from 60%, to 80%, whereas, it is 25% to 30% when only siblings form the donor pool.
3. Research funding, is substantial in the West. In the past few decades funding for various research activities has significantly increased from agencies such as the Dept of Biotechnology, Dept of Science and Technology, Indian Council of Medical Research etc. In the Armed Forces there is healthy funding for research.
4. There is a shortage of established laboratories to carry out molecular studies for chimerism. This is vital to monitor non-myeloablative HSCT for donor chimerism and decision making regarding donor lymphocyte infusion (DLI)
5. Availability of blood components especially single donor platelets (SDP) harvested by aphaeresis was not freely available in India. However, with awareness and the need, the situation has vastly improved with many blood banks having quality aphaeresis units.
6. All cellular blood products have to be irradiated to prevent transfusion associated GVHD. However, availability of blood irradiators is limited.

Patient and Methods

Patient selection

Once a patient with haematological/ genetic disorder was identified for an allo-HSCT, then a search was done amongst the siblings/parents to find a HLA matched donor, who ideally should have a 6/6 HLA antigen match. Since there is no functioning donor registry in India yet, no unrelated allo-HSCT have been performed at our center. Most transplants have been myeloablative (n=98) wherein, the marrow was ablated using high dose chemotherapy referred

to as 'conditioning'. The age limit for such transplants has been 55 years. Non-myeloablative allo – HSCTs (also called reduced intensity conditioning or 'mini' transplants) have been done in older patients (n=21). In the latter procedure lack of myeloablative conditioning is compensated by the 'graft versus leukemia (GVL) effect' for an effective tumor kill.

HLA matching

Initially, HLA was done by serology and later by molecular typing using sequence specific primers (SSP) which is a low resolution DNA based typing for HLA using PCR technology. HLA loci A, B and D R was done in all patients and of late, we are also doing HLA C, DP and DQ on a case to case basis for doing mismatched HSCTs

Donor screening and pre-HSCT work-up

All donors were thoroughly screened for any underlying disease and they were screened for CMV, hepatitis B and C virus and herpes virus by PCR methods . They were also screened for malaria and VDRL.

Pre-BMT workup of the patient involves a thorough search for any focus of infection, echocardiography for left ventricular ejection fraction, pulmonary function test, ENT and dental check up for any occult focus of infection.

Venous Access

Venous access in all HSCTs was provided by inserting a double lumen Hickman broviac catheter under general anesthesia in children and under local anesthesia in adults. This procedure was carried out by a member of the hematology team in conjunction with the interventional radiologist or anesthesiologist. We have a well trained team of nurses who carry out dressing of these catheters as per laid out SOPs. Each central catheter remains in situ for a period of 1-3 months for blood sampling and transfusion support required post HSCT.

Conditioning

In our center all cases are administered chemotherapy based conditioning as radiation based conditioning is in the process of being established. For acute and chronic leukemias and MDS we used the Busulphan (Bu)-Cyclophosphamide(Cy) regimen proposed by Tutschka (5). Bu in a total dose of 16 mg/kg over a period of 4 days and Cy, 120 mg/kg over two days. For aplastic anemia a combination of fludarabine, Cy and anti-thymocyte globulin (ATG) and in Fanconi's anemia a modified protocol with Cy, ATG ± low dose Bu was used.

Stem cell harvest

PBSC was harvested using a COBE Spectra cell separator. Bone marrow was collected from the donor under general anesthesia from the posterior superior iliac crests using Jamshedi bone marrow aspiration needles. Harvested marrow was collected into a blood bag containing anticoagulant, citrate phosphate dextrose adenine (CPDA) solution without using a filter. The donor is administered 25 units/kg of heparin (UFH) intravenously prior to commencing bone marrow harvest. A minimum nucleated cell dose of 3 x 10^{8}/kg recipient weight is aimed at and harvest volume is accordingly calculated.

Total parentral nutrition

Total Parentral nutrition (TPN) was given to patients when oral intake was compromised because of mucositis. In our center, ready to use commercially available TPN, (kabiven of Fresineus Kabi) was used which contained 19% dextrose, amino acids, electrolytes and 20% lipids. In adults 1026 ml was infused through the central catheter which provided 900 kcal in 24 hours. In children, pediatric formulation of the same was used. No TPN filters were used. Trace elements (chromium, copper, iodine, manganese, selenium, zinc and molybdenum) were administered separately. TPN was discontinued once patient started taking oral fluids. The average period for TPN was 10-14 days.

Blood component therapy

All cellular blood products (RBC, single and random donor platelets and rarely granulocytes) are irradiated (25 Gy per bag) prior to transfusion. RBCs were leucodepleted at collection by the Armed Force Transfusion Center, Delhi Cantt. SDP were harvested using a COBE Spectra (Model: 950000-902) cell separator with leucodepletion using LRS Turbo version 7.0.

The ABO type of blood components transfused after transplantation was defined by the blood groups of the donor and recipient. Patients undergoing major or bi-directional ABO-incompatible transplants received RBCs of blood group O although group A or B recipients of cells from group AB donors could receive donor-type RBCs. This transfusion support was maintained until donor ABO type was demonstrable. Patients undergoing minor ABO-incompatible transplants were also transfused with group O RBCs although group AB recipients of cells from group A or B donors could receive donor-type RBCs (6).

The preferred ABO-type of platelet was that of the donor for major ABO-incompatible transplants and of the

recipient for minor ABO-incompatible recipients. Bidirectional ABO incompatible received plasma-depleted platelet components of donor ABO type. Plasma depleted platelet components of other ABO types were also infused for those patients if a component of the desired type was not available (7).

Prophylaxis for haemorrhagic cystitis

Prevention of hemorrhagic cystitis secondary to use of high dose cyclophosphamide was done by administering continuous infusion of MESNA (2-Mercaptoethanesulfonic acid sodium salt) starting 12 hours prior to and continuing 12 hours following cessation of cyclophospahamide along with hydration. Despite meticulous hydration plus MESNA infusion ,five cases had HC which responded to conservative management. In all these cases a thorough search was made for GVHD and viruses such as BK, CMV and Adeno virus, when hemorrhagic cystitis occurred after engraftment.

GVHD prophylaxis

GVHD prophylaxis in most cases was done using cyclosporine (CSA) with methotrexate (MTX). Intra-venous (IV) CSA was administered beginning Day-3 and continued till mucositis settled and oral fluids were tolerated, following which oral CSA was started (usually double the dose of IV-CSA). CSA levels were done weekly, targeting a level of 250 ng/ml. In cases of CSA intolerance, mycophenolate mofetil/ tacrolimus replaced CSA.

Gluksberg's scale was used to stage acute GVHD. For the treatment of grade-III/IV acute GVHD, intra-venous methyl prednisolone was administered in a dose of 2 mg/kg per day in two divided doses. In steroid non-responders, alternative treatment with ATG (ATGAM-Pfizer), dacluzimab (MoAb against IL-2), or infliximab (MoAb against TNF-á) was resorted to.

Antimicrobials

None of the patients received any gut sterilization with oral quinolones. Oral fluconazole was used as antifungal prophylaxis in the initial transplants. However, it was noticed that colonization by non-albicans candida species occurred in these patients following which fluconazole prophylaxis was discontinued. Intravenous ganciclovir is given as CMV prophylaxis starting on Day-10 continued till Day-2 in a dose of 5 mg/ kg daily. Intravenous acyclovir, 5 mg /kg Q8h was started on Day-1 and continued till patient commenced oral feeds when a switch to oral acyclovir was made. This was continued till immunosuppressives

were used and longer if GVHD occurred (8). A well laid out algorithm to manage febrile neutropenia based on the microbial sensitivity was followed. Usually a third generation cephalosporin with anti-pseudomonal spectrum and an aminoglycoside is the initial therapy followed by addition of anti-staphylococcal (vanomycin/ teicoplanin) and antifungal agents if fever persists. A high index of suspicion for fungal infections was kept and antifungal therapy was instituted early with voriconazole / amphotericin.

HSCT Program in Non HEPA Settings

The first 18 allo-HSCTs were done in single air-conditioned side rooms of the general Hematology Ward (May 1998 – Dec 2001) Indications included 10 CML; 04 AML; 02 ALL; 01 CLL and 01 thalassemia major. The median age was 26.5 (4.5-39) years and median cell dose administered was 6.1 x 10^8 MNC/ kg (1.7-15). Acute GVHD was noted in 9 (50%) and chronic GVHD in 3 (16%) patients. Out of these eighteen transplants, eight (44%) are disease free on a median follow-up of 11 (9-11) years. Ten (56%) patients died, of which three died of grade-III/IV acute GVHD, three of extensive chronic GVHD, three of sepsis and one of VOD and pneumonia. The high mortality was attributed to the non-HEPA environment, patient selection and initial learning curve (9)**.** The HLA typing in these patients was done by serological typing.

HSCT Program in HEPA Settings

The next 119 Transplants between February 2002-March 2010 were done in a positive pressure HEPA filtered units, each having all intensive care backup facilities. The air-quality was monitored regularly for bacterial and fungal spore contamination using appropriate settle plates (9).

We allow one attendant, who is usually the mother/father in case of pediatric patients and spouse / relative in case of adults. Foldable couches in addition to the patient bed are provided in all rooms for use by the attendant. In our experience the presence of a family member as an attendant has been a huge success and provides great moral support through this labour intensive procedure, which keeps the patient in isolation for a period of 3-4 weeks. We also have an attached common kitchen in the transplant unit, wherein, the attendants are allowed to cook. All food is pressure cooked and no raw / uncooked food is allowed for consumption. Only filtered water is used, which is also boiled for drinking purposes. We also have a dedicated air conditioned, non-HEPA filtered, 4-bed step-down unit, where patients are shifted once neutrophil

Table 1: Baseline Data and Outcomes in 114 allo-HSCT patients

BMTS	AML (n=30/33)	ALL (n=13/13)	CML(n=19/20)	AA(n=15)	Thal Maj (n=27	Miscellaneous(n=10/11)
M/F	23/7	11/2	8/11	9/6	18/9	7/3
Median Age (years)	24(5 - 54)	19 (9 - 32)	27.5(7 - 46)	22(12 - 46)	6 (2.5 - 13)	16(2 - 60)
Median Follow-up (months)	58.5 (7- 84)	.50.77 (76 - 92)	59(2 – 95)	16 (2- 56)	35 (2-92)	42 (2-58)
Stem Cell Source BM+PB: 1	BM:5; PB:28	BM:2, PB:11	BM:5; PB:14 BM+PB:1	PB: 15	BM:26, BM+CB:1	BM:4; PB: 5
Median Cell Dose (MNC x 108/kg)	7(2.33 - 9.2)	6.4(1.9 - 7.5)	5 (2.5 - 8.2)	6.75(3.1 - 7.7)	5.3 (2.2 - 9.7)	5.2 (2.9 - 8.6)
Median Neutrophil Engraftment (days)	11(8 -16)	12 (9 - 14)	11(9-14)	10 (8 - 12)	13 (9 - 14)	11(7 - 20)
Acute GvHD	3(10%) Gr III/IV:3	4(30.77%) Grade I:1 grade II: 1 Grade III:2	4(21.05%) Gr III/IV-04	1(6.67%) Gr II:1	3(11%) Gr I:1, Gr III/IV :2	2(18.18%) Gr II:1 Gr IV:1
Chronic GvHD	5(16.67 %) Ext: 3 Ltd: 2	.4(30.77%) Ltd: 2, Ext: 2	8(42.11 %) Ext: 3 Ltd : 5	3 (20.00%) Ltd: 1 Extd: 2	2(7.5%) Ltd: 2	2(20%) Ext: 1 Ltd: 1
VOD	4(13.33%)	3(23.08%)	3(15.79%)	Nil	2(7.41%)	3(30%)
Infections	5(16.67%)	3 (23.08%)	4(21.05%)	3(20.00%)	5(18.52%)	1(10%)
Mortality & causes of death	53.33% (16/30) Relapse: 6; Pneumonia+ Sepsis:4 VOD:1 Ac GVHD IV: 2 Others: 3	46.15% (6/13) Relapse: 2, Acute GVHD:2, Sepsis: 1 Ch GVHD with CMV:1	31.58%(6/19) Relapse: 1 Septicemia:1 VOD 1 Ac GVHD :2 Ch GVHD:1	20% (3/15) Infections : 2 pericardia effusion: 1	22.22% (6/27) Severe VOD: 2 Acute GVHD: 2 Sepsis:1 Ch GVHD with CMV :1 infection	30% (3/10) Relapse: 1; Ch GVHD +(BOOP): 1 Ac GVHD-IV):1
DFS/EFS*	46.77%(14/30)	53.85% (7/13)	68.42%(14/19)	80% (12/15) *	77.78% (21/27)*	70% (7/10)*

engraftment has occurred. A year-wise distribution of allo-HSCTs carried out at this centre between March 1998 to February 2010 is shown in Fig. 1 and the distribution of various indications in Fig. 2.

Results

The base line data and outcomes are outline in Table 1.

Acute myeloid leukemia

Total of thirty-three allo-HSCTs

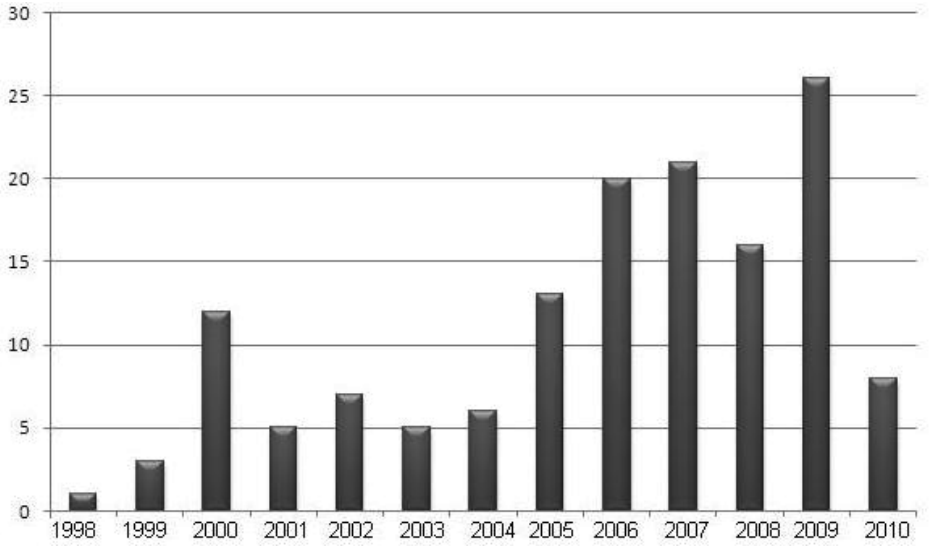

Fig. 1: Allo-HSCTs at AHRR between March 1998–Feb 2010

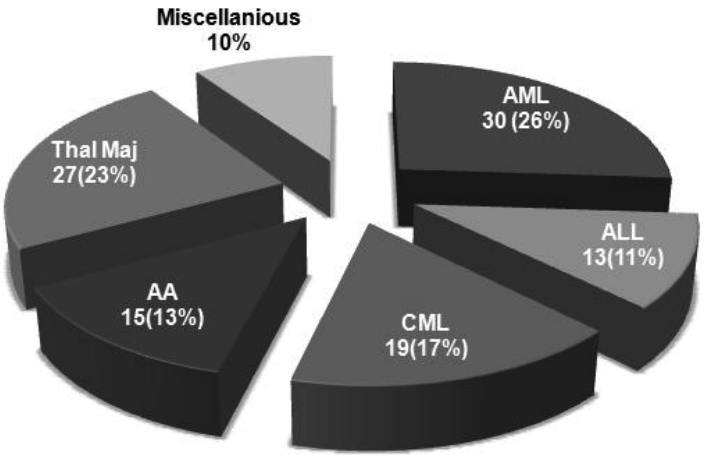

Fig. 2: Indications for allo-HSCT at AHRR between Jan 2002-Feb 2010

were done in thirty patients of AML, where twenty-eight were PBSCTs and five were BMTs. Three patients who underwent a second transplant were all PBSCTs. Out of 30 patients, 25 were in complete remission (CR)1, four in CR2

and one patient who underwent a second transplant was in CR3. Median age was 24 (5-54) years and male-female ratio was 23/7. Grade-III/IV acute GVHD was noted in three (10%) patients and chronic GVHD in five (16%) patients, three of whom had extensive chronic GVHD (2 skin & liver; 1 lung). Fourteen (47%) patients are alive and disease free on a median follow-up of 25.5 (2-59) months. Sixteen (53%) patients died including, six of relapse (4 of CR1; 1 of CR2 and 1 of CR 3); six of pneumonia with sepsis; two with VOD; one of grade-III/IV acute GVHD and one due to chronic lung GVHD.

Acute lymphoblastic leukemia

Thirteen patients underwent HSCT of which eight were in CR1 with high risk disease including three Ph+; and five were in CR2. There were eleven males and two females with a median age of 19(9-32) years. Seven (53.85%) patients are alive and disease free on a median follow-up of 50 (3-99) months. Grade-III/IV acute GVHD was seen in two patients and chronic GVHD in four (31%) patients (two limited; two extensive). Out of six (46.15%) deaths, two patients died due to grade-III/IV acute GVHD; one due to extensive chronic GVHD; three due to relapse(2 with CR2 and one with Ph+ disease).

Chronic myeloid leukemia

CML was one of the most frequent indications for allo-HSCT in the pre imatinib mesylate era. However, after the introduction of imatinib mesylate the number of transplants done has been negligible due to the excellent response to imatinib mesylate. Nineteen patients in the first chronic phase (CML-CP-1) underwent 20 allo-HSCTs out of which 14 were PBSCT and 6 were BMT. The median age was 27.5(7-46) years with a male-female ratio of 8:11. Grade-III/IV acute GVHD was seen in four (20%) patients and chronic GVHD in 8 (40%) patients, of which three had extensive chronic GVHD (one skin & liver, two lung). Thirteen (68%) patients are alive and disease free on a median follow-up of 59 (2-95) months while 32.58%(6/19) mortality was observed. One patient transplanted using PBSC developed grade-II acute skin GVHD which was successfully treated with CSA and a short course of steroids. One-year post-HSCT, this patient developed significant weight loss, extensive oral ulcers and severe cholestatic jaundice. Skin and mucosal biopsy confirmed chronic GVHD (Fig. 3). The liver biopsy revealed portal fibrosis with lymphocytic infiltration. There was loss of bile duct with cholestasis consistent

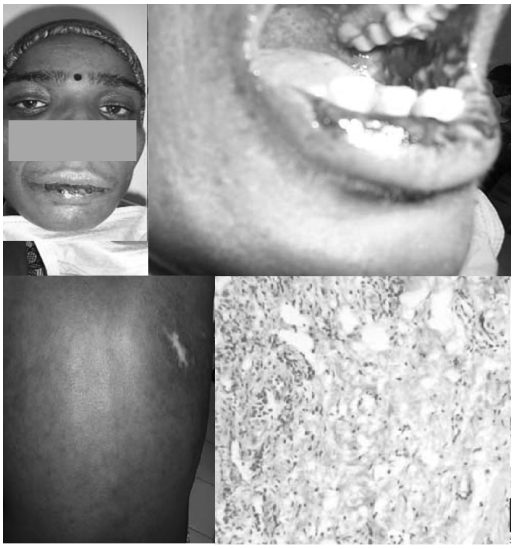

Fig. 3: Extensive chronic GVHD in patients underwent allo-HSCT (vanishing bile duct syndrome)

with a diagnosis of 'Vanishing bile duct syndrome'. This patient died due to severe extensive chronic GVHD (10). The other causes of death included VOD in one; grade-III/IV acute GVHD in two; septicaemia in one and relapse in one.

Thalassemia major

Total of twenty seven thalassemia major patients underwent transplant using CB and BM in one and BM in 26 patients. The median age was 6 (2.5-13) years with a male-female ratio of 2:1. All patients were risk stratified using Lucarelli's criteria, which proposed three classes based on, evidence of good iron chelation, hepatomegaly and presence of portal fibrosis on liver biopsy. Class-I were patients with good iron chelation, no hepatomegaly or portal fibrosis. The presence of one or two factors was classified as class-II and presence of all three factors as class-III. In our series 4 patients belonged to class-I, 15 to class-II and 8 to class-III (Table 2). The only patient who underwent CBT rejected the graft but was successfully transplanted a second time, using BM from the same sibling donor. Three out of four class-I patients died, two due to infection and one due to severe VOD; one in class- II died due to grade-III/IV acute GVHD, and sepsis. Two patients died in class-

Table 2: Results as per Lucarelli's classification

Outcome variables	**Class I (%)**	**Class II (%)**	**Class III (%)**
No. of patients	4(14.81%)	15(56%)	8(30%)
Severe VOD	1(25%)	3(20%)	2(25%)
Acute GVHD (III/IV)	Nil	1(6.7%)	2(25%)
Chronic GVHD	Nil	1(6.7%)	1(13%)
Infections	2(50%)	2(13%)	2(25%)
Mortality	3(75%)	1(6.7%)	2(25%)
EFS	1(25%)	14(93%)	6(75%)

III; one due to grade-III/IV acute GVHD and another due severe VOD. Event free survival (EFS) was noted in 77.7% (21/27) patients on a median follow-up of 35 (2-92) months.

Acquired severe aplastic anaemia

Fifteen patients of severe aplastic anemia underwent allo-HSCT and all were PBSCT (11). The median age was 22 (12-46) years with a male-female ratio of 3:2. Twelve (80%) patients are alive, free of disease on a median follow-up of 16 (2-56) months. Only four (26.5%) patients had GVHD including one acute GVHD (grade II) and three chronic GVHD (one limited and two extensive) without any fatal outcome. Three (20%) patients died, two due to sepsis and one due to massive pericardial effusion with cardiac arrhythmia.

Miscellaneous

In this group, there were three patients of Fanconi's constitutional anaemia who underwent four transplants. Two were males and one female who underwent a second transplant due to graft rejection. The median age of this cohort was 14 (8-16) years. Regimen related toxicity was seen in one patient in form of VOD and one also had extensive chronic skin GVHD. All three patients in this group are surviving and are disease free on a median follow-up period of 42 months. There were 2 patients of JMML, both infants, where a combination of Bu/Cy as conditioning regimen and CSA+MTX as GVHD prophylaxis was given. Out of these two patients, one patient relapsed and died 5- month post-BMT and the second

child is surviving, disease free, on a follow-up period of 42 months. There were two patients of MDS, one male and one female, where combination of Flu, Bu and ATG was used as conditioning regimen and CSA+MTX was given for GVHD prophylaxis. Out of these two patients, one patient developed acute GVHD and second patient developed chronic GVHD of lung with septicaemia and succumbed to these complications, 11 months post-BMT. There was one patient of PNH who developed grade-III/IV acute GVHD and died 5-months post-BMT. Two patients with congenital PRCA were transplanted of which one patient also had Duchenne muscular dystrophy (DMD) (2). Though, HSCT was done only for transfusion dependent PRCA, outcomes are suggestive of transplant being of some benefit in DMD also. The child is transfusion free and interestingly has not shown any deterioration in his muscular power 39 months post HSCT. His CPK levels have reduced from 20,000 U/L pre BMT to 350 U/L. The muscle biopsy (biceps) done two years post-HSCT has shown 8% cells of donor origin. Sequential chimerism studies, using whole blood, established trilineage engraftment with 100% donor chimerism. However, immunostains for dystrophin I were markedly reduced, while dystrophin II and III were absent. The other patient with PRCA, developed mild-VOD and hemorrhagic cystitis which responded to conservative treatment and is disease free four years post-transplant.

Infections

There were 130 documented infections in 114 transplants. This included 61% bacterial, 24% viral, 14% fungal and one parasitic infection. There was no difference between patients transplanted for malignant and non-malignant indications.

Bacterial infections

In 76 patients there were 92 documented bacterial infections, 53 % of which occurred in the first 30 days, post–HSCT. Gram negative bacteria (GNB) were isolated in 77% and gram positive in 23% of cultures. The common GNB isolated included, E.coli (48%), P. aeroginosa (13%), non-fermentative Gram negative bacteria (12%), Enterobacter (2%) and K. pneumonia (2%). The gram positive organisms were, S.aureus (13%) and coagulase negative Staphylococcus (8%).

Positive bacteriological cultures were obtained from blood (65%) followed by urine (12%), sputum (8%) and catheter related infections (5%).

Viral infections: In all, 26 of the

transplant patients had 34 documented viral infections. The common pathogens were CMV, transfusion related hepatitis viruses and herpes group of viruses. CMV was detected in 13 patients (CMV-DNA by PCR) and Hepatitis B infection in 3 patients ,all three detected post BMT. Hepatitis C virus infection was seen in one patient.

Fungal infections: fungal infections were documented in 20 transplants and the common fungi identified were, Candida spp (57%), Aspergjllus, spp (33%) and Zygomycetes (10%). For infections due to Aspergillus, all patients fulfilled the CDC criteria of either proven or possible fungal infection. Majority of the infections were seen in the first 100 days post transplant and the most common site was the lung (52%). The other sites included the CNS, para-nasal sinuses, gastrointestinal tract, skin, catheter related, isolation from blood (Candida) and disseminated forms.

Parasitic infections: Only one transplant patient showed seropositivity post transplant for toxoplasma. There were no infections with tuberculosis or malaria.

Discussion

The Hematology unit of AHRRR was set up over a 2 year period starting in February 1994. The minimum infrastructure to carry out a HSCT was in place by 1997 except for a blood irradiator. In 2002, a Gamma Cell 1000 elite (NORDION), blood irradiator with a cesium 137 source was procured by the Armed Forces Transfusion Center which supplies our blood components. Till 2002, irradiation of all cellular products was carried out in the Radiotherapy department of our hospital. The more difficult part was to train a team of nurses to carry out the following tasks,

1. Identify venous access
2. Care of venous catheters, specially central catheters
3. Administer chemotherapeutic agents and blood components
4. HES sedimentation for major mismatched transplants
5. Stem cell harvest, both BM and PBSC
6. Familiarize with various transplant protocols and supportive care

In addition the blood bank and laboratory assistants had to be trained in,

1. Cryopreservation of stem cells and thawing protocols
2. Cell viability studies and
3. Flowcytometry

HLA typing was initially outsourced and then set up in-house using serological methods. In 2002 molecular typing

by SSP, was established and in 2005, chimerism studies were also set up.

From a single consultant to start with, we have come a long way, and now have four consultants with a fully trained nursing team. Now we have a good, effective HSCT program which offers transplant for all hematological disorders including genetic diseases, catering for all Armed Forces personnel and their dependants.

In the non-HEPA filter setting, a overall survival (OS) of 44.4% (8/18) was noted, whereas, OS of 65.8% (75/114) was noted in the HEPA filter setting. Though this is not statistically significant (p=0.139) as the numbers were very small in the non-HEPA group (n=18) compared to the HEPA group (n=114), the trend is clear, with an advantage in the HEPA group (Fig. 4). Taking into account the environmental

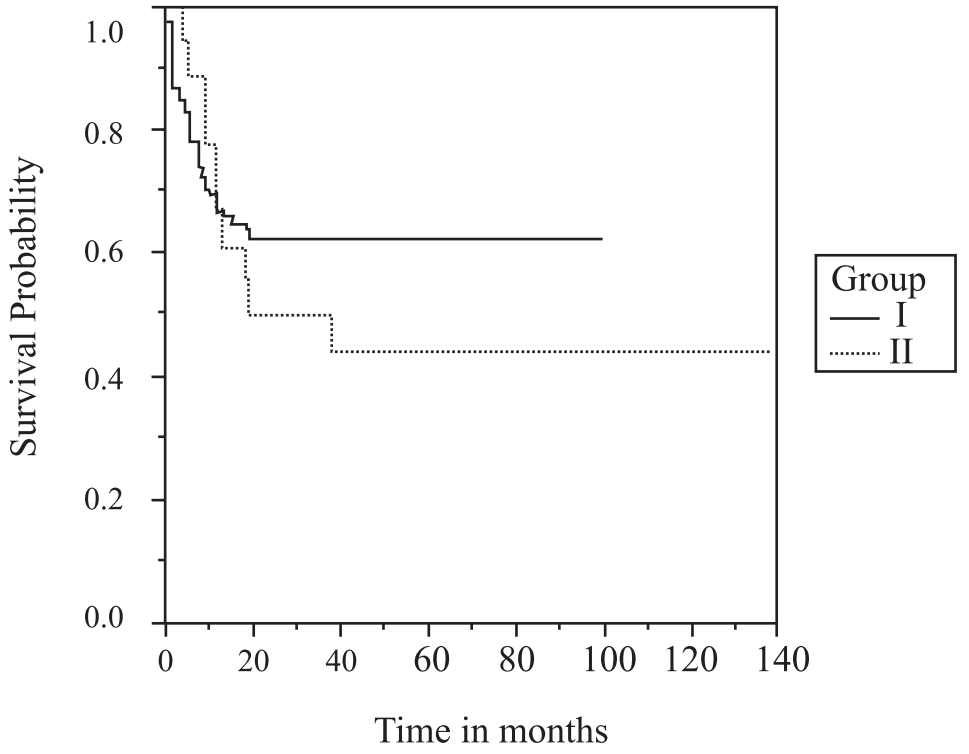

Fig. 4: Kaplan –Meier probability of survival for allo-HSCTs in Non-HEPA (1998-2001) versus HEPA (2002-2010) setting

conditions with a high incidence of antimicrobial resistance we believe that it is prudent to perform HSCTs in a HEPA filtered unit despite the feasibility of doing the same in clean side rooms, albeit, with inferior results (9).

For all our thalassemia major transplants, BM was used as the source of stem cells, except in one case, where BM+CB were used. In most cases of hematological malignancies and bone marrow failure syndromes, PBSC was used. In all pediatric donors only BM was harvested as PBSC harvest was not feasible in this age group. The donor was educated about the merits and demerits of BM versus PBSC, and allowed to make a choice as and when possible.

There was no significant difference in the incidence of acute GVHD between the BM and PBSC groups. Acute GVHD was seen in three (6.98 %) out of 43 BMTs and in 14(18.42%) out of 76 PBSCTs (p=0.142). However, the incidence of chronic GVHD was significantly higher in patients transplanted with PBSC. Chronic GVHD was noted in 6.98% in the BMT group where as it was noted in 30.70% in the PBSCT group (p=0.0058). This is as observed by other workers.

Twenty-six patients with thalassemia major underwent BMT and one was given BM plus CB. A EFS of 77.7 % was noted on a median follow-up of 35 months (Fig. 5). A 93% EFS was observed in 15 patients of thalassemia major classified to Lucarell's class-II. These results are comparable with that of Pessaro, Italy and with CMC, Vellore (12, 13). Both these centres have amongst the largest series for thalassemia major transplants in the west and in India, respectively. Three of our four Class I patients died, two of sepsis and one of severe VOD which emphasizes the unpredictability of sepsis and regimen related toxicity in HSCT (13).

In 15 patients with severe aplastic anemia with a median age of 22 years a EFS of 80% was noted on a median follow-up of 16 months (Fig.5). Though the number of transplants are small, results are superior to those published from India (13) and the west. This is because most of our patients were diagnosed early and minimally transfused given the network of more than 100 armed forces hospitals spread all over India. A delay in diagnosis and multiple transfusions, pre-transplant, negatively impacts transplant outcomes in this group of patients.

Fourteen (46%) out of 30 patients with AML are disease free on a median follow up of 25.5 months. Out of 13 patients with ALL, seven (54%) are alive

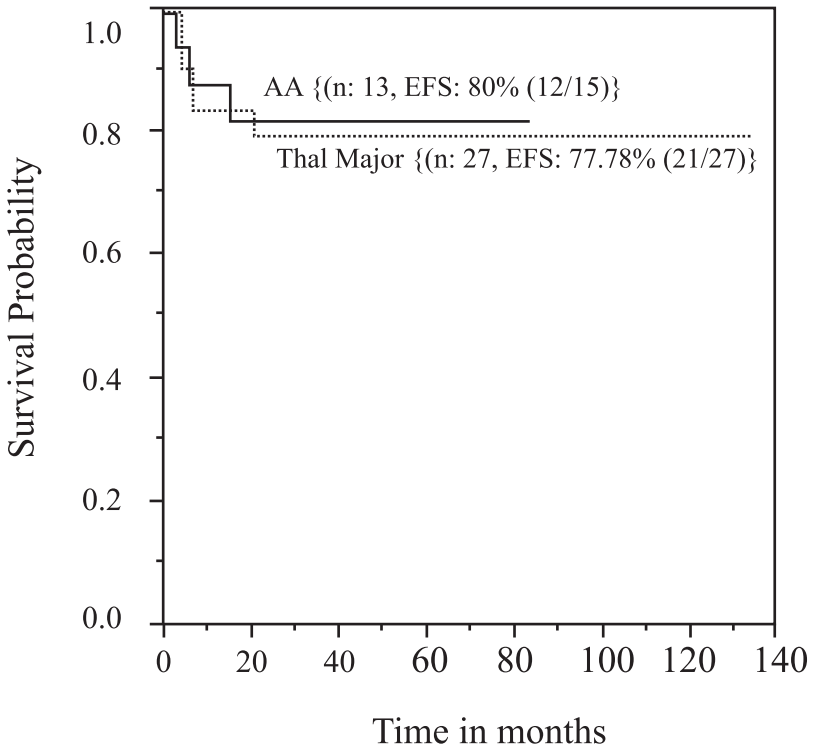

Fig. 5 : Kaplan-Meier probability of survival for patients with Thalassemia Major and Aplastic Anaemia

on a median follow up of 50 months. Results for both AML and ALL are comparable with those published from India and the west.

CML is the commonest leukemia in India, and was the most frequent indication for allo-HSCT at our center, till the advent of Imatinib mesylate. Imatinib mesylate has revolutionized the treatment of CML, with long term molecular responses noted, as long as the patient is on the drug. However, HSCT remains the only curative treatment and may be a cost effective option, if done early within the first year of diagnosis, in younger patients (<30years), especially in developing countries. This is more so because, Imatinib mesylate has to be continued long term and maybe life long, which adds to the cost. In our series a DFS of 68% was noted in 19 patients in CML-CP1, over a median follow up of 59 months (Fig.6).Our results are superior to those published from India and the west. The American Society of Hematology panel reported 50%

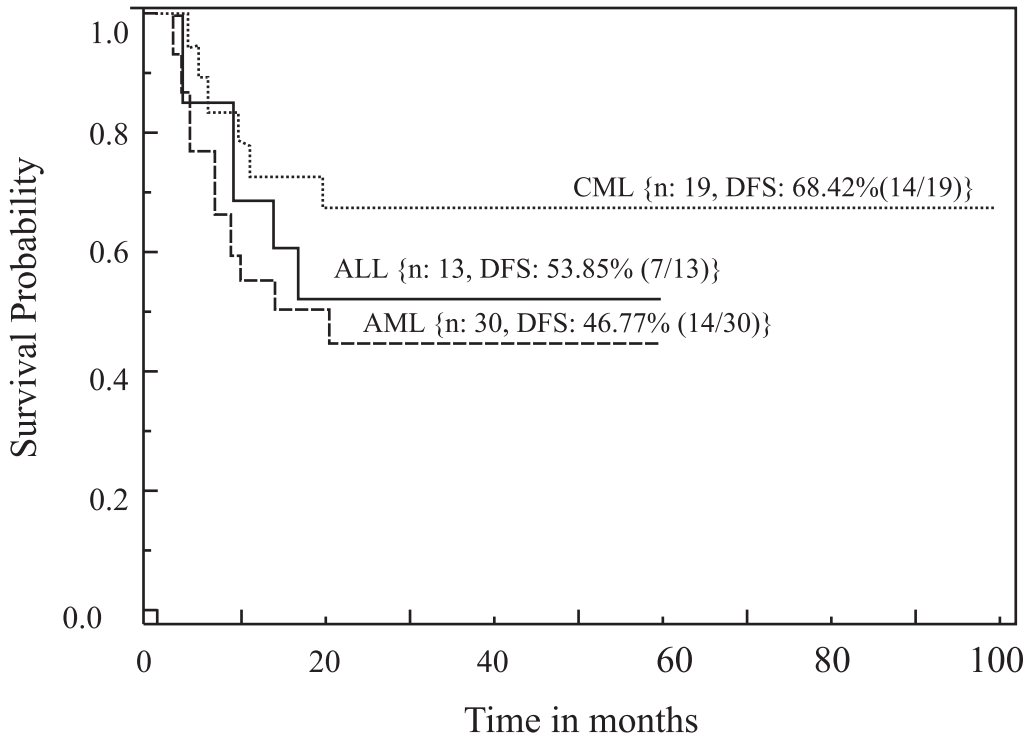

Fig. 6 : Kaplan-Meier probability of survival for ALL, AML and CML.

leukemia free survival after 5 years in CML-CP following a matched related allo-HSCT. The cumulative incidence of relapse at 18 years was 25% in these patients (14). Hence a long term close follow up is warranted in these patients with BCR-ABL monitoring for relapse and early intervention with donor lymphocyte infusion or Imatinib mesylate. The European bone marrow transplant registry reported a 10 year survival of 65 to 70% in children (15). Early diagnosis and transplant within one year of diagnosis in our patients with CML-CP1 with a median age of 27.5 years have largely contributed to our excellent results (15).

Three patients with Fanconi's constitutional anemia and two with congenital PRCA are disease free on a median follow up of 42 and 48 months respectively. Interestingly, one of the PRCA patients also suffered from DMD, which is a fatal form of muscular dystrophy. Though the transplant was

successfully performed, primarily for transfusion dependent PRCA, it seems to have also benefited DMD, as evidenced by a static clinical course over the last 39 months. Muscle biopsy has also shown 8 % donor cells on DNA typing with 100 % donor chimerism in the blood in this patient (2).

The cost of an allo-HSCT in private hospitals is approximately 10 to 12 lakhs Indian rupees (US $ 20-24,000).The cost in our hospital is half of this while the same in the West can cost between 150 to 400,000 US $. This vast difference in cost opens up a huge scope for medical tourism.

Hence, it has been possible to establish an excellent HSCT centre and program over the last 12 years. A wide range of indications for HSCTs has been transplanted successfully with outcomes which have been comparable/ superior to published data from India and the west**.** In the armed forces, health care is a priority and all efforts are made to ensure delivery of the recommended 'standard of care' in our transplant program. This coupled with an excellent tracking system ensures a near 100% follow up, which has helped us in achieving good outcomes. Our future plans include starting HLA matched unrelated donor transplants and setting up a haploidentical transplant program.

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