Hematopoietic Stem Cell Transplantation

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Hematopoietic stem cell disorders such as severe aplastic anemia, myelodysplastic syndrome, paroxysmal nocturnal hemoglobinuria and clonal disorders, e.g., acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, lymphoma and multiple myeloma are fatal conditions. Secondaries from solid tumors, e.g., neuroblastoma, Wilms' tumor and rhabdomyosarcoma in children involving the hematopoietic system are also fatal. High dose chemotherapy or radiotherapy either alone or in combination, not only eradicates the tumor cells but also destroys the hematopoietic stem cells. Reconstitution of hematopoietic stem cells offers a definitive therapy for a variety of disorders and it has been a focus of intense research over the last 3-4 decades (1-5).

Hematopoietic stem cells were initially obtained from the bone marrow of a donor and were infused intravenously into recipient. This procedure is commonly known as bone marrow transplantation (BMT). The hematopoietic stem cells can be obtained either from genetically identical twin (syngenic) or from an HLA identical matched sibling (allogenic). Another source of hematopoietic stem cells is from the peripheral blood used for stem cell transplantation (PBSCT). The probability of getting a HLA matched donor is about 30-35% (4). It is becoming increasingly difficult to find suitable HLA matched sibling donors as the family sizes are getting smaller. To overcome this problem, bone marrow registries have been established in the developed countries from volunteer donors to find suitable HLA matched unrelated donor (MUD) for those patients who do not have matched sibling donor.

HLA Matching

Success of hematopoietic stem cell transplantation is dependent upon accurate HLA matching. Standard serological method using alloantisera for class I (HLA-A, B) and class II (HLA-DR-DQ) antigens along with family haplotype segregation studies are essential to determine the genotypic identity. Recently a number of molecular techniques have been developed using dimensional isoelectric focusing for identification of HLA-A, B and C antigen variants. DNA molecular studies have been employed to identify a better match for the HLA-DR and DQ alleles (6). Some centers use polymerase chain reaction for fingerprinting the HLA-DR matching. These advances have helped to identify the perfect HLA matching. Mixed lymphocyte culture assays have been used to determine the functional significance of HLA disparity. However, the results of mixed lymphocyte culture studies are unable to predict the acute graft versus host disease (GVHD) (3). To overcome the disparity of mixed lymphocyte culture studies, functional assays
using dilution assay of cytotoxic T-lymphocyte and T-helper lymphocyte precursor cells have been developed(7,8). These studies have been successful in predicting the development of acute GVHD.

HLA studies have revealed that matching of minor histocompatibility antigens is equally important besides matching for the major histocompatibility complex. Acute GVHD in HLA identical sibling transplants develops due to the mismatching of minor histocompatibility antigens. Minor histocompatible molecules which are recognized by the T cells of the donor. The reactivity of donor T cells against recipients minor histocompatibility antigens can be evaluated by using dilution studies(9). All these studies have helped in identifying an accurate HLA match and in predicting the possibility of acute GVHD.

**Hematopoietic Progenitor Cell Assays**

Definition of stem cells has been a subject of controversy and presently it is based upon the long term hematopoietic reconstitution. Various methods have been developed to identify the hematopoietic progenitor cells.

**Colony Cultures Assays**

Colony forming assays were developed initially to identify hematopoietic progenitor cells. The number, growth rate and morphological features on the semisolid agar plates were the basis of the culture assays in the presence of growth factors. Every colony, belonged to particular cell lineage. However, these studies were not well standardized, difficult to interpret and were time consuming. It is now believed that long term culture-initiating cells should form the basis of progenitor stem cells(10).

**Phenotypic Characterization**

A monoclonal body against KG-Ia Cells has been developed to identify the CD-34 antigen as a marker for hematopoietic cells(11). One to three per cent of normal bone marrow mononuclear cells were identified as immature progenitor cells(12,13). CD-34 cells, when correlated with the colony forming assays generated multipotent granulocyte-erythroidmacrophage-megakaryocyte colony forming units (CFU-GEMM), burst forming erythroid units (BFU-E), erythroid colony forming units (CFU-E), granulocyte-macrophage colony forming units (CFU-GM) and megakaryocyte colony forming units (CFU-MEG)(14,15). Further studies revealed that more immature subpopulation of CD34+ progenitor cells which constitute 5-20% of bone marrow cells express multiple lineage specific antigens (CD 5, CD 10, CD 33, CD 71)(16,17). These observations indicate that CD-34 antigen identifies hematopoietic progenitor cells in various stages of maturation and serves as a better indicator for identification of hematopoietic progenitor cells.

**Cell Compartments**

Cells from the bone marrow, peripheral blood and umbilical cord generate almost equivalent number of CFU-GEMM, CFU-E, CFU-GM and CFU-MEG colony forming units(12). It has been observed that CD-34+ cells in the peripheral blood are always in equilibrium with the bone marrow and usually do not exceed 10% of fraction in the bone marrow(13). Umbilical cord blood is more comparable to adult bone marrow in the hematopoietic progenitor cell number(16,18).

Quantitative determination of CD 34+ cells subpopulation is often difficult. Peripheral CD 34+ cells may express myeloid CD 33 and panleucocyte CD 45 antigens more often than bone marrow. Similarly CD-71 transfusion receptor antigen on the
The disadvantages of PBSCT include: (a) central vascular access; (b) need of large volume transfusions; (c) need for a long period for adequate harvest; and (d) adverse effects of chemotherapy and cytokines.

Cell Mobilization

It is a process of transient shift of hematopoietic stem cells from the bone marrow to the peripheral blood which enables better PBSC collection. Normally the progenitor cells (CD 34+) constitute nearly 0.1% of the peripheral blood which is nearly 10% of the bone marrow concentration. Many drugs such as cyclophosphamide, daunorubicin, cytosine arabinoside and etoposide have no cytotoxic effect on the stem cells and result in increase in number of stem cells during recovery from the myelosuppresion. Cyclophosphamide (4-7 g/M^2) is administered intravenously and stem cell collection is done when total leucocyte count reaches 1000/µl(6). Three to six aphareses are essential for desired number of stem cells(22).

Use of cytokines like granuiocyte macrophage-colony stimulating factors (GM-CSF) or granulocyte-colony stimulating factors (G-CSF), in the doses of 5-10 µg/ kg/day increases the CFU-GM number by 50-100 folds between 5-7th day of therapy (23-25). It allows harvesting of sufficient number of CFU-GM (1x10^5/kg) for most patients. Recently, the relative efficacy of various growth factors has been evaluated(26). It was observed that combination of interleukin-3 (IL-3) and GM-CSF has maximum effect on the cell mobilization. Mega-karyocyte progenitors cells were not mobilized by G-CSF. Combination of cytokines have resulted in the increase of trilineage committed progenitor cells. The procedure of collection and preservation of hematopoietic stem cells have been reviewed recently(27-29).
Tumor Cell Contamination of PBSC

It was believed that PBSC collection will have lesser number of tumor cells as compared with the autologous BMT. However, the studies by sensitive techniques of detection have revealed that contamination of PBSC with malignant cells is commoner than envisaged(30). On the contrary, studies by other workers have failed to demonstrate the significant stimulation of tumor cells by growth factors(31,32). Recently, the gene on the stem cells has been marked to identify whether stem cells used in the autologous BMT contain tumor cells which may be responsible for relapse(33). They have suggested that effective purging will be essential to improve the event free survivors for diseases requiring autologous BMT. Multiple purging protocols using drugs such as VP-16 and 4-hydroperoxycyclophosphamide (4 HC)(34) or use of monoclonal antibodies specific to various malignancies have been successful for separating the malignant cells(35).

Cord Blood Stem Cell Transplant (CBSCT)

HLA matched suitable donor for allogenic BMT or PBSCT is usually available in nearly 30% of cases. In the absence of suitable donor, BMT centers in the developed countries make an effort to search a list of 2 million volunteer registry for National marrow donor programme. Inspite of these efforts, one is able to find a unrelated suitable donor in less than 40% of cases(36). Placental blood which is universally discarded is rich in hematopoietic progenitor cells. Attempts to capitalize this source have been fruitful, as evident by the success of over 200 CBSCT in the World(36). CBSCT have not only been successful with complete HLA matched sibling but even with partial match (difference of single antigen or allele). Success of CBSCT from unrelated donors has been documented (37). In addition, it was observed that CBSCTs that differed from recipients even by three HLA antigen or allele were successfully engrafted and produced all three cell lines without any significant graft versus host disease (GVHD). It is believed that cord blood stem cells from a fetus will be sufficient for a child weighing up to 30 kilograms. However, success has been reported in engrafting of cord blood stem cells from an unrelated donor in a 26 year old woman weighing 55 kg with chronic myeloid leukemia(38). She only received a relatively low dose of 10 million nucleated cells per kilogram of her body weight. It is possible that cord blood stem cells have greater capacity for proliferation and self renewal than bone marrow(39). Further studies are needed to assess the GVHD and use of CBSCT in adults. It is important to study whether the absence of GVHD will influence, in any way, the survival of patients with leukemia.

It is justified to establish cord blood banks to expedite CBSCT. These banks would need to maintain good control on the quantity of collection, processing and cryopreservation (40). Further, they will need to maintain the universal standards to enable the exchange of supply of cord blood units to other centers. With the development of cord blood banking, proper testing of HLA and screening for various infections and genetic disorders will become essential. It is anticipated that the number of CBSCT will outnumber the BMT or PBSCT in the near future.

Clinical Application

Hematopoietic stem cells transplantation using bone marrow or PBSC has been in practice for over a decade for multiple indications in children (Table I). Besides
these indications, the transplantation in adults have been undertaken for several diseases such as chronic lymphocytic leukemia, multiple myeloma, breast cancer, ovarian cancer and germ cells tumors which have been reviewed elsewhere along with the complications of BMT(3,41-44). Studies on hematopoietic stem cells transplantation generally include both adults and children and therefore in the present review the overall outcome of the BMT has been considered.

**Acute Myeloid Leukemia**

With the current chemotherapy protocols, cure is possible only in 20-25% of patients. Thus allogenic and even autologous BMT have been undertaken to improve the event free survival (EFS). Allogenic BMT have resulted an overall EFS in 50% of patients in first remission and 20-30% of cases in patients with second or third remission.

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In three randomized clinical studies, the results of BMT have been compared with chemotherapy(45-47). In one of them, the results were similar(45) while in other two patients undergoing allogenic BMT had significant better EFS(46,47). In a prospective study(48) the projected EFS at 4 years was 55% and 48% in patients with allogenic and autologous BMT while it was 30% for patients on intensive chemotherapy. However, the authors concluded that overall survivals after successful complete remission were similar in the above three groups. Another study has also reported that the results of allogenic BMT were better when compared with the chemotherapy group(49). On comparing the results of PBSCT in 28 patients with the data of autologous Bone Marrow Transplant Registry of 683 matched patients, it was observed that EFS were identical in these two groups of patients(50).

There is a theoretical risk that the autologous BMT may be contaminated with residual leukemia cells. Purging of bone marrow by mafosfamide has improved the survivals in the European study(51). Recently EFS of 57% has been observed in patients who received purged BM (4 HC and/or etoposide) as compared with 32% in patients who received unpurged marrow. It is expected that with further improvement in purging techniques, the results of autologous BMT or PBSCT are likely to improve further.

**Acute Lymphoblastic Leukemia (ALL)**

Currently the EFS in children with ALL varies between 70 to 80% for the newly diagnosed cases. Presently patients at high risk are considered for BMT or PBSCT and both these procedures have resulted in frequent relapses(52). Recently polymerase chain reaction (PCR) has been utilized to identify the minimal residual disease(53).
Presently allogenic BMT is recommended for: (a) children in second or third remission; and (c) young adults with high leucocyte count at presentation. In a recent study from International Bone Marrow Transplant Registry (IBMTR) of 38 patients with resistant ALL, BMT resulted in successful remission in 89% of patients with EFS in 23% \((54)\). Therefore allogenic BMT may successfully cure some patients who are resistant to chemotherapy \((54,55)\).

In a French multicentric study, of 572 evaluable adult patients with ALL, 436 (76%) subjects achieved complete remission \((56)\). Of 116 patients who underwent allogenic BMT, 43% of them had 3 years of EFS while EFS in 96 patients, each, with chemotherapy and autologous BMT was 32% and 39%, respectively. Thus all these studies have shown that allogenic BMT offers better EFS in patients with high risk ALL.

**Chronic Myeloid Leukemia**

Presently for patients \(<45\) years of age, allogenic BMT, if undertaken within one year of diagnosis offers cure in 50-70% of patients. However, results of allogenic BMT in patients with accelerated phase and in blast crisis vary between 15-25% and 15% of cases, respectively \((57,58)\). The outcome of autologous BMT in 200 patients over 8 centers has been reviewed. All twenty eight cases with blast crisis died within 3 years of transplant while the median survival in 30 patients with accelerated phase was 39.9 months. In the 142 patients transplanted in chronic phase, the median survival had not been reached within seven years of follow up \((59)\). PBSC harvested at diagnosis has been used to transplant 14 Philadelphia positive patients during chronic phase. Thirteen patients engrafted and survived for more than one year, three for several years not requiring further therapy and two became Philadelphia negative \((60)\). Progress on cell identification and separation techniques will permit for vitro selection, better purging and expansion of normal hematopoietic stem cells for BMT/PBSCT \((61,62)\). With these studies it is expected that one will be able to use purified PBSC for the treatment of CML.

**Hodgkin’s Disease**

Bone marrow is frequently involved in refractory Hodgkin’s disease. In a study, 72 patients were treated with high dose chemotherapy and unmobilized PBSC for transplantation \((63)\). Complete and partial remission was observed in 50% and 35% respectively of the 68 evaluable patients. In 33% of survivals there was no progression of the disease at 46 months. Similarly high dose chemotherapy followed by autologous BMT has resulted in complete remission in 50% of patients with EFS of 20-30% at 2-3 years \((63)\). Autologous BMT if undertaken when the disease is minimal \((i.e.,\) soon after relapse) has resulted in improved survivals as against those in whom multiple chemotherapeutic regimens have failed \((65)\).

**Non-Hodgkin’s Lymphoma (NHL)**

Results of autologous and allogenic BMT in patients with NHL resistant to chemotherapy are identical \((66,67)\). However, the results are better in those patients who are chemosensitive with minimal disease and with good performance status \((67)\). Paired hematopoietic stem cells have been cultured from the bone marrow and PBSC and tumor cells were observed in 36% and 5% of cases, respectively. Patients treated with PBSCT fared better than those who underwent BMT \((3)\). In a study of 158 patients with intermediate or high grade NHL, results indicate an EFS at 3 years in 70% of patients \((n=53)\) undergoing PBSCT as compared with 32% of cases \((n=105)\).
who underwent BMT(68). Complete remission in 51% of patients with actuarial survival of 57 months in 41 (39%) of patients with bone marrow involvement has also been documented(69). Thus it is expected that PBSCT will outnumber BMT in most centers for NHL as the results with PBSCT are better because of lesser contamination by tumor cells.

**Solid Tumors**

BMT is being undertaken more frequently for solid tumors with bone marrow infiltration. The results of 25 children who underwent high dose chemotherapy along with autologous BMT have been recently reported(70). Seventeen (68%) children achieved complete EFS between 14-90 (media 34) months. Non-randomized studies of high dose chemotherapy with autologous BMT have revealed EFS in 20-40% of children with Stage IV neuroblastome(71,72). There is a need of controlled studies to evaluate the results from multicentric centers on myeloablative therapy supported by autologous BMT or PBSCT versus conventional chemotherapy in children with various solid tumors.

**Aplastic Anemia**

The International Bone Marrow Transplant Registry (IBMTR) has reported results of 595 patients with aplastic anemia following allogenic BMT from HLA identical siblings. Five year survival was observed in 69% of patients receiving cyclosporin-A and 56% of those patients receiving methotrexate for GVHD. Presence of infections and multiple transfusions before BMT, use of multiparous or multitransfused women donors, and older patients were associated with a poor prognosis(73). Experience with family donor transplants (other than HLA sibling donor) is limited. IBMTR, in a study of 60 patients, reported that the probability of EFS for 2 years was 27% for related family donor transplants as compared to 67% for HLA identical sibling donor transplants. Risk of BMT failure increased significantly with HLA disparity(74). These studies have revealed that BMT is the treatment of choice for young patients (<40 years of age) with severe aplastic anemia and should preferably be undertaken soon after the diagnosis. Patients who are old or who do not have HLA matched sibling donor should be treated with alternative methods of treatment.

**Thalassemia**

BMT is being done at multiple centers for Thalassemia. However, the maximum number of children have undergone BMT in Italy. Three distinct prognostic factors have been identified (hepatomegaly of more than 2 cm, portal fibrosis and poor chelation therapy). Class I children were those who did not have any of the above factor, Class II children had one or two of the above factors while Class III children had all three risk factors. In 484 children, the authors observed EFS in 94%, 84% and 67% of children for Classes, I, II and III, respectively(75). Results from other studies are not as good. However, the results between various centers cannot be compared as selection criteria and classification of various subgroups are not identical. BMT at present offers complete cure for those children who have HLA matched donor, are young and well chelated.

**Sickle Cell Anemia**

Sickle cell anemia is another life threatening inherited disorder associated with sickle cell crisis. Allogenic BMT offers complete cure. Thirty six of 42 symptomatic children without chronic organ damage had successful allogenic BMT. All these children became asymptomatic and had normalization of the hemoglobin electro-
Bone marrow rejection was observed in 5 cases and two of them engrafted again following second BMT. Multicentric controlled studies are essential to evaluate the results of BMT in a larger number of patients which need to be compared with prophylactic hydroxyurea therapy.

**Immunodeficiency Diseases**

Currently BMT offers complete cure for immunodeficiency diseases such as Severe Combined Immunodeficiency Disease, Wiscott Aldrich syndrome, or Chediak Higashi syndrome. European Bone Marrow Transplant Group (EBMTG) have reported encouraging results for these groups of patients and have observed that presence of infection prior to BMT, absence of protected environment, and use of female donors for male patients were bad prognostic factors.(76,77)

**Hematopoietic Stem Cell Transplantation in India**

In India at present, there are only five centers where BMT or PBSCT are being performed, such as All India Institute of Medical Sciences, New Delhi, Tata Memorial Cancer Hospital, Mumbai, Christian Medical College (CMC), Vellore; Apollo Hospital, Madras; and Adeyer Cancer Hospital, Madras. Till date over 225 transplants have been undertaken and half of these have been done at Vellore. Majority of transplants at Vellore have been undertaken for children with thalassemia, while at other centers, BMT is being done for a large variety of indications. Till date PBSCT have been done in nearly 25 cases. All these centers have a long waiting list of over two years for non-malignant cases. Lack of space, trained staff, and the will of the institutions and the Government have been the main stumbling blocks in the development of centers for BMT. The cost of BMT in the developed countries varies between 20-60 lakhs. In our country, patients have to spend between 4-6 lakhs, besides the expenses borne by the hospital or institutions. Such a high cost is beyond the reach of the majority. Therefore, it is essential that the Government and voluntary organizations should come forward to help these patients. The Government should also provide financial assistance to institutions to develop BMT or PBSCT facilities which will benefit a large number of patients.

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