REMI Healthcare

Blood Bank Chronicles

The Transfusion Medicine Update

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'Transfusion and Transplant'

Editorial

Role of Blood Transfusion Services in Transplants

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The number of organ transplantation has been growing in recent years across the country; more so in bigger cities and towns. Though kidney transplantation still accounts for the vast majority of transplants, other solid organs, besides autologous and allogenic peripheral blood stem cells (bone marrow transplants are done largely for pediatric patients) transplantation are now well established procedures. Stem cell transplants are performed for curative treatment of certain hematologic malignancies and even few benign disorders like Thalassemia major. Liver transplants are being performed in increasingly large number in various large hospitals with better patient and graft outcomes; followed by other solid organs including, heart, lung and pancreas, at least in select centers.

Blood Transfusion Services (BTS) play a critical role in hospital transplantation programs. The role of BTS spans from pre-transplant tests like HLA typing, histo-compatibility, Infectious Disease Markers (IDM) tests, CD-34 cell counts, harvests by apheresis, manipulations in stem-cell product, cryopreservation & infusion, and providing blood component support to the patients. BTS functions also involve providing specialized blood components, solving complex immuno-hematological problems, and facilitating what is now widely used ABO-incompatible (ABO-i) solid organ transplants. ABO-i transplants have opened a new avenue for patients on the waiting list of kidney and liver transplants. This is particularly relevant to our country where deceased-donor program is yet to take off and the organ transplantation act allows only near-relatives to donate an organ/part of organ (live-related-donor program). My colleagues have done a fabulous job of putting this all together. I would like to briefly mention this here as follows:

Dr. Ankit Mathur in the section on 'Preparation before transplant is the key' has wonderfully elaborated on the Human Leukocyte Antigen (HLA) system, its nomenclature, and methods of testing; serological and molecular. In the molecular tests he dwells upon techniques like Sequence specific primers (SSP), SSO (Sequence Specific Oligonucleotide) and Sequence Based Typing (SBT) giving varying resolutions of typing. He also discusses the pre-transplant testing algorithm which may be different for each geographic region, hospital, and even individual patient.

Dr. Shashank Ojha focuses very well on Peripheral Blood Stem Cells (PBSC) transplants which may be autologous or allogenic; their indications; the process from donor selection, mobilization, apheresis, processing/manipulation and finally infusion. He also writes about the importance of conditioning in the patient before he receives the 'PBSC gift-of-life'

Dr. Mohit Chowdhry talks of setting transfusion protocols in various solid organ transplants. He eloquently speaks how the Blood Transfusion Services (BTS) not only provides blood and blood components but also specialized blood components, solves complex serologic problems, and facilitates ABO-incompatible transplants. He elaborates on the specialized products like CMV-negative/safe blood, irradiated blood and leukocyte-reduced components.

Dr. A. Surekha Devi describes beautifully as to how there have been various phases in blood transfusion policies, swinging from liberal transfusions to avoidance of transfusions, since the time solid organ transplantation began. Improved HLA matching, immuno-suppression to prevent and treat rejection, prompt diagnosis and treatment of early rejection and better understanding of whether blood (in general and use of pre-transplant donor-specific transfusion) has declined would benefit or harm has led to current understanding and protocols that would first of all avoid transfusions; and prefer leuco-depleted components if transfusion becomes inevitable.

Pre-transplant tests, testing algorithms, transfusion protocols in various transplants and PBSC transplant process form the 'core' of over all role of BTS in transplants. This role shall grow and expand further in years to come

For References :

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Effect of blood transfusion on - subsequent organ transplantation

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Organ transplantation is an established speciality for treatment of multiple disorders. Since the beginning of clinical transplantation, there has been four phases in blood transfusion policies, swinging from liberal transfusions to avoidance of transfusions, followed by a repeat cycle of deliberate transfusions and again returning to abstinence. Pre-exposure to alloantigens has been discovered to have a dual effect: it is detrimental in some cases, while in others, it prolongs the graft survival.

History of blood transfusion effect in clinical transplantation

Most patients with end-stage renal disease awaiting renal transplantation, sustained by dialysis machines, become anemic and benefit symptomatically from blood transfusions (Carpenter, 1990). In 1973, Opelz et al reported that pretransplantation blood transfusions were associated with improved cadaveric renal graft survival. Transfusions from prospective living kidney donors became a common practice to induce tolerance(at the cost of some recipients becoming sensitized to their potential donor). A similar trend in recipients of heart allografts was also noted(Caves, 1973). Some studies showed that transfusions were beneficial mainly if the donor and recipient shared one HLA-DR antigen. However, leucocytes in blood components were thought to mediate this immunosuppressive effect, as evident by the fact that frozen-thawed RBC transfusions did not improve graft survival. Sensitization to transplantation antigens could be prevented by leucodepleting blood components that are to be used in pretransplantation transfusions. Thus leucocyte poor red cells and frozen deglycerolized red cells were assumed to have low incidence of HLA alloimmunization(Sanfilippo 1985, Polesky 1977). The incidence of alloimmunization was also low when stored units, rather fresh units of blood were used for transfusion(Light 1982).

Improved immunosuppression by cyclosporine in 1980s and tacrolimus(FK506) has neutralized much of the beneficial effect of transfusions. Renal transplant centers in the Netherlands discontinued pre-transplant transfusions after a retrospective multicenter comparison of random, donor-specific, or HLA-matched(one HLA-A and –B, one HLA-DR) transfusions in nonimmunized female candidates showed no advantage in transplant outcomes over nontransfused controls. A French multicenter trial randomly assigned renal transplant candidates to receive no pretransplant transfusion, one random-unit transfusion, or a one-HLA-DR-match transfusion, and found no difference in graft survival or acute rejections.

Current general practice is not to transfuse organ transplant candidates intentionally. In addition, increased concern about transfusion-induced infections and alloimmunization has dampened enthusiasm for this approach. The use of erythropoietin has greatly reduced the need for transfusions in renal failure. Van der Mast et al reported a case of fatal transfusion-associated graft-vs-host disease(TA-GVHD) after donor-specific leucocyte transfusion before kidney transplantation. These formed clear opinions to move away from use of pre-transplantation blood transfusions. This led to a policy, a return to the withholding of blood as possibly unnecessary, for the improvement of graft survival.

Some recent studies show that pre-transplant transfusion may cause severe acute rejection(Wanders 2003). Patients with end stage liver disease are being treated by drug (octreotide), variceal banding, sclerotherapy, transjugular intrahepatic portosystemic shunt placements to relieve the effects of portal hypertension in order to have less gastrointestinal bleeding and to avoid pre-transplant transfusion(Calcutti 2002). Recent evidence suggests that the blood transfusion causes rejection episodes and possibly the prospects of tolerance induction.

Possible mechanism of beneficial effects of pre-transplant transfusion

- Pre-transplant blood transfusions may cause early immunization of some recipients to select HLA antigens. This enables the pre-transplantation crossmatch to detect those cases where rejection of donor organ would be most likely to occur. Preformed HLA antibodies are a major contraindication to transplantation.
- Transfusions cause immunosuppression which may occur through enhancement of suppressor T cell activity or induction of immune tolerance by some unknown mechanism.

Allogeneic blood transfusion and HLA sensitization

- 30% of transfused individuals develop anti-HLA antibodies, with the incidence being higher in previously pregnant females(Opelz 1981). In non transfused multiparous females, about 10% develop such antibodies.
- Some responders have a highly selective immune response directed to one to four HLA antigens, while others show sensitivity to 95% of a reference panel.
- The degree of sensitization is expressed as panel-reactive antibody(PRA). PRA testing evaluates who is at risk of hyperacute or humoral rejection. Patients with a PRA of > 10% are considered as sensitized and those with > 80% as highly sensitized. Different centers use different PRA cut-offs for determining sensitivity(Cecka 2010).
- PRA is determined by complement dependant cytotoxicity(CDC) test, Enzyme-linked immunoabsorbant assay or flow cytometry. Commercial kits include Flow PRA and Luminex tests.
- Since the introduction of more potent immunosuppressants to prevent and treat rejection, the use of pretransplant donor-specific transfusion has declined. Other factors such as prompt diagnosis and treatment of early rejection and HLA matching play a role in the decline observed in the transfusion effect.

Nonspecific immunosuppression after transfusion

- Allogeneic blood transfusions produce generalized immunosuppression in the recipient. This is due to decreased function natural killer cells, macrophage migration to sites of injury, lymphocyte proliferation, and cutaneous delayed hypersensitivity. Donor leucocytes in allogeneic blood may play a role in suppressing cellular immune function.
- Allogeneic blood transfusion increase the incidence of postoperative infection and tumour recurrence rate. This is attributed to immunomodulatory effects of blood transfusion, which can be prevented by routine use of leucodepleted blood components.

Antigen specific immune-suppression

- The final objective of transplantation is the induction of specific unresponsiveness, or tolerance, so that patients do not need to take anti-rejection medications indefinitely. This unresponsiveness is specific for donor antigens, i.e the recipients produce perfectly normal response to cells bearing other HLA antigens.
- When a kidney recipient receives single-donor blood transfusion from the potential kidney donor, this is known as "donor-specific transfusions"(DST), (which share an HLA haplotype, or at least one DR antigen with the transfused recipient) do not produce an increase in cytotoxic T cells precursor frequency or cell mediated immunity. If such recipients do not develop a positive cross-match, they are reported to have superior graft

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Transfusion Medicine Chronicles

1950 : Carl Walter and W.P. Murphy Jr. develop the plastic bag for blood collection.

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Introduction

Stem Cells are pluripotent undifferentiated cells that have the ability to divide and differentiate into other cell types, capable of self renewal and multi lineage differentiation

Hematopoietic Progenitor Stem Cells (HPSC) are multi potent cells with no capacity of self-renewal, which differentiate and proliferate according to their pre-programmed destiny into either common myeloid progenitor or common lymphoid progenitor cells. There are approximately 1 to 4 HPSC per one lac nucleated cells in the bone marrow. Hematopoietic stem cells in the adult reside most prominently in the marrow, spleen and blood. In blood their number is increased by-

1. Hematopoetic stresses (anemia, endotoxin, infection)
2. Cytotoxic therapy (cyclophosphamide)
3. Administration of various cytokines (G-CSF, KIT ligand, FLT-3, IL-8, GM-CSF, or thrombopoietin)

Hematopoietic Progenitor Stem Cells Transplantation (HPSCT)/ Hematopoietic Stem Cells Transplantation (HSCT)/ Bone Marrow Transplantation (BMT)

A bridge procedure in the form of hematopoietic rescue that allows chemotherapy and/or radiotherapy for the treatment of malignancies and/or other non-malignant conditions

HPSC Source

Based on Product : - Bone Marrow Harvest (BMH) / Peripheral Blood Stem Cell (PBSC) / Umbilical Cord Blood (UCB)

Based on Donor : - Autologous Donor(Self) / Syngeneic Donor : (Identical twin) / Allogeneic Donor (Related/unrelated donor (HLA matched))

Indications for HPSCT

a) Marrow Malignancies: Acute leukemias (AML, ALL), Lymphomas (Hodgkin's Disease , Non hodgkin's), Multiple myeloma, relapsed CML etc

b) Metastatic tumor

c) Recurrent Solid tumor: Neuroblastoma, Wilm's tumour, Breast cancer Ovarian and Testicular cancer

d) Non Malignant diseases: Thalessemia, Sickle cell Disease, Severe combined immunodeficiency disorder(SCID) etc

Various factors to consider before HPSCT

1. Selection of HLA matched donors (related or unrelated) for allogeneic HPSCT
2. Donor/Patient (for autologous HPSCT) assessment
3. Mobilization of donor/patient (for PBSC harvest only)
4. Collection/harvest by apheresis
5. Processing of harvest product
6. Cryopreservation of harvest product
7. Administration of preparative regimen for transplantation
8. Transfusion support

HLA TYPING IN HPSCT

Family members typed with patient for HLA A, B and DR. Likelihood of 6/6 or 5/6 match depends on frequency of recipient HLA haplotype. Likelihood of unrelated donor match is related to haplotype frequency in general population. Some HLA combinations are more frequently found among certain ethnic groups (Ethnic sequestration phenomenon).

DONOR/ PATIENT ASSESSMENT

All Patients/Donors planned for HPSC harvest are counseled and assessed along with relatives for:

- Procedural details and information regarding risks/ benefits
- Physical examination including venous access

Review Patient Chart for diagnosis, prior chemo treatments, past medical history, serology and other laboratory reports including CMV status and consent.

MOBILZATION

The goal of PBSC collection is to collect an adequate number of CD34+ cells in as few apheresis collection procedures as possible. Usually by stimulating the donor/patient with either hematopoietic growth factors, or chemotherapy and growth factors, a sufficient number of circulating stem cells for marrow rescue can be collected in one to three apheresis procedures. The PBSC mobilization regimen for autologous patients can be cytokines alone or cytokines combined with chemotherapy. Allogeneic donors are generally mobilized with daily subcutaneous injections of G-CSF 10 μg/kg for 5 days in single or divided doses. Recently plerixafor is also being used in concert with G-CSF for poor mobilizers.

Factors Associated with Inadequate Mobilization of CD34+ Cells in HPSCT Donors

Autologous

a. Disease type: aggressive Leukemias and indolent lymphomas (compared to Multiple myeloma)

b. Marrow involvement with disease

c. Age >60 years

d. Prior radiation therapy to marrow-producing areas

e. Numbers of previous chemotherapy cycles and different cytotoxic regimens, especially previous therapy with particular stem-cell-toxic agents: Melphalan, carmustine, dacarbazine, platinum analogs, fludarabine, lenalidomide

f. Low platelet counts at time of mobilization

g. Inadequate mobilization regimen:

i). Chemotherapy alone (without cytokine)

ii). Suboptimal chemotherapeutic agents

iii). Cytokine alone (compared to chemo-mobilization)

h. Prior failed chemo-mobilization attempt

I. Maximal peripheral blood CD34+ cell count <5 × 106/L

Allogeneic

a. Older age (>38 to 55 years old)

b. Female gender

c. Small body weight or body mass index

d. Lower cumulative G-CSF exposure (related to dose, schedule, and/or body weight)

e. European ethnicity (compared to Hispanic, African, and Asian/Pacific)

f. Lower baseline blood platelet count, hemoglobin, and/or mononuclear cell count

g. Lower pre-apheresis blood CD34 positive cell count

COLLECTION : - HPSC Harvest

Adult stem cells are obtained by large volume apheresis (processing of peripheral blood of patients/healthy donors) after stimulation with blood cell growth factors (e.g. G-CSF)

Cord blood stem cells obtained at delivery after tying the cord; by emptying

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1958 : Jean Dausset & Rose Payne discovers blood compatibility due to HLA on the blood cell.

Process Excellence

Setting Transfusion Protocol For Various Transplants

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INTRODUCTION

Transfusion support remains an integral part of solid-organ transplantation. Blood Transfusion Services (BTS) not only caters to the needs of the blood and blood components but also specialised blood components, the complex serologic problems, and the immunologic effects of transfusion on both the allograft and the recipient.

BLOOD COMPONENT UTILISATION

Liver Transplant (LT): In addition to surgical procedure, abnormal bleeding typically occurs during LT as a consequence of severe haemostatic dysfunction. Important variables affecting transfusion requirements include the preoperative Prothrombin Time (PT), history of abdominal operations, and factor V levels. The Child classification and the Model for End stage Liver Disease (MELD) classification are used to assess the severity of the disease. Blood use in liver transplantation has declined dramatically over the last decade mainly due to improved surgical technique, organ preservation, anaesthetic management, as well as better intraoperative monitoring of coagulation status and pharmacologic treatment of fibrinolysis.

Two Studies were done at our centre (Apollo Hospital, New Delhi) to evaluate the preoperative predictors of blood component transfusion in Live Donor Liver Transplant (LDLT) from 2006 to 2008 and March 2010 to February 2011.

The results of both the studies have been tabulated as below:

		Study 1 (2006-2008)[1]	Study 2 (2010-2011)[2]
Blood Component		**Mean**	**Mean**
1	RBC	8.84	8.48
2	Cryoprecipitate	2.03	2.19
3	FFP	7.1 units	2025 ml
4	Platelets (SDP)	2.36	0.93
5	Platelets(RDP)	1.83	0
MELD SCORE		**ND**	**19.7**

Kidney Transplant: Since the beginning of Kidney transplantation, there have been four phases in blood transfusion policies, swinging from liberal transfusions to avoidance of transfusions, followed by a repeat cycle of deliberate transfusions and again returning to abstinence.[3] The majority of patients receiving kidney transplants do not require blood. The Maximum Surgical Blood Order Schedule (MSBOS) for a Kidney Transplant in our institute consists of 3 units of cross matched PRCs, 3 units of FFP and 3 units of Platelet concentrates.

Heart transplantation: is associated with lower blood requirements than that of approximate blood usage observed in complex cardiopulmonary bypass procedures.

Lung Transplant: Blood usage in lung transplantation varies by the type of lung transplant procedure. More than 2/3 of single-lung transplant recipients do not require any transfusions. Double-lung transplant procedures typically require more red cells than heart transplant procedures.

STRATEGIES FOR SELECTION OF COMPATIBLE COMPONENTS

For solid-organ transplant procedures other than liver, transfusions frequently exceed the available supply of ABO identical red cells, antigen-negative red cells, or compatible plasma, and thus require blood group switching protocols to optimally use available blood resources.

PATIENTS WITH CLINICALLY SIGNIFICANT ALLOANTIBODIES

Pre-existing potentially clinically significant red cell alloantibodies are found in approximately 6% of liver transplant candidates. In our practice we try to provide antigen negative blood after performing various adsorption/elution techniques depending upon individual patient's need. This strategy requires close communication between the anaesthesiologist and Transfusion Medicine specialist.

INDICATIONS FOR SPECIALISED BLOOD COMPONENTS

1. CMV-Negative/Safe Blood Components

CMV infection is the most frequent infectious complication following solid organ transplantation. In seropositive solid organ recipients, reactivation of latent virus represents the major risk for CMV infection. Leukocyte reduction by filtration is effective in reducing the risk of CMV transmission from blood specially for a country like India where CMV sero-positivity is very high.

2. Irradiated Blood Components

Transfusion associated Graft Versus Host Disease (TA-GVHD) is very rare in solid organ transplant recipients. These few cases do not support a policy of routine irradiation (no routine irradiation in our Institution) of cellular blood components for organ transplant recipients.

3. Leukocyte-Reduced Blood Components

Alloimmunization to HLA antigens is of considerable importance in organ transplantation. HLA alloimmunization has clearly been shown to be associated with decreased graft survival in renal transplantation and more recently in heart and/or lung transplantation. However, liver allograft survival is not adversely impacted by HLA alloimmunization.The liver graft may neutralize existing antibodies by Kupffer cell action by the secretion of soluble class I MHC antigens that neutralize or alter the antibodies and/or by poor expression of class I antigens on hepatocytes. The dual blood supply is also advantageous as the microvascular thrombosis and intense vasoconstriction associated with humoral events only develop in the arterial tree. The enormous mass of the liver may offer protection against antibody-mediated graft destruction.[4]

Thus, leuko-reduction to prevent allo-immunization provides benefit to kidney, heart, and lung recipients.

In a retrospective study done at our centre, CDC-AHG CROSSMATCH and Panel Reactive Antibody (PRA) test (wherever available) tests were done from July 2013 to January 2014 for 136 liver transplant recipients. A Positive CDC-AHG lymphocyte cross-match was observed in 3 patients (2.2 %); included 2 (1.47%) allo-cross-match positive and 1(0.73%) auto-cross-match positive. Negative CDC-AHG lymphocyte cross-match was observed in 133 patients (97.8%). There was no statistical difference in graft

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Transfusion Medicine Chronicles

1960 : Alan Solomon & John L. Fahey develop plasmapheresis, for separating plasma and RBC

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survival rates for patients with positive and negative cross-matches at transplantation. A high PRA at the time of LT also not associated with decreased graft survival.

4. ABO blood group system in organ transplantation

The ABO system is clinically important in two aspects of solid-organ transplantation: first, as a transplantation antigen that influences graft survival, and second as an antigen-antibody system implicated in immune hemolytic anemias in ABO non-identical organ transplant recipients.

ABO system as a Transplantation Antigen

Transplantation across ABO lines will typically cause hyperacute rejection of kidney and heart transplants, although exceptions do exist. Successful transplantation of kidneys and hearts across ABO lines has been accomplished by removing ABO antibodies in the recipient or by taking advantage of the variable expression of ABO antigens such as in A2 individuals or neonates. ABO major mismatch liver allografts are used in 6.9% of pediatric and 2.4% of adult liver recipients due to organ shortages. Liver allografts are felt to be resistant to hyperacute rejection when transplanted across ABO barriers although reports of hyperacute rejection do exist.

ABO-incompatible liver transplants are reserved for patients with fulminant liver failure in whom death is imminent without transplantation and when an ABO-compatible organ is not available. Plasmapheresis to remove recipient isohemagglutinins and/or splenectomy is of benefit. There is a lack of a consensus regarding the absolute titre at which the transplants need to be done. There have been studies suggesting the titres under 16 to be the cut off for performing the transplant.[5] In our Institute a titre of 16 is considered as the cut off for performing the ABO-incompatible liver transplant.

ABO-incompatible Renal Transplants are being done in many centres of the world. Fully ABO-incompatible kidney transplants have been performed using desensitisation protocols with extra immuno-suppression, removal of pre-existing ABO antibodies by Therapeutic Plasma Exchange (TPE), blockage of antibody production, and frequent monitoring of ABO antibody levels. These programs are most often established in living donor transplants, when the date of transplant can be scheduled in advance, in contrast to unscheduled deceased-donor organ. Use of TPE to reduce the titres of anti-A and anti-B is the central feature of desensitisation therapy to reduce titres of anti-A and anti-B. Orlin and Beckman[6] identified that one plasma volume exchange reduce the titre 63% below the initial titre and that the IgM antibodies are more susceptible to removal than IgG antibodies as they are intravascular.

There is a lack of a consensus regarding the absolute titer at which ABO incompatible transplantation is less likely to face antibody mediated rejection. Tyden et al [7] aimed to achieve IgG titer of <1:8 on the day of transplantation, whereas Tanabe et al [8] had accepted an upper limit of 1:32 for IgG and IgM titers. Both studies reported good graft survival rates despite the difference in titers. At our center, a titer of </=8 is the target titer for renal transplant.

In our centre, we found a significant role of TPE procedures for the desensitization in ABO incompatible transplants. Newer techniques like antibody specific immunoadsorption columns are available as an alternative to non-specific apheresis methods like TPE. But they are of limited use when the patient has core-chain-dependent A/B antibodies which do not get adsorbed by these columns. [9,10] The procedures like TPE does not differentiate between the type of antibodies and help reducing the titers like in case of core-chain-dependent A/B antibodies. Similarly, procedure like Double filtration Plasmapheresis which is claimed to be better option [11, 12] as it uses lesser amount of replacement fluid, is found to be ineffective in preventing the rebound of antibodies after the transplant in comparison to plasma exchange [13,14].

Immunohematologic complications

Passenger lymphocytes transplanted with the donor organ are capable of producing ABO antibodies and hemolysis in ABO mismatched organ recipients. A positive Coombs test +/- hemolysis is typically observed 7-10 days after transplantation.

For References :

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survival, close to that of an HLA identical donor.

Effect of in-utero(feto-maternal) transfusion in adult renal transplant

· Many people behave as if they are clonally deleted for the HLA antigens of their mothers which they did not inherit. This was observed in patients having very high PRA, but having no antibodies against a small number of HLA antigens. This unresponsiveness was found to be due to a failure to respond to non-inherited maternal HLA antigens.

Conditioning with blood transfusions for tolerance induction

· Studies of renal and heart transplant recipients have shown a reduced rejection frequency and better graft survival when the only blood received prior to transplantation was 1-3 units from donors marked for one DR antigen with the recipient. Anti-HLA antibody production was also diminished in the one DR matched transfused group. This immunization effect could be due to suppression via some antigen-specific immunoregulatory pathway.

Effect of blood transfusions on subsequent liver transplantation

Many studies in liver transplantation has shown that increased transfusion requirement for RBCs was independently associated with patient and graft survival. While transplantation without transfusion of intra-operative RBCs was associated with superior patient and graft survival. Some studies showed moderate transfusion needs are associated with longer hospital stay, and higher needs result in a negative impact on survival. Allogeneic blood transfusion has been associated with an increased incidence of postoperative bacterial infections. Measures like pre-operative normalization of hemoglobin level and placement of an intraoperative portocaval shunt can diminish the need for red blood cell transfusion during liver transplantation. Transfusion-free transplantation is gaining more attention because of concerns surrounding transmission of diseases. Successful decrease in blood use is an upshot of an advanced transfusion-free program.

For References :

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1964 : Judith Pool discovered a method of concentrating clotting factors from FFP for hemophilia patient.

Expert Speaks

Preparation Before Transplant Is The Key

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The HLA system

The Human Leukocyte Antigen (HLA) system comprises of antigens encoded by a group of genes on the short arm of chromosome number six (6p21.3), which form the Major Histocompatibility Complex (MHC) in humans.

HLA antigens are broadly classified into two groups that differ in expression, structure and function. The two groups are Class I and II as detailed below:

· Class I antigens which include classical loci A, B and C (other loci e.g. E, F, G, H, J, K, L, exist but are less important) are expressed on all nucleated cells.

· Class II antigens which include loci DR, DQ and DP are expressed on select immuno-competent cells – B cells, macrophages and dendritic cells, activated T lymphocytes and endothelial cells.

Pre-transplant testing

The objective of pre-transplant testing in the HLA context is to minimize the complications of transplantation through:

· Advising on the best match through determination of the HLA type of the patient and donors; where there is a choice of donors or recipients as in bone marrow registries and organ allocation programs.

· Assessing the risk of rejection with regard to a particular donor recipient pair and categorizing it for e.g. high-risk where the transplant would be contra-indicated, intermediate risk, and low or normal risk.

The tests that help in making these decisions are

1) HLA typing
2) Antibody screening
3) Cross-match

HLA typing

Each person inherits two alleles of HLA at each locus, one from the father and one from the mother.

HLA typing can be done by following methods:

-by complement dependent cytotoxicity (serological typing) or

-by methods that characterize the genes (molecular typing).

The number of assignments defined by serological typing is only a few hundreds, whereas by molecular typing, thousands of alleles are defined. This is because molecular methods allow detection of even single nucleotide differences between genes, whereas serology can detect only the proteins that has been expressed on the cells. Nowadays, most laboratories perform molecular typing, with serological typing being a secondary methodology used in special circumstances; to resolve ambiguities.

A standard nomenclature is used to define alleles as shown below:

e.g. HLA - A*02:101:01:01

DNA typing techniques vary in resolution depending on the degree to which they can differentiate molecules. Low resolution techniques e.g. detect only major differences corresponding to an antigenic or allele group level i.e. the first 2 digits e.g. serology, Sequence specific primers (SSP), SSOP. High resolution techniques detect the subtype within the allele group i.e. at least 4 digit level e.g. High resolution SSP, SSOP, SBT. Intermediate level resolution techniques assign a group of subtypes or alleles e.g. Intermediate-resolution SSP and SSOP. The method we choose depends upon our applications; if the typing is being done in the context of solid organ transplantation then low resolution typing is sufficient. However, where typing is being done for Bone-marrow transplants (BMT)/Hematopoietic Stem Cell Transplant (HSCT) using unrelated donors or cord blood high resolution typing is essential.

Antibody screening

The main objective of pre-transplant antibody screening is to avoid rejection. The screening test results inform us about:

· The presence of antibody

· The nature of antibody (complement fixing or not, anti HLA or other, IgG or IgM, donor specific or other)

Generally, clinically significant antibodies are IgG, anti HLA, donor specific and complement fixing allo-antibodies, while the significance of auto-antibodies, IgM antibodies, and non-complement fixing antibodies is unclear, but they are generally regarded as less capable of acute damage.

Antibody screening platforms can be categorized based on the nature of the antigenic target into those using membrane bound antigen e.g.

a. Complement Dependent Cytotoxicity (CDC)

b. Flow cytometry cross-match

c. Solid phase assays like ELISA and Luminex.

a. The Complement Dependent Cytotoxicity (CDC) cross match is a screening assay, based on lysis of lymphocytes in the presence of HLA antibodies and is done for all prospective transplant cases. As a functional assay it strongly depends on the availability of isolated donor lymphocytes and in particular on their viability. It can give rise to "false positive" results if the lymphocytes are rendered more fragile due to fever, drugs like Rituximab and other medical conditions in the donor as well mishandling during the process of cell isolation. Low levels of HLA antibodies can also be missed, thus giving false negative results. This test is also labor intensive and cumbersome. The various modifications of the CDC include those that aim at increasing sensitivity such as extending incubation times to enable further interaction between antigen, antibody and complement, a triple wash that aims at removing any complement inhibitors in the serum, and the aforementioned Anti-Human Globulin (AHG) –CDC that increases the sensitivity by increasing the total number of Fc receptors available for interaction with complement.

b. A flow cytometric cross-match uses a fluorescent dye coupled to antihuman globulin to detect antibody bound to the cell surface. Fluorescence is detected and quantified on a flow-cytometer. This test offers greater sensitivity and more than one parameter can be assessed simultaneously. For example, to differentiate T and B cells without physical separation or to discern cytotoxic antibodies from non-cytotoxic antibodies.

c. Solid phase assays: The Luminex assay on the other hand uses a panel of fluorescent micro-beads as a medium to which antigens are attached. The micro-beads comprise a panel of approximately 500 beads each with its own fluorescent signature. The beads are incubated with serum and fluorescent AHG is used to detect antibody binding. The reaction is read by a dual laser flow system and quantified in terms of mean fluorescent intensity. The solid phase assays are definitely more sensitive than CDC. Plus they are easily standardized, objective and free you from the constraints of cell viability. They are also specific for the detection of anti-HLA antibodies which are the antibodies we are interested in. Because

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1968 : Rh Immune Globulin, discovered to address the differences between -ve & +ve blood types

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antigens are isolated or produced in the lab, they can be physically separated and used in formats that can identify the class and the specificity of the antibody with greater definition. The relative sensitivity of these platforms goes like this. The standard CDC is the least sensitive. The AHG CDC is two to three times as sensitive. And flow cytometry and Luminex are the most sensitive.

Screening algorithms

A desirable antibody screening algorithm would involve consideration of the priming history. It would include an initial screen of CDC cross-match with a more sensitive technique. It would also include a solid phase assay for confirming anti-HLA specificity. Antibody screening should ideally be repeated once in 3 months, and following each priming event. The final cross-match should be done using current and historical sera (i.e. the sera with maximal titre) and at a level of sensitivity at least equivalent to the initial screen. However, each centre should develop its own algorithm based on the nature of its patient population.

Conclusion

Pre-transplantation testing today has evolved into structured formulae that apply advanced, objective, highly sensitive and specific tests and an accumulating knowledge of the factors that affect graft survival. However, algorithms have to be tailored to fit into contexts that are unique for each geographic region, centre, and even patient.

*For References : Contact the author on Email Id: ankit@bmstindia.org

....Continue from Pg No. 3

umbilical cord and placental blood into blood donation bag in a sterile manner.

Timing of collection for PBSC harvest by apheresis

Collection is generally started after adequate mobilization in case of PBSC donors /patients. The adequacy can be judged by either of the below mentioned parameters:

- Total Leucocyte Count > 1 × 109/L
- CD 34 positive cell count >20/ul

However CD 34 positive count by flow cytometry is the more objective way to assess adequacy of mobilization. If CD 34 positive cell count is less than 10/ul there is failure of mobilization.

Target collection

Number of CD 34 positive cells correlates with speed of engraftment. Generally 2 – 2.5 x 106 CD 34 positive cells / kg is considered acceptable for engraftment. Few studies have suggested dose of 5 x 106 CD 34 positive cells / kg for more rapid engraftment and lower incidence of graft failure.

PROCESSING

HPSCT harvest product can be infused un-manipulated, minimally manipulated (only removal of contaminating cells by centrifugation) or more than minimally manipulated (selective removal/expansion/enrichment by purging, negative or positive selection).

Aims and advantages of manipulation

- To enrich the target cell population for better engraftment
- To deplete T cells in allogeneic harvest and tumor cells in autologous harvest to minimize graft versus host disease and tumor relapse respectively
- Reduction of volume of the product results in reduced storage space , less amount of cryoprotectant related toxicity during infusion and prevention of circulatory overload in pediatric patients
- Removal of contaminating cells (red cells, granulocytes, platelets, plasma proteins etc) to minimize related adverse events during infusion

CRYOPRESERVATION OF HARVEST PRODUCT

Cryopreservation is among the most critical manipulations performed in clinical cell processing laboratories. Techniques vary slightly among institutions; however, in all cases, the objective is to adjust the volume to the container of choice, add a cryoprotectant solution and then freeze the cells at a controlled rate, in a manner that preserves cell viability and proliferative potential after thawing.

Aims

- To preserve pluripotent HPSC obtained from bone marrow (BM)/peripheral blood/cord blood
- To make possible the resuscitation of these cells with a high degree of viability.
- To maintain functional integrity
- Preserve these cells in a form suitable for administration without undue toxicity.
- Volume reduction of the BM collection is essential since this will then lower volumes of cryoprotectant thereby reducing its risk and severity of toxicity.

Thawing and Infusion of harvest product

Thawing of cryopreserved HSPC is done at 37ºC to 40ºC water-bath and re-infusion is done as early as possible after thawing. All solid clumps are thawed and infusion is done by gravity drip through central venous catheter. Care has to be taken to maintain sterility of the infused product.

Quality Control of Harvest product

CD 34 positive cells/kg, Total nucleated cell count, Mono Nuclear Cell Content (%),CFU-GM, viability, sterility testing etc. can be done to assess post transplantation reconstitution capacity.

PREPARATIVE REGIMEN FOR TRANSPLANTATION

Preparative have been used with the dual aims of disease eradication and host immunosuppression for acceptance of the allografts. Preparative regimens for HPSCT have been termed myeloablative, reduced intensity, and non-myeloablative depending on dose of irradiation and type of chemotherapy. Choice of preparative regimen depends on recipient co-morbidities, underlying condition, disease status and risk of rejection.

Generally non-myeloablative conditioning regimen reduce the morbidity and mortality associated with high-intensity regimens and extend HPSCT to include older patients and those with comorbidities. However in patients with hematologic malignancies or hyperplastic marrows (eg, thalassemia) where eradication of the malignant or abnormal hematopoietic stem cells is primary goal myeloablation is preferred. In contrast, patients with severe aplastic anemia or certain immunodeficiency states (eg, SCID) requiring only immunosuppression to allow engraftment non-myeloablative regimen is preferred. Similarly allogeneic HPSCT with greater rejection risk due to HLA disparity require myeloablative conditioning.

Summary

HPSCT is a complex multidisciplinary activity requiring involvement of transplant, transfusion medicine and other laboratory units. For successful outcome of any transplant program several factors have to be taken into consideration in coordination with all stakeholders.

*For References : Contact the author on Email Id: sojha@actrec.gov.in

1969 : Scott Murphy and Frank Gardner develop a method for storing platelets at room temperature.

Quiz

Q. Hematopoietic stem cells in the adult reside most prominently in the marrow, spleen and blood. In blood their number is increased by?

a) Hematopoetic stresses (anemia, endotoxin, infection)
b) Cytotoxic therapy (cyclophosphamide)
c) Administration of various cytokines (G-CSF, KIT ligand, FLT-3, IL-8, GM-CSF, or thrombopoietin)
d) All the above

To enroll yourself for the lucky draw, Send us the Mail to us on contat.hcd@remigroup.com

you have to type the following

1. Mention the subject = Lucky Draw Registration
2. Type the correct option in the mail
3. Mention your mobile no., Blood Bank Name & Contact details

Send the Answer for the question to us to win lucky draw (3 Nos) : - Last date of enrollment : 31st Oct 15

Last Quiz Winners

Dear Customer,

We are happy to announce that lucky draw winners of last issue quiz are as follows: -

1. Dr. Anju Verma , Rotary Blood Bank, New Delhi
2. Mr. Ramesh Kumar, Life Line Blood bank & Research Centre, Tirunelveli
3. Mr.Vishvakanth servankar, Fortis Hospitals Blood Bank, Mumbai

Congratulations to All the Winners!!!
Your gift will reach to you in next 1 month.

Blood Bank Chronicles Gallery

Transfusion Support for Patients Undergoing ABO-Mismatched Allogeneic HPC Transplantation

			Phase I	Phase II			Phase III
Recipient	Donor	Mismatch Type	All Components	RBCs	1st Choice Platelets	Next Choice Platelets	FFP	All Components
A	O	Minor	Recipient	O	A,AB	AB;B;O	A,AB	Donor
B	O	Minor	Recipient	O	B,AB	AB;A;O	B,AB	Donor
AB	O	Minor	Recipient	O	AB	A;B;O	AB	Donor
AB	A	Minor	Recipient	A,O	AB	A;B;O	AB	Donor
AB	B	Minor	Recipient	B,O	AB	B;A;O	AB	Donor
O	A	Major	Recipient	O	A	AB;B;O	A,AB	Donor
O	B	Major	Recipient	O	B	AB;A;O	B,AB	Donor
O	AB	Major	Recipient	O	AB	A;B;O	AB	Donor
A	AB	Major	Recipient	A,O	AB	A;B;O	AB	Donor
B	AB	Major	Recipient	B,O	AB	B;A;O	AB	Donor
A	B	Minor & Major	Recipient	O	AB	A;B;O	AB	Donor
B	A	Minor & Major	Recipient	O	AB	B;A;O	AB	Donor

*Platelet concentrates should be selected in the order presented. Modified from Friedberg et al.
Phase I = the time when the patient/recipient is prepared for HPC transplantation.
Phase II = from the initiation of myeloablative therapy until:
a. For RBC—DAT is negative and antidonor isohemagglutinins are no longer detectable (ie, the reverse typing is donor type).
b. For FFPs—recipient's erythrocytes are no longer detectable (ie, the forward typing is consistent with donor's ABO group).
Phase III = after the forward and reverse type of the patient are consistent with donor's ABO group.
Beginning from Phase I, all cellular components should be irradiated and leukocyte reduced.

*1. Cytotherapy. 2002;4(6):551-5,
2. Transfusion. 2013 Jan;53(1):85-90. doi: 10.1111/j.1537-2995.2012.03669.x Epub 2012 Apr 27
3. Am J Hematol. 2007 June; 82(6): 463–472. doi: 10.1002/ajh.20707