Umbilical Cord Blood Transplantation: Newer Trends

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Abstract
During last ten years, over 4000 umbilical cord blood transplantations have been performed worldwide. The interest in this modality of transplantation has been growing as this provides easy access to an alternative source of stem cells for treating cancer and serious genetic disorders with otherwise fatal outcome or immense morbidity. Umbilical cord blood is a commonly discarded source of useful stem cells. The outcome of transplantation using cells from this source in children mirrors the results of unrelated donor transplantation and hence the procedure is widely accepted by paediatric transplant community. Results are, however, hampered in adults due to low cell dose. Newer techniques, such as pooled or sequential cord blood transplantation, may help to increase progenitor cell numbers and improve immune reconstitution. In near future, non-haematopoietic uses will make this even more exiting area. In this write-up, we will review this treatment including cord blood banking issues and the ethical concerns. We will discuss both paediatric and adult transplantations including certain new indications.

The availability of haematopoietic stem or progenitor cell donors is a global challenge. Even in the western world, despite over 9 million registered donors, over 50% of patients needing HLA matched donors are unable to get one. Also, the time taken from initiating search to transplantation is 6 months or more.

Thirty years ago, investigators had shown that the umbilical cord blood contained a high number of granulocyte - macrophage progenitor cells, adequate enough for reconstituting marrow in irradiated subjects. Such engraftment was subsequently shown to be durable in human beings. These observations paved the way for clinical umbilical cord blood transplantation and for the growth of umbilical cord blood banking. These cord blood cells can be preserved for years and used for:
1) Allogeneic transplants
2) Autologous transplants and even
3) Tissue repair (!!!) - as these cells have the ability to differentiate into other tissue types

Allogeneic umbilical cord blood transplant is an established entity while the autologous one is still an investigational tool.

The immunologic properties of stem cells from cord blood differ from mature marrow or peripheral blood stem cells. Cord blood contains higher proportion of T-cells expressing the CD45 RA+ / CD45 RO- , CD62 L+. These cells are immunologically naïve leading to decreased graft-versus-host disease (GVHD). The chemokine receptor CCR5, expressed by T-helper 1 T-cells, is less abundant among cord blood compared with adult T-cells.

CORD BLOOD BANKING

The first successful related umbilical cord stem cell transplant was carried out in France in a patient with Fanconi’s anaemia in 1988 and this was a trans-Atlantic collaborative effort. First unrelated umbilical blood transplant was done in USA in 1993.

The first cord blood bank was established in early 1990s in New York. Subsequently, over 35 cord blood banks have been established in 21 countries. As of today, over 150,000 cord blood units are available. Majority of these have been typed for HLA A, B and DR; 75% have molecular typing for class II and 50% have molecular typing for class I. Cord blood banking involves: recruitment, consent, testing of maternal donors, collection, processing, cryopreservation, testing, and releasing cord blood unit to transplant centre. Protocols have been established for these. Guidelines exist while standards are evolving. Cord blood banks operate under strict guidelines instituted by NETCORD, FACT (Foundation for Accreditation of Cellular Therapy) or the AABB (American Association of Blood Banks).

Cord blood banking is of two types i.e. public and private. A woman can donate cord blood for unrelated recipient to public banks (unrelated allogeneic transplantation). Private (commercial) banks, on the other hand, offer expectant parents the option to store cord blood for possible future use by that same child (autologous transplantation).
There is already a debate whether commercial cord blood banking is scientifically and ethically justified. Questions are being raised whether the commercial banking exploits the emotional vulnerabilities of parents for financial gain? Parents often believe or are made to believe that the child’s own stem cells are the best. It is also possible that the private industry might overlook important safety issues. The investigational nature of autologous umbilical cord stem cell transplant may not be openely discussed. That the genetic disorders cannot be cured by autologous transplants may not be emphasized. The duration for which the cells can be stored is questionable. Benefits are often overstated. Untruthful advertising is common. Discloser of uncertainties is rare. Cost involved is large and full discomber of financial aspects is often not made. Worldwide, very few of the stored cord blood units have been used and the likelihood of recruitment may actually be just 1 in 1,000 to 1 in 200,000.

Large number of organizations have started discouraging autologous banking. These include American Academy of Paediatrics, American College of Obstetrics and Gynaecologist, French National Consultative Ethics Committee and European Group on Ethics in Science and New Technologies. In fact, Italy has completely banned autologous and private banking.

**Asian Scenario**

Table 1 gives the picture of umbilical cord stem cell banking and transplants in Asian countries.

<table>
<thead>
<tr>
<th>Cord blood bank</th>
<th>Inventory</th>
<th>Transplanted Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beijing</td>
<td>5,800</td>
<td>44</td>
<td>26</td>
</tr>
<tr>
<td>Tianjin</td>
<td>5,400</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Seoul</td>
<td>32,000</td>
<td>84</td>
<td>80</td>
</tr>
<tr>
<td>Taipei</td>
<td>6,700</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Ho Chi Minh</td>
<td>1,076</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Bangkok</td>
<td>315</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tokyo</td>
<td>5,563</td>
<td>277</td>
<td>105</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>56,854</strong></td>
<td><strong>437</strong></td>
<td><strong>238</strong></td>
</tr>
</tbody>
</table>

**Indian scenario**

In India, there are no public umbilical cord blood banks available, although, attempts have been started at AIIMS, New Delhi and PGI, Chandigarh. In the private sector, there are a few of them. The public banking, as and when it develops, will be more useful as it would benefit all, it would be cheaper as the operating costs are likely to be provided by government agencies and it may also be useful for the minor ethnic groups. Private banks, on the other side, are useful for an individual who has to bear all the costs and hence it is expensive. It is not available to others including minor ethnic groups.

Reliance Life Sciences (RLS) established the country’s first umbilical cord blood bank (cord blood repository - CBR) in 2002. It has two programmes i.e. Relicord A which collects donor umbilical cord stem cells for unrelated allogeneic transplants and Relicord S which collects the same for sibling transplants. In addition, there are banks at Bangalore, Chennai and other places.

It must be remembered that education status of mothers in India is poor. Most of the women are not involved in decision-making and hence legally valid consent remains a rarity. Maternal mortality is 540 per 100,000 liver births. Only 60% of rural women and 86% of urban women receive antenatal check ups. Over 50% of rural women have no access to health facilities as they live in rural India. Only 34% of deliveries occur within the infrastructure of health facilities and skilled personnel attend to only 42% of deliveries.

**Recruitment and Consent of Donors**

Pregnant women are recruited as donors prior to delivery. Majority of cord blood banks are governed by FDA. Consent from the mother is required for collecting, processing, freezing the units, for maternal infectious disease testing, and for storage of both maternal and cord blood samples, for possible future genetic and infectious disease testing. Criteria similar to marrow donor are applied. In western world, an attempt is made to increase “minority” donation as it is difficult to find donors amongst “minority” through the national marrow donor programme.

**Collection**

The cord blood can be collected either in utero, before the delivery of the placenta, or ex-utero, after placental delivery.

**Processing and Freezing**

In early years, the cord blood was frozen as whole blood in 10% DMSO (Dimethylsulphoxide). Space considerations became paramount. Subsequently, the volume was reduced by removing red cells and plasma. Long-term storage is done at a temperature of less than -180°C. Recovery has been studied up to 12 years and it has been over 90%.

Blood from the maternal donor is tested for syphilis, HTLV-1, HIV, hepatitis B, hepatitis C and CMV antibodies. The unit is cultured as well. HLA typing is done using molecular methods. A nucleated cell count is performed before and after processing. CD34+ testing is done. About half of the units collected are frozen, the commonest reason being a “low-volume product”. The mother and baby are reevaluated at six months after delivery. Detailed medical history and repeat infectious disease tests are obtained.

**Release of Units to Transplant Centres**

Possible HLA-matched units are found via computerized registries. An advantage of cord blood is the speed of the search process as there is no living donor to contact and retest. The units are thawed using a different technique called dextran / albumin wash.
Clinical Studies

The indications for umbilical cord stem cell transplant can be divided into malignant and the non-malignant haematopoietic disorders. The first group includes AML, ALL, CML, JCML and MDS while the second group includes aplastic anaemia, haemoglobinopathies (thalassaemia and sickle cell disease), immunodeficiency disorders and metabolic disorders.

PAEDIATRIC STUDIES

Related Donor Transplantation

This is an uncommon use of cord blood. Cord blood has a low risk of GVHD as it is a naturally T-cell-depleted product. Hence, cord blood is well-suited for treatment of non-malignant diseases, where graft-versus-leukaemia (GVL) is not needed. Eurocord group reported 44 children with thalassaemia or sickle cell disease who were transplanted using related cord blood with zero mortality on day-100, four patients developing grade II acute GVHD and 8 subjects having ill-sustained donor engraftment with 2-year event-free survival of 80% for thalassaemia and 90% for sickle cell disease. Same group reported 102 children with acute leukaemia, 45% of whom had a related donor transplant of which 1/3rd received a mismatched graft. Two-year event-free survival was 40%.8

Related Cord Blood Transplantation in Patients with Thalassaemia and Sickle Cell Disease23

Allogeneic BMT from HLA-identical siblings is an accepted treatment for both thalassaemia and sickle cell disease (SCD). However, it is associated with risk of both transplant-related mortality (TRM) and chronic GVHD. Forty four subjects with median age of 5 years (range, 1-20 years) received allogeneic related umbilical cord blood transplant for either thalassaemia (n=33) or SCD (n=11). No patient died. 36/44 children remain free of disease with a median follow up of 24 months (range, 4-76 months). 7/33 patients with thalassaemia and 1/11 patient with SCD did not have sustained donor engraftment. 3/8 of these had sustained donor engraftment after BMT from the same donor. Four patients experienced grade II acute GVHD. Only 2/36 patients at risk developed limited chronic GVHD. The 2-year probability of event-free survival is 79% and 90% for patients with thalassaemia and SCD respectively. Authors concluded that related UCBT for haemoglobinopathies offers a good probability of success and is associated with low risk of GVHD.23

Unrelated Donor Transplantation

The first 25 such cases were reported in 1996.9 Event-free survival at one year was 50%. The data suggested that the engraftment could occur despite mismatching at two loci, that the risk of severe GVHD was low, and that a higher cell dose is important. The New York Blood Centre reported 562 cases with 82% being children, who underwent transplantation at different centres with differing conditioning regimens and GVHD prophylaxis.10 Engraftment occurred in 80%. The risk of grades III to IV GVHD was 20%. Younger age and a higher cell dose infused predicted improved engraftment and survival. The median nucleated cell dose/kg was 5.0 x 10^7. In a group of children with AML, transplant related mortality was 20% and 2-year disease-free survival was 40% for children in complete remission 1 (CR1), 50% for children in complete remission 2 (CR2) and 20% for refractory patients. Severe acute GVHD occurred in 10% while chronic GVHD also occurred in 10%. The low incidence of GVHD raises the question of whether a detrimental decrease in GVL effect occurs. Rocha et al have compared related cord blood recipient with related bone marrow recipients.11 There were patients with both malignant and non-malignant diseases. GVHD was lower among cord blood patients. Mortality was similar. Engraftment was delayed in cord blood group. There have been no randomized studies comparing outcomes after unrelated bone marrow or unrelated cord blood transplantation.

Cord blood transplantation has also been shown to be effective in metabolic storage diseases e.g. Hurler syndrome. The data indicates that the transplants are successful, even if they are mismatched at two antigens. Therefore, the potential donor pool is considerably increased.

Table 2 gives the results of paediatric unrelated cord blood transplants.

Adult Unrelated Cord Blood Transplantation

Although initial studies were in children, during last few years, several investigators have published the results of cord blood transplantations in adult subjects. The disorders have included leukaemia, lymphoma and myelodysplasia. Majority have received units which were mismatched at two or more HLA antigens. The engraftment is delayed, especially for the platelets. Grade III to IV acute GVHD occurred in 20% and chronic GVHD in 30%. Transplant-related mortality has been high - almost 50% by day-100 (infection being the leading cause followed by regimen-related toxicity). Twenty five
percent of recipients are alive and disease-free by the end of two years. A higher number of CD34+ cells in the graft correlated with superior outcome.12

The International Bone Marrow Transplant Registry (IBMTR) has reported the results of a study comparing survival after unrelated cord blood transplantation to survival after unrelated marrow transplantations.13 Cord blood transplants were 1- or 2-antigen-mismatched while marrow transplant were matched or 1-antigen mismatched. Deaths due to infection were highest in the cord blood recipients while acute GVHD was less likely. The 3-year leukaemia-free survival was 30% for HLA-matched marrow transplant, 25% for cord blood transplant and 20% for 1-antigen-mismatched marrow transplant. In all studies, early transplantation-related mortality secondary to infection is high while the cell dose invariably correlated with the final outcome.

Four strategies have been developed to improve the outcomes:

1) Pooled or sequential cord blood transplantation
2) Cord blood expansion
3) Combined cord blood and haplo-identical bone marrow transplants
4) Non-myeloablative or reduced intensity conditioning regimens with 1, 2 or 3

**Pooled or Sequential Cord Blood Transplantation**

This is done to increase the cell count, improve engraftment and immune reconstitution. The results appear promising in terms of neutrophil recovery and a low risk of GVHD but more work is needed. Reports have suggested that crossed immunologic rejection does not occur.14

**Cord Blood Expansion**

There have been many preclinical studies trying to expand the cord blood cells using a variety of cytokine mixtures.15 A combination of stem cell factor, G-CSF and megakaryocyte growth and development factor has been used. One approach is to use a continuous perfusion device. The ex vivo-expanded cells are well-tolerated; however, the engraftment rate remained unchanged. The 3-year probability of event-free survival was 40%.

**Combination Cord Blood and Haplo-identical Bone Marrow Transplants**

The Spanish group has pioneered this unique approach. Neutrophil engraftment occurred at 12 days (range, 9-36 days). 5/11 had disease-free survival at 6-43 months.16

**Future Trends**

What could be the future of umbilical cord blood transplantation during next one decade? I suppose, more and more centres would perform pooled or sequential cord blood transplantations for adults. More trials would be performed to define the best application of cord blood transplant. There may be a role for cord blood transplant in place of matched unrelated transplant in elderly patients who have high risk of GVHD. Additional experience in benign diseases is anticipated. One application could be autoimmune diseases where there has been some success with autologous transplantation and the low risk of GVHD makes cord blood transplantation attractive. Another application is in HIV disease, in which the possibility of gene transfer to a haematopoietic stem cell reservoir may eventually be possible. In theory, an allogeneic stem cell vaccine may replace haematopoietic stem cells infected with HIV with uninfected umbilical cord blood cells.

Exciting applications of cord blood transplantation are coming up and these include repair of damaged myocardium or neural tissue. Cord blood cells have better capacity than marrow cells for multi-lineage differentiation.17 Recently, cord blood cells have been shown to improve functional recovery in rats who have been subjected to strokes, by middle cerebral artery occlusion.18 Infarct volume was reduced and behavioral performance increased.

**Conclusion**

It may be concluded that the patients who do not have a matched sibling donor, should search simultaneously from unrelated bone marrow and unrelated cord blood. Patients without time to find an unrelated bone marrow donor, or who do not have a 10/10 or 9/10 unrelated adult volunteer donor should be considered for cord blood transplantation. The goal should be to procure a safe donor source, either bone marrow or cord blood, in a timely fashion so that none is denied cure by transplantation.

Umbilical cord blood cells have the advantages of relative ease of procurement, higher progenitor count and proliferative potential and lesser risk of severe acute GVHD while the disadvantages include expensive banking, relatively small number of mononuclear cells and delayed engraftment.

The problem unique to cord blood transplantation is early infection. The reason for this is delayed immune reconstitution. Over 50% experienced bacteremia, 60% developed CMV infection while 10% had documented fungal infection. Recommendations for management include prophylaxis against viral, bacterial and pneumocystis infections; frequent monitoring of CMV antigenemia; and replacement of gammaglobulin for immunoglobulin G levels below 500 mg/dl.

An additional problem is that the original donor cannot be re-contacted for donor lymphocyte infusion or more cells. Back-up donor sources should be reviewed prior to cord blood transplantation. Table 3 summarizes certain issues unique to cord blood transplantation.

India has to make serious efforts to establish transplant machinery. Transplant centres, registry and stem cell
Table 3: Unique issues related to cord blood transplantation

<table>
<thead>
<tr>
<th>Feature</th>
<th>Cord blood</th>
<th>Unmanipulated BMT / PBSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Availability</td>
<td>Limitless</td>
<td>Limited</td>
</tr>
<tr>
<td>Waiting period</td>
<td>Small</td>
<td>Large</td>
</tr>
<tr>
<td>Donor attrition</td>
<td>None</td>
<td>Significant</td>
</tr>
<tr>
<td>Matching</td>
<td>4 / 6 or other</td>
<td>10 / 10 or 9 / 10</td>
</tr>
<tr>
<td>Collection procedure</td>
<td>Simple</td>
<td>Complicated</td>
</tr>
<tr>
<td>Risk of collection</td>
<td>Negligible</td>
<td>More</td>
</tr>
<tr>
<td>GVHD risk</td>
<td>Less</td>
<td>High</td>
</tr>
<tr>
<td>Cell yield</td>
<td>Less</td>
<td>More</td>
</tr>
<tr>
<td>Engraftment</td>
<td>Slow</td>
<td>Fast</td>
</tr>
<tr>
<td>Risk of infection</td>
<td>Higher</td>
<td>High</td>
</tr>
<tr>
<td>Graft durability</td>
<td>Up to 16 y</td>
<td>Up to 30 y</td>
</tr>
<tr>
<td>Donor</td>
<td>None</td>
<td>Available</td>
</tr>
</tbody>
</table>

banks have to develop across the country. Perinatal care providers have to be made knowledgeable about umbilical cord blood banking and transplantation. Safe management of obstetric delivery must not get compromised. Public and private cord blood banking must strictly adhere to standardize policies and procedures. Indian government should establish registration, regulation and accreditation of blood bank centres and banks. Indian Council of Medical Research (ICMR), Department of Biotechnology (DBT) and Drug Controller General of India (DCGI) have important role to play.

REFERENCES