Optimization of the Inventory Size of the Public Cord Blood Program – The Indian Context

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Abstract

Background: There are reportedly more than 250000 cord blood units [CBU] available in cord blood banks worldwide, yet, there are many who are unable to get a suitable match. Being the pioneer to set up the first and only public cord blood bank in India, we needed to strategize the pool size that will meet the transplantation needs of the people of Indian origin, worldwide.

Purpose: To define the optimum size of this public cord blood repository [CBR] that will give at least one 5/6 HLA match to a defined number of cord blood graft requests.

Methods: We checked the trend of human Leukocyte antigen [HLA] match graft offerings, to 112 random requests from a database of 1800 CBUs. The HLA match function was performed using an ‘in house’ built and validated software. The pattern of availability of the matches was used as the basis of our study. We then performed a probability analysis to check the probable pool size that would offer at least one acceptable match to all the 112 requests.

Results: With an inventory of 1800 units, we could offer 4/6 matches to about 99% and 5/6 matches to approximately only 29% and 6/6 matches to only 7% of the 112 random requests. Thus, acceptable matches were offered to about 30% of the requests received in the period and database considered for this study.

Conclusion: By employing probability analysis, we concluded that, by doubling the size, we will probably offer at least one 5/6 match to each requester, and from a pool size of about 55500 grafts, we may offer a full house (6/6 match) to the same number of requisitions. Genetic homology between the recipient and donor base, increases the probability of match availability. A good ethnic representation of the Indian population in our CBR plays a significant role in match availability.

Introduction

Seventy percent of patients of Indian origin, who need a bone marrow transplant cannot find a match within their own families and will have to find an unrelated matching donor. But the representation of this population in the existing world bone marrow registries is abysmal.1 In India, around 1000 patients require bone marrow transplants (BMT), every year, but only around 200 patients are receiving this treatment annually probably due to non-availability of suitable matches.

Cord blood is a widely accepted source of progenitors for haematopoietic stem cell transplantation, (HSCT). Unrelated public UCB banking programs have been initiated in many countries around the world, with more than 250,000 units banked for public use and an estimated more than 10000 unrelated UCB transplantsations having been performed till date.2

Although there have been cases where the patients found matching donors from people outside their ethnic group, the most likely match is the one from his or her own racial or ethnic group. Umbilical cord blood has substantial logistic and clinical advantages, such as much faster availability of banked cryopreserved umbilical cord blood units (within 2 weeks after starting search); lower frequency and severity of acute graft versus host disease (GVHD); lower risk of transmission of infections by latent viruses and absence of risks to donors. A cord blood transplant can be performed with a 4 out of 6 HLA allele match (considering the HLA-A, -B and –DRB1 genes) but as per majority of reports, the unit should be matched for at least 5 out of 6 HLA alleles and should contain at least 2.5 x 10^7 nucleated cells per kg body weight of the recipient.3

Racial and ethnic groups vary in the diversity of their human leukocyte tissue antigens (HLA) haplotypes. In groups where many members have similar HLA types, not as many potential donors are needed. In groups with wide polymorphism among their HLA types, relatively more donors are needed.4

Both culturally and biologically, India is one of the mega diversity countries of the world. This pertains to human genomic diversity as well. Although less explored, HLA is no exception. HLA profile varies based on regional, caste, community and ancestry, migration pattern etc. Different regions and populations of India have their own HLA profile, and “more related caste groups” have more similar profiles. The entire Indian populations have not been extensively studied for HLA polymorphism and there is sporadic literature on exact allele frequencies as on date.5

Thus, there is a wide difference between the genetic types of people from state to state. In recent times, due to marriages across different communities, racial mingling has taken place and the type of haplotypes observed in the offspring is unique. It poses a challenge, when such people need an HLA match for transplantation, as the donor base is also required to be the same. Availability of genotypes with blank and homozygous alleles in the donor and recipient populations will also have a major bearing on the magnitude of the matches.

India has crossed the billion mark in population. In the
US alone, there are over 1 million Indians. Yet the number of potential bone marrow donors is relatively low for the South Asian community, showing as low as 44000 South Asians (Indians, Pakistanis, Sri Lankans, and Bangladeshis) on the NMDP registry.

Our public cord blood program involves cord blood donated voluntarily by the mothers from various birthing centres. Mumbai is a highly cosmopolitan city with larger pockets where concentrations of certain communities are common. Such settlements are in existence for decades. We used this to our advantage to bring about proper representation of the donor base in our cord blood bank by selecting birthing centers from these areas. Thus, the ethnic representation at the cord blood repository was strategically planned.

Aims and Objectives

We wanted to evaluate and analyze the match frequency obtained by the search function, for all requests received from Dec. 2003 to Jan 2009. Also, we needed to check the impact on availability of matches, by increasing the pool size and relaxing the match criteria.

Finally, we estimated the probable size of the repository that might find at least one 5/6 or 6/6 match for these graft requests.

Material and Methods

Database

We initiated our analysis based on the assumption that both donors and recipients have equivalent HLA haplotype frequencies. Only 1800 CBUs were selected for the match search function out of a total of 3600 banked units. The selection was based on a certain cell count and viability. All selected CBUs on an average have cells enough to treat at least 30 kg individual calculated at the rate of 20million per kg body weight.

Patient Demography

Each request contained the HLA type and major community designation, [self declared] for whom a cord blood graft was sought. All patient requests were from Indian origin.

Search Function

The HLA data base pool in the repository was increased in batches of 450. We have put in a system wherein if we did not get a graft of choice in the first search, it will automatically search every time the pool size is increased, until the patient withdraws the search request. It is assumed that he is still interested in getting a good graft. Interim manual searches are also possible. Thus, several requests had a chance to be “searched” several times in the last few years. The number of units we have issued for transplantation is very few and hence for the ease of calculation, we have decided to retain it in the data base.

HLA typing

The HLA typing of the banked CBUs was performed and approved by our CAP and NABL accredited laboratory. We looked for matches as a function of three levels of HLA matching (4, 5 and 6 out of 6 alleles) by considering HLA A, B and DRB1.

The method followed for typing the HLA A, B and DRB locus is the low resolution molecular SSP method using the GenoVision, SCORE™, Olerup SSP® kit (Qiagen, USA). High resolution is performed only if a graft is selected for transplantation, and not at the search level.

Estimate of probability of finding an unrelated donor match/mismatch

We estimated the probability of match availability using the method where, m(k) denotes the probability of a match for at least k out of six antigens for a patient with phenotype {AaBbDd}, where k could be 4, 5, or 6. Thus m(6) (AaBbDd) would represent the probability of a complete match for phenotype {AaBbDd}, m(5) (AaBbDd) the probability of a one antigen mismatch, and so on. In the definition of what constitutes a 4 or 5 out of 6 match, we assume that there is matching for at least one antigen at each locus. Thus, for a 4 out of 6 match we insist that there is matching for one HLA-A antigen, two HLA-B antigen and one HLA-DR antigen (but not necessarily on the same haplotype).

Exact formulas were determined for each of these probabilities. For example:

\[ m(6) = p(AaBbDd) + p(AXBbDd) + p(AaBbXd) + p(AaBbDXd) - 5p(AaBbDd) \]

where X denotes an arbitrary antigen at the locus. Thus, p(AaBbDd) includes possibility that X is A1, A2, A3, A11, A23, and so on. This philosophy was incorporated in the HLA search module of our software called ‘Regen’.

This was built over Windows 03 server and based on dot net frame work. Regen starts running the text based “search” activity over the database and completes the process in less than 1 minute with 100 percent perfection. Regen has a “SQL injection attack” prevention system and is able to distinguish the match category as 4 of 6, 5 of 6 and 6 of 6 alleles or none. Each HLA antigen is treated as a character, irrespective of the positioning in the excel sheet. Thus the search looks for all the possible combinations of the antigens in the database and hence is termed as a ‘wild search’. Fig. 1 shows the snapshot of our representative search screen.

Results

We observed that CBR’s public cord blood program had a good ethnic representation of the Indian population as planned.7 We have a concordant donor and recipient pool. Table 1 shows 80.5% of the units stored at the repository were from Hindus, 13.4% from Muslims, 0.9% from Sikhs 0.8% from Buddhists and remaining consisted of smaller sub-sects like Mahars, Kunbhis, Marwaris, Kutchis, Jains, Lohanas, Parsis, Christians etc. which is reflective of India’s ethnic population distribution.

An analysis of 112 requests shows that most search requests were from the patients with Thalassemia (46.4%) as can be seen in Table 2. On an average, the absolute total nucleated cell counts

Fig. 1 : R-eGen Search screen
of the cord blood grafts varied from $44.31 \times 10^7$ to $202.48 \times 10^7$ million cells, with a median total nucleated cell count of $87.60 \times 10^7$ million cells and a viability of 94.71%.

We could offer 4/6 matches to about 98.2% of the requests while the percentage dropped to 53.6% when the stringency increased to 5/6 match level as shown in Table 3. There were 4 cases for which we provided a full house match [6/6]. There were only 2 requests out of 112 requests, for which we could not offer a single match.

Figure 2 shows the frequency of graft availability as we increased the pool size. It can be seen that a 6/6 match was offered only to two patients when the pool size was 450 and to 4 patients when it was increased to 1800. Of these, two patients were offered two full house matches each. However, more than 100 people did not get a single full house match from this pool. But by lowering the stringency to 5/6 and 4/6, there was a clear improvement in the chances of availability of matches. It is to be noted that most patients were offered more than one match, in some cases even as many as three to four matches to choose from. Yet, 52 of the 112 requesters did not get even a single 5/6 match from 1800 grafts.

By applying the probability formula on the existing data, keeping the number of requests constant, we could plot the projected pool size that will potentially throw out at least one 5/6 match for each request [Fig. 3].

**Discussion**

The phenotypic frequency at serological level of various populations in the Indian subcontinent is not available comprehensively. There is no extensive study on HLA polymorphism in the said population who forms our donor pool. Sporadic reports however, are available for research purposes.

Since the probability of a patient of Indian origin finding a match on any of the current international registries is very low, one of the basic goals in setting up the public cord blood repository in India was to cover most of the Indian ethnicities so that it can address cord blood graft requests from people of Indian origin. Congenital, hematological, abnormalities was one of the key unmet medical needs, that the cord blood program was intended to address. Thalassemia is more prevalent in Indian communities like Sindhis, Lohanas, Kutchis, Marwaris, Kunbhis, etc. Hence, in a well represented CBR like that of ours, there is a definite theoretical chance of an affected individual from any of these communities, finding a suitable cord blood graft.

We have analyzed a total number (n=112) of unrelated HLA match requests received by us from Dec. 2003 to Jan. 2009. Out of 112 requests, 49% were from males, and 51% from female patients. The majority of the requests (65%) were from the age group of <10yrs and 25 % were in the 10 - 20 yrs age group. This gave us an idea of the approximate body weights and helped us with the probable dose requirement calculations.

The ethnic representation of the donor pool at the CBR and the Indian population are similar as intended. We had recruited voluntary donors through appropriate selection of the community birthing centers. Our observation on the community breakup of our donor base is reflective of the findings of cord blood banks in the west which constitute 43% Caucasian, 30% African-American, 17% Hispanic, and 10% Asian-American cord blood units, representative of their population base.8

The Japanese community has a high level of common haplotypes, which naturally means that a Japanese patient seeking cord blood matches will have a very high chance of success from the Japanese cord blood banks.

The allele frequency of the different donors at the CBR revealed that HLA A1, A2, A3, A11, A24, B5, B35, B40, B44 were the most frequent alleles while HLA A28, A36, A69, B14, B16, B38

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**Table 1: Ethnic representation of units stored at the CBR**

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Percentage population</th>
</tr>
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<tbody>
<tr>
<td>Hindus</td>
<td>80.5</td>
</tr>
<tr>
<td>Muslims</td>
<td>13.4</td>
</tr>
<tr>
<td>Sikhs</td>
<td>0.9</td>
</tr>
<tr>
<td>Buddhists</td>
<td>0.8</td>
</tr>
<tr>
<td>Jains</td>
<td>0.6</td>
</tr>
<tr>
<td>Others</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Table 2: HLA requests, based on clinical diagnosis, figures in parenthesis are absolute numbers**

<table>
<thead>
<tr>
<th>Clinical Indications for Cord Blood Requests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age wise</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>&lt;10 Yrs</td>
</tr>
<tr>
<td>10-20 Yrs</td>
</tr>
<tr>
<td>&gt;20 Yrs</td>
</tr>
</tbody>
</table>

**Table 3: Match frequency at different levels**

<table>
<thead>
<tr>
<th>Degree of match</th>
<th>6/6</th>
<th>5/6</th>
<th>4/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of matches available</td>
<td>6</td>
<td>147</td>
<td>1767</td>
</tr>
<tr>
<td>No. of patients to whom it was offered</td>
<td>4</td>
<td>60</td>
<td>110</td>
</tr>
<tr>
<td>Total no. of requests in the study period: 112</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 2**: The incidence of matches with increasing pool size at various match levels

**Fig. 3**: Probability graph for obtaining 100% match requests.
were the less frequent alleles, which was almost reflective of the observations made by Kankonkar et al.9 and Shankarkumar et al.10

Contrary to the earlier perception that a bank should have at least 50000 units for a population of 60 million inhabitants to address the medical needs, our match rates with the present data base were better, perhaps due to planned ethnic representation.11 Apart from HLA matches and the degree of match, other important factors considered for transplantation were dose availability, whether or not the unit is red blood cell (RBC) depleted, it’s CD34+ content, HLA-A and -B match at high resolution, match at HLA-C locus, availability of an attached segment for confirmatory typing, the accreditation status of the cord blood bank.

Most banks prefer to collect only the largest units of more than 70 ml so as to be able to address at least 2×10^7 nucleated cells/kg.15 Eurocord has recommended that units less than 3×10^7 TNC/kg were not to be used.12 The cell content of our grafts are in comparison with those available with the leading international cord blood banks as we have also applied similar stringent criteria.13

The probability analysis on the data and observations made in the current inventory shows that if we double our pool size, we could offer at least one 5/6 match to each request, and a 6/6 match to about 7% of them. But, if we increase our pool size by 15 fold i.e. between 55500 to 60000, there is a good probability of providing 6/6 matches to 100% of the requesters (Fig. 3).

This perhaps is due to the fact that all the matches are for Indian patients and the pool is very Indian in nature. We foresee no problem in getting matched unrelated grafts for most of our population who otherwise do not stand to get a good match elsewhere. This, in our mind addresses a very important unmet medical need. These calculations are based on phenotypic profiling of HLA using molecular methods of low resolution. The number of alleles that can be identified by higher resolution is expected to be definitely higher. Due to the phenomena of linkage disequilibrium, donors that are HLA matched at the antigen level may be more likely than chance alone to be also matched at the allelic level. Thus, these projected numbers could be considered as underestimates of the size of pool required, or conversely, overestimates of the probability of finding an acceptable donor.

Conclusions

The use of umbilical cord blood, which can be cryopreserved and hence available indefinitely on an ‘on call’ basis, has raised hopes of expanding allogeneic stem cell applications. Preliminary data show that the stringency for HLA-matching might be relaxed for a cord blood donor due to the immunologically naive state of cord blood cells.11 Thus, if a 4 of 6 matched unrelated cord blood graft is acceptable; the size of cord blood registry required would be in the low thousands.

Our public cord blood repository is a national treasure. We have been able to find good matches at acceptable levels, for the Indian patients. Homogeneity of the donor and recipient population determine the probability of finding a match and the time taken to achieve this probability is a function of size of the repository. Increasing the size of the repository to two or three times will increase the probability of finding a full house match albeit, very marginally for the present level of requests. However, increasing the size to fifteen times will probably make a significant difference. By conforming to International Standards such as those prescribed by the AABB, FACT etc, our aspirations are to offer increasingly diverse source of high-quality grafts to worldwide transplant community for their patients of Indian origin.

Our experience, expertise and knowledge has received recognition that has awarded us the AABB accreditation for our quality systems, and we hope to make this resource available globally. The great and long ‘Indian Heritage’ that has resulted in diversified cord blood units from different ethnic and racial groups, may need to be better understood by considering several other aspects too. We might then have answers to most of the transplantation needs in India.

Acknowledgement

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