Indian J Hematol Blood Transfus 25(3):111–117

ORIGINAL ARTICLE

India's first public cord blood repository – looking back and moving forward

Chandra Viswanathan · Preeti Kabra · Vanita Nazareth · Manisha Kulkarni · Arunava Roy

Received: 15 June 2009 / Accepted: 18 August 2009

Abstract Umbilical cord blood is an established source of stem cells useful for hematopoetic reconstitution. The first clinical transplantation in France by Eliane Gluckman in 1988 using HLA matched umbilical cord blood from a sibling on a 6-year-old boy with Fanconi's anemia is an example of a successful transplantation. So far, more than 8,000 patients worldwide have been treated for malignant and inherent blood disorders [1, 2]. Our cord blood repository (CBR) was established as the part of the Life Sciences initiative, almost 7 years ago. The cord blood program consisted of developing a good network of obstetricians and social workers, develop manpower in various aspects of the banking activity, develop methods of process and analysis and above all, increase the level of awareness among the medical, paramedical fraternity and the general public on the cord blood program. The present paper gives a detailed account of our experience as we set up the repository.

C. Viswanathan · P. Kabra · V. Nazareth · M. Kulkarni · A. Roy
Reliance Life Sciences Pvt. Ltd.
R-282, DALSC, Thane - Belapur Road,
Rabale, Navi Mumbai,
India

C. Viswanathan (✉)
E-mail: Chandra.Viswanathan@ril.com

Keywords Cord blood unit · Human leukocyte antigen · Total nucleated cell count · Cryopreservation · Repository

Introduction

India is a country unified by its differences. As the second most populous nation on the globe, India has 28 states, 7 union territories, 22 national languages with more than 2,000 total ethnic groups where 6 of them are major as shown in Table 1 [3].

The present requirements of hematopoietic stem cells for our subcontinent are not met by the banks in the rest of the world as these banks cater to different population and ethnicities. The implementation and operation of an unrelated umbilical cord blood bank is analogous to the establishment of a new blood banking program.

Worldwide, the growth of autologous and related hematopoietic stem cell transplantation (HSCT) appears exponential; however, growth in the allogenic setting is relatively lesser due to lack of availability of suitable human leukocyte antigen (HLA) compatible grafts for patients [4]. The hardship faced by Asian patients in general, and Indians in particular, while awaiting a suitable bone marrow match turned them towards 'cord blood' as a ray of hope.

The healthcare provision for minority ethnic groups of India and Asia spread across the globe and the large, young population of ethnically and genetically diverse individuals in our country, is a very important consideration in the set up of this initiative.

Our public cord blood banking facility, licensed by the Indian FDA, is now operational for a little over 7 years. We have about 3,500 voluntary samples stored with us in the public cord blood banking facility, available for clinical use. We have gradually extended this banking service to families

Table 1 Ethnic representation of the grafts at the CBR

Ethnicity	Percentage population
Hindu	80.5
Muslim	13.4
Sikh	1.2
Buddhist	0.8
Jain	0.6
Others	0.4
Religion not stated	3.1

as well, to store cells for their own future use. We narrate our experience on this initiative.

Aims and objectives

Our objective was to establish various components of the cord blood program in order to produce cord blood derived stem cell products of requisite quality that would address clinical needs.

The establishment of this program consisted of laying down a quality management system that includes quality assurance, a robust donor education and recruitment, cord blood collection, transport, processing, cryopreservation, donor data management and HLA search request processing. It was not in our scope to set up our own transplant center as part of this program. This paper also includes challenges, opportunities and the progress made by us so far.

Observations

General awareness

Though the clinical applications on the use of cord blood was gaining momentum in the other countries, the level of awareness among the masses, the medical and paramedical professionals on the potential uses of cord blood was relatively low in this country, at the start of the program.

The collection of umbilical cord blood is a multi-step procedure that begins with donor recruitment. We required an informed consent from donor mothers well before delivery. There were several levels of resistance to accept the fact that cord blood, which is normally a bio waste, can be put to fruitful use. To bring about a paradigm shift in the understanding of this new subject, we had to learn it ourselves initially and generate interest among the medics and paramedics. This had to be done on a continuous basis. Sadly, it was not easy to convince the clinicians and paramedics as there were no success stories on the use of cord blood in this country then. Most clinicians of the earlier generation were happy practicing traditional medicine, rather than wanting to move to newer tools that are less tried and tested. In the Indian context, the senior physicians were extremely skeptical on the use of cord blood. In sharp contrast, the younger generation of physicians, were more open, receptive, and willing to learn by being participative. Interestingly, the internet access to information and overseas training programs taken up some of the doctors, added value to the program.

Establishment of collection centers

India has a high birth rate; childbirths are conducted by large corporate hospitals, public hospitals, private nursing homes, not for profit foundations, etc. It may be interesting to note that even today, some mothers deliver at home in rural India. With such complex healthcare delivery systems, we were looking out for birthing centers that will meet our quality requirements so as to enroll them as our collection facility. We found extreme resistance to enrollment, although many of them appeared cooperative and interested. We were keen on signing up with birthing centers catering to certain specific communities to ensure adequate ethnic representation. Antenatal practices at the hospitals and the track record of the clinicians and their capability to handle high-risk pregnancies and above all, their level of interest in the cord blood program were our important parameters. We signed up only with those collection centers that were keen on this program and rejected those that perceived it as an additional burden on the existing manpower and financial resources. It has taken us tremendous efforts to reach out to them on a continuous basis, keep them motivated, and send our counselors to talk to the mothers including putting up information to the doctors through our information booklets. Finally, we have been able to develop and maintain smooth working relationships with obstetricians, nurses, delivery suite personnel and hospital administrators at each of our participating collection centers. Further, these centers had to consent for being audited by the regulatory agencies at short notice.

These activities even now require immense efforts in terms of organizing numerous meetings and adequate communication and ongoing supportive efforts to maintain proper collection of cord blood in an area, which is not routinely designed or designated for such work.

Consenting

The cord blood consenting process was an experience worth mentioning. Trained staff or the physician himself administered consenting. The issues faced varied depending upon the birthing centers and the socio financial status of the customer base they catered to. We came across mothers with varying levels of literacy, education, and motivation. With a little orientation talk from our trained staff, most mothers were willing to see the importance of the program and participated and consented instantly. Apart from the mother, in certain communities in India, there were other decision-making members who mattered. Provisional consenting

was administered to all expectant mothers in a language they understood best, before a final formal consent was obtained. Our aim was to make sure that no woman would be pestered for consent by any of our trained staff without giving adequate decision making time to the donor and her family.

While this challenge is reasonably overcome today, we have started depending more on the obstetrician and his trained team because, in the Indian context, the patients have more faith on her family obstetricians rather than a counselor who is an outsider. Often, unremunerated donation of cord blood to a public cord blood program especially run by a private limited company invited several questions, which had to be addressed appropriately from our end. The medical history form was developed and approved by the institutional ethics committee, and is based largely on the format laid down by the Foundation for Accreditation of Cellular Therapy (FACT) and the Indian Council of Medical Research (ICMR) guidelines [5, 6].

Personal and medical history

Needless to say, that the final product processed from the cord blood needs to be very safe for human use. It is expected to provide long-term sustained hematopoietic recovery after transplantation. The medical history of the mother and laboratory testing for mandatory infectious disease markers form important inclusion criteria. These are similar to those applied to blood and marrow donation. History of potential risk of genetically inherited diseases from the donor to the recipient can be avoided if a detailed parents' family history is taken. Though the documentation process of the medical history of the donor and the family was very lengthy, exhaustive and cumbersome, it is a regulatory requirement [5, 6]. Eliciting history of promiscuous behavioral tendencies when a woman comes to an obstetrician for a physiological process like birthing was not socially acceptable; hence we depended on the obstetrician alone to fill in these details. Today, this task appears less cumbersome, but the challenge of following up on the baby's progress for a period of 6 months is still quite daunting. Migration of families and change in contact numbers adds to this problem, and is yet unresolved.

Training needs

Our role in organizing educational and motivational sessions over the last 7 years has been very significant. Today, this is evident by greater willingness of doctors and paramedics to participate in this program. Training them for aseptic cord blood collection, documentation, etc. was quite difficult due to the fact that a new practice had to be introduced additionally into an already existing system. It called for extra planning, protocol development and training of all those involved in the program. We had to invest time and efforts to keep monitoring the effectiveness of our training, which we continue to do even today. Above all, an elaborate documentation is the part that they disliked most. Busy practitioners normally would like to leave it to their trained assistants. Consistent efforts and persuasion from our side to get the documents right and get the samples transported on time has rewarded us with much better compliance.

As a part of learning and competency development for all the employees, training needs are identified and relevant training programs are provided on specific areas like cell culture techniques, good manufacturing practices and validation practices, etc. Understanding and implementing quality policies, new test methodologies, proficiency testing, and participation in external quality programs, etc. have become a regular activity.

Collection

There are several methods to collect the placental cord blood. It could be either while the placenta is *in utero* or after it is expelled out or a combination of both in order to get started with good volumes and good cell counts. For an experienced obstetrician or a nurse, cord blood collection as described by Rubeinstein et al. is easy. Every cord blood must be accompanied by a maternal sample. Compliance in this regard is very satisfactory now as compared to earlier.

In the first year of our operations, volume of blood collected was as low as 30 ml in about 40% of our collections, perhaps due to external or negative pressure in the umbilical vein. With better training, we have been able to improve collection volumes, which meet our inclusion criteria (a minimum of 60 ml). Our continuous efforts are to educate the collection staff to minimize the variability in the quality of the cord blood collected. Thus our rejection rates on account of volume have come down relatively from a 40% then, to about a 10% today. The other important inclusion criterion is cell counts.

Of all the units stored, more than 80% were from Hindus, constituted by Maharashtrians, Bengalis, Marwaris, Gujaratis and Sindhis, about 13% from Muslims and the remaining from other communities (Table 1), which is reflective of India's ethnic population distribution (Table 2) [3].

Logistics and transportation

Costeffective biological couriers, with capabilities to transport samples under stringent transit conditions were not easily available. India is a tropical country and not all cities are well connected by air. Hence, we continue to depend on more than one mode of transport to get samples from rural and remote areas. Therefore, the public program had to make do with nearby birthing centers where logistics could be better managed.

Airport authorities especially due to the recent security threat issues do not generally permit movement of

Table 2 Showing population distribution religion wise in India as per 2001 Census

Composition	Hindus	Muslims	Christians	Sikhs	Buddhist	Jains	Others	Religion not stated
% total of population 2001	80.5	13.4	2.3	1.9	0.8	0.4	0.6	0.1

biological samples without the mandatory security screening. While we spent time in the early years to convince the authorities not to screen the live cells, recent literature on this subject, points out that the dose emitted is very negligible for causing any harm to the cord blood or the final product, whatsoever [7]. A well-defined and validated logistics process planning has been developed that brings cord blood to the facility at temperatures between 4°C and 22°C within 36 hours of collection.

Processing infrastructure

In India, regulatory requirements in cord blood are yet unclear. Yet, we applied the traditional cGMP and GLP knowledge available in the area of biologicals. We applied our learnings of sterile biological processing to our processes and often referred to the international guidelines to evolve our own processes and policies [8].

But as a pioneer in establishing this project on a production scale in this country, developing and validating the process protocols was indeed a new challenge. The technical expertise to support our specific process had to be obtained through a well structured training process for all those involved in the program right from technical staff, quality control, the engineering and instrumentation, the data management staff, the molecular medicine, health to safety staff. We set up in – process checks and balances including cell based assays for monitoring the quality of the product. Standards and controls were not easily available, and so, in-house standards and protocols were developed. Seven years now in this business, we have an excellent quality management system and are participating regularly in external quality control programs. Such quality requirements are absolutely essential, if one plans to send the products to other geographies.

Process

Units that meet our stiff inclusion criteria are considered for processing. Setting the requisite acceptance criteria on the cord blood had to be made by a process of learning. Today, we have set very stringent acceptability standards in order to arrive at a qualitatively superior product that will match transplantation needs. We follow the Rubinstein's process of volume reduction, erythrocyte removal, followed by enrichment of nucleated cells [9]. After addition of hydroxy ethyl starch, the sample undergoes 1 hour of sedimentation. Sedimented red blood cells are removed and the remaining leukocyte rich plasma is then centrifuged. After centrifugation, plasma is expressed and the leukocyte rich fraction is transferred to the freezing bag. It undergoes controlled rate freezing and is then stored at –196°C in liquid nitrogen in the Bioarchive system (Figs. 1–6). Cell based products are not handled like traditional pharma products, in terms of batch size, retention, sampling and storage. The challenge of bringing about consistency in production, QA approvals of raw materials prior to production, "stability data" generation especially for non-traditional products was a challenge to our quality group. Great emphasis is laid on adhering to the Standard Operating Procedures (SOPs), which cover all necessary details of the process, testing, rejection, storage, retrieval, etc. A greater challenge was to achieve highest recovery without customizing the process protocol.

Fig. 1 Receipt and accessioning of the cord blood units

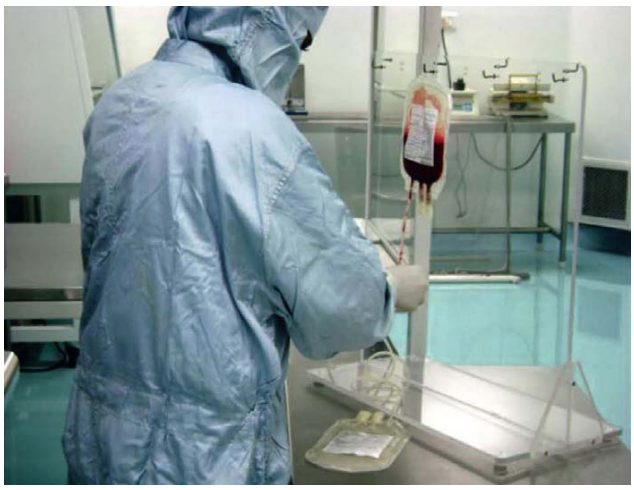

Fig. 2 Processing of cord blood units in a clean room

Fig. 3 Processing bag set

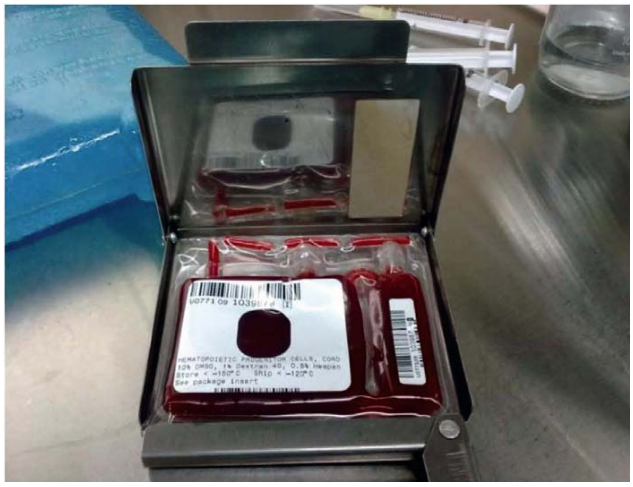

Fig. 4 Processed cord blood unit in SS canister

Fig. 5 Freezing and storage of cord blood units

Storage

While finalizing the freezing protocol, we were confronted with issues such as the ideal final product volume. There were as many presentation volumes as the number of cord blood banks. As there was no regulation on them too, differ-

Fig. 6 Cryoshipper for transportation of the final product

ent bankers had the choice and liberty to follow their own practices and storage conditions. We finalized our protocols after understanding the impact of improper freezing arising out of different storage bag designs, and thus have a fixed final product volume, with fixed levels of cryoprotectant, namely 25 ml final product that includes 5 ml of Di Methyl Sulphoxide (DMSO).

Stem cell products cannot be compared with traditional pharma and biopharma products in the matter of determining shelf lives. Stem cell enriched cord blood is a very stable product for extended periods of time in special subzero temperature conditions for durations as long as 21 years, as per literature reports. Stability experiments continue to be performed by cryobiologists, to understand this further. Yet, it was a tough challenge to convince doctors and patients that the cells were viable for that long in liquid nitrogen. Our own real time studies of product stability at –196°C for 7 years are comparable to the observations by others in the field.

Requests for transplantation

We received several requests for HLA matched grafts not only from within the country, but also from outside. We have been able to offer cord blood grafts with 5/6 matches to most of the patients and even 6/6 matches to 5 of them, although not all of them were transplanted. This is quite contrary to our earlier understanding that the chances of HLA match are proportional to the number of cord blood units banked. We had received 123 requests from January 2008 to May 2009 for the cord blood grafts stored at our repository. Table 3 shows the number of 6/6, 5/6 and 4/6 matches made available to these patients. Table 4 shows the age-wise and disease-wise distribution of these patients, most requests coming from patients <10-year-old as the cord blood cell dosage is sufficient mostly for pediatric age-group. Thalassemia is more common in certain communities. As these communities are well represented in our bank, we could provide matches to patients with thalassemia.

As shown in Table 5, the viable nucleated cell count ranged from 320.6–2004.1 million cells with a mean of

Table 3 HLA matches offered from the CBR for 123 requests. Further lower matches have not been considered for discussion

Degree of match	6/6	5/6	4/6
No. of matches available	7	82	571
No. of cases to whom this was offered	5	36	122

Table 4 Breakup of requests age-wise and disease-wise

Age		Disease conditions	
<10 yrs	(80) 65%	Thalassemia	(57) 46.40%
10–20 yrs	(30) 25%	Leukemia	(16) 12.9% (ALL)
			(23) 18.5% (AML)
>20 yrs	(13) 10%	Lymphoma and myeloma	(27) 22%

An analysis of all the requests received.

Table 5 Graft characteristics including viability and cell counts

	Viable nucleated cells (million)	Viability (%)
Minimum	320.6	72.35
Maximum	2004.1	98.98
Mean	829.7	94.71

829.7 million cells per graft, which is an adequate dose for patient weighing up to 40 kg. The mean viability of the cells was 94.71%.

So far, we have issued 9 cord blood samples for transplant from our cord blood repository (CBR). In India, the Tata Memorial Hospital performed the first UCBT in 1996 [4]. There is no published data available on the total number of cord blood transplants performed in India. Lay press reports indicate that about 15–20 cord blood transplants have been conducted [10].

Other challenges, from the organizational viewpoints include high investments for the establishment and running of this program. Equipment needed for storage and retrieval, high end tests performed on all the processed cord blood units and maternal units with no assurance of when it would be taken up for medical use, setting up of quality systems, processes and procedures, and yet aiming to make them available to the needy at an affordable price is indeed a greater challenge.

Yet, the opportunity to serve the medical community in general and to address the unmet needs of the patient in particular was more compelling than the uncertainty associated with the probability of usage of the final product.

Discussion

The implementation and operation of an unrelated umbilical cord blood bank is analogous to the establishment of a new blood banking program. The objectives and goals were based on the target number of units to be banked. As there was no perfect method to arrive at an optimum size of the cord blood bank, we decided to continue banking activity by setting high quality standards at all levels of the program with a periodic review.

The CBR has been operational for 7 years now, and looking back we feel, that we have made significant improvements, which have a direct impact on the final product as well as patient care. While we started with 50 cord blood units banked in the first year of our operations, we now have almost 3,500 ‘ready to use’ cord blood units in our public CBR. We are happy to state that the Indian ethnic groups are well represented as planned (Tables 1 and 2).

All employees continue to undergo relevant training programs, which is an inherent part of career development. Training evaluation is done objectively, with a scoring system.

Regulatory requirements for cell therapy products demand a validated production process under GMP environment. Each cord blood unit is a batch and each unit has a good traceability right from the donor to the recipient of the graft. The (ISBT 128) unique bar code labeling system philosophy ensures total traceability.

While at the beginning of the process, we had relatively less stringent inclusion criteria; we realized the need to elevate the bar on this in order to improve the final product standards. This was with a view to meet the demands of the transplantation physicians, the international trends and cell dosage. At the start of the program, we had kept 60 ml as the inclusion to start process. We have now stepped up the requirements to ensure at least 800 million total nucleated cells (TNC) in the collected cord blood in addition to the volume criteria. Although the rejection rates are very high due to this self-imposed stringency, we feel that it will add immense value to the available grafts.

Each and every cord blood unit has its own freezing profile with documentation followed by continuous temperature recording throughout the storage period and shipment that ensures good viability. Each cord blood stem cell enriched graft’s donor mother sample is tested for transfusion transmissible disease markers and viruses by high-end tests. We specify total cell counts, CD34+ counts, viability, colony forming ability, HLA type, sterility status, ABO blood type, etc. on each and every graft.

We have the experience of working with both automated and semi-automated storage systems. All personnel involved in the program are proficient in the science and art of cryopreservation and the associated regulatory requirements. A continuous QA oversight helps maintenance of quality in all aspects of production, infrastructure, documentation, training, equipments and safety. Over the years, we have been steadily improving our standards for both raw material and final cord blood products to be able to comply,

excel and exceed the regulatory and accreditation requirements. Stem cell products from our facility are shipped under a closely monitored, controlled condition, as per the transport validation guidelines to ensure product integrity at the bedside [8].

Our prior knowledge in the area of blood products and aseptic processing helped us to form our early quality systems. There have been continuous improvements in all areas of activity guided by the other standards available from the American Association of Blood Banks (AABB) and FACT.

The AABB Accreditation certification is an additional step towards improvement and maintenance of quality in all aspects of our cord banking program. We believe that receiving such accolades truly puts our product in the international market, which is the larger objective of this program. Moving forward, the repository will aim at process improvements, innovation, and cost optimization.

We have graduated from collection of cord blood samples from our own neighborhood to our country's neighborhood, breaking the geographical borders for the family-banking program. This program has been gaining momentum. Now, there are about 7–8 cord blood banks operating in our country, which provide only family banking services. Families with medical problems should be encouraged to avail these banking services [10]. The rich experience and learning, over the last few years, has been very rewarding. This has given us an immense confidence that the objectives of setting up this repository to address many unmet medical needs will be a reality in the immediate future.

Conclusion

We faced several challenges as we set up the first public cord blood bank in India. The journey continues to be an experience, and we still have a long way ahead. The usage of cord blood stem cells for hematopoietic reconstitution is slowly increasing as is evident from abundant literature reports. In the Indian context, as more cord blood banks are getting established, we hope to see more cord blood transplants taking place in the future.

The significance of other cells in the cord blood is also of increasing importance. Therefore there is no doubt that we are on the edge of a major stem cell breakthrough where these cells are predicted to provide costeffective treatment for diabetes, some form of blindness, heart attack, stroke, spinal cord damage and many other degenerative disorders.

Thus, increasing inventory of grafts and improving the chances of available matches from our CBR, for Asians and Indians anywhere in the globe, for both haematopoietic and regenerative medicine applications, are our immediate imperatives.

Acknowledgements This repository is set up and run by Reliance Life Sciences Pvt. Ltd., Mumbai, India. We thank the management for their advice and support, from time to time in the furtherance of this project. We also thank the other fellow researchers from the Regenerative medicine and Molecular medicine group for their constant help.

References

1. Gluckman E, Broxmeyer HE, Auerbach AD, et al. (1989) Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical cord blood from an HLA-identical sibling. N Engl J Med 321:1174–1178
2. Broxmeyer HE, Kurtzberg J, Gluckman E, et al. (1991) Umbilical cord blood hematopoietic stem and repopulating cells in human clinical transplantation. Blood Cells 17:313–329
3. http://www.censusindia.gov.in/2001
4. Sastry PSRK, Bakshi AV, Bhagwat R, Parikh PM (2005) Hematopoietic stem cell transplant activities a the Tata Memorial Hospital, Mumbai Hematopoietic stem cell transplant working group, Department of Medical Oncology, Tata Memorial Hospital, Mumbai. Indian J Transplant 1:25–29
5. Food and Drug Administration (2001) Eligibility determination for donors of human cells, tissues, and cellular and tissue based products; inspection and enforcement. Fed Regist 66:1507–1559
6. Ethical guidelines for biomedical research on human participants. Indian Council of Medical Research, New Delhi, 2007
7. Petzer AL, Speth H-G, Hoflehner E, Clausen J, Nachbaur D, Gastl G, Gunsilius E (2002) breaking the rules, X-ray examination of hematopoietic stem cell grafts at international airports. Blood 99:4632–4633
8. Committee for medicinal product for human use [CHMP] Guidelines on human cell based products, September 2008
9. Rubinstein P, Dobrila L, Rosenfield R et al. (1995) Processing and cryopreservation of placental–umbilical cord blood for unrelated marrow reconstitution. Proc Natl Acad Sci USA 92:10119–10122
10. Nasim S, Momaya KK Cord Blood Banking – Opening New Vistas for Medical Tourism Industry in India