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# Cord blood - from a disposable byproduct of human birth into a precious source for life saving therapies

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**Abstract**

The review article concerns the transplantation of hematopoietic stem cells (HSCs) derived from cord blood (CB). This approach was previously used in pediatric settings. In partu procedures of CB HSCs harvesting, along with the routine methods of their quality control (i.e., HLA typing, testing for infectious pathogens) are listed in brief. Ca. 250,000 CB units are now stored in 35 blood banks in 21 countries worldwide. Some ethical problems with application of CB cells could arise during their long-term storage. The authors point to the controversies associated with the development of private cord blood banks (capacity is estimated at 600,000 CB units), due to indefinite and/or indefensible terms of their storage for eventual transplants. The specific potential of CB HSCs is limited by small sample volume; however relatively low numbers of HSCs with high proliferative activities, along with lower counts of T lymphocytes and their higher immunological tolerance enable HSC transplants at reduced rejection risk and lower GvHD rates.
Clinical experience with CB HSC transplantation is compared for different centers, where the high efficiency of this approach is shown, being, however, associated with longer terms of hematopoietic recovery when compared to bone marrow transplants. A minimal acceptable HSC CB dose is estimated as1.5-2.5x$10^7$ nucleated cells per kg body mass of a patient. The main areas of CB HSC transplantation are described, i.e., related or unrelated transplants, performed in non-cancer and malignant disorders. The authors point to scarce data comparing the efficiency of HSCs derived from cord blood versus bone marrow samples.
Special attention is paid to CB HSC transplantation in non-malignant conditions with bone marrow aplasia associated with unacceptably high non-engraftment risk. Good results of CB HSCT are demonstrated in hemoglobinopathies and mucopolysaccharidoses. When administering CB HSCs to adult patients, non-myeloablative conditioning regimens are proposed, despite the poorly defined efficiency of such an approach. An opportunity for simultaneous transplants of two or more HSC units is considered, including a unit of CB HSCs. An option of intraosseous CB HSC injection is also discussed. In vitro techniques of CB HSC expansion are under development, in spite of scarce data on their proliferative rates and differentiation ability. As an additional stimulus, injection of mesenchymal stem cells together with CB HSCs was recently proposed. In conclusion, the possible usage of normal CB HSCs to correct genetic deficiencies in children is described. CB HSCs' pluripotency may be also applied to the repair of various tissue lesions, e.g., myocardial infarction, or vascular defects.

**Keywords:** Cord blood, hematopoietic stem cells, harvesting, storage, transplantation, review

## Introduction

The birth of every newborn human produces a precious byproduct. In the expelled placenta there is a sufficient amount of Cord Blood (CB), in which there is abundance of hematopoietic elements. In1988 Elaine Gluckman showed that a Hematopoietic Stem Cell Transplantation (HSCT) can succeed by using CB as a source for the graft [Gluckman$_a$]. Gradually thousands of such transplantations began to be performed all over the world. Up until recently most transplantations with CB had been done in children; however the main progress in the field in the last 2 years has been achieved in adults with hematological malignancies. But even though HSCT is still the major indication for using CB, there is a growing interest in finding it as a source for non-hematopoietic stem cells (SC) for regenerative medicine, gene therapy vector, and other potential uses.

## Collection of cord blood in the delivery room

CB's collection is done in the delivery room. The blood is drawn from the umbilical vein, before or after the expulsion of the placenta.

The main advantage of the collection process of CB is its simplicity. It poses no danger and causes no pain to the laboring mother or the newborn. Before the collection itself consent is given by the mother. Some centers also addend a short interview, and others use questionnaires for identification of high risk mothers [Elchalal].

**Cryopreservation**

After its collection CB undergoes cryopreservation. The most widely used method is the one reported by Rubinstein et al [Rubinstein]. It is based on red blood cell depletion and volume reduction. At the end of the process the total volume of each unit is 25 milliliters.

**Cord blood public banks**

Before freezing, CB samples undergo several tests. Every unit is screened for infectious agents, and in some banks, for relevant inherited diseases. HLA typing is usually done for A, B in serology, and DR in DNA. Some diversity exists between banks with regard to the routine tests that each unit undergoes. Additional samples are maintained in small plastic segments attached to the frozen unit in case future tests are needed.

It is estimated that today there are about 250,000 CB units frozen in 35 banks in 21 countries [Bone marrow Donors, Annual report]. CB banking is facing some challenges. The first is the scarcity of space, which is dealt with by volume reduction methods and selection of presumed optimal units, usually with higher volume. Another issue is the uncertainty regarding the period of time which units can be preserved without damage to the viability of the cells. The banks also face ethical issues. For example, if an adult disagrees with the usage of CB that his parents gave consent for donation for decades ago, what is the value of such consent? Another concern is the fate of the information that is stored in CB banks regarding donors' infectious status or the presence of genetic diseases. Is this sensitive data protected as it should be? The need for follow up is understandable, but it could also affect the diversity of ethnic pool of the donors. The ability to detect the donors might also put their families under pressure to donate more cells when the need for it might come.

The importance of public CB banking gained an official acknowledgment when the American congress decided to add $30 million for collection of an additional 150,000 units.

Apart from the above, one of the most controversial issues is private CB banking.

**Private cord blood banks**

This is an ever growing trend that emerged in the early 90s. These private firms offer storage of CB units against a future need for autologous or related allogeneic transplantations. Questions have been recently being raised about whether overanxious parents are truly aware that there are no indications today for autologous cord blood transplantation (CBT). Are they informed about the slim chances for a family of ever needing a sibling CBT, and do they know about the lack of knowledge regarding the how long CB's hematopoietic SC preserve their viability while frozen?

The pace of collection in the private banks exceeds the one in the public banks. It is estimated that approximately 600,000 units are frozen privately. These facts raise questions about whether this limited source of hematopoietic SC should not be solely in public hands.

**The unique properties of cord blood**

Aspirates of bone marrow (BM), or the more recently used Peripheral Blood Stem Cell Collection (PBSC) product, have been traditionally used as sources for HSCT. CB has a few different qualities.

It had long been acknowledged that the more nucleated cells in a graft, the better the chances of engraftment. When taking CB into account as a hematopoietic SC source for transplantation, it is evident that it has fewer nucleated cells than other sources. Each aspirate of BM yields 750-1000 milliliters. This volume usually gives a nucleated cell dose of $2x10^8$/kg for an average weight adult. The product of PBSC yields similar number of SC. The volume of a typical CB unit is usually only 75-150 milliliters. The nucleated cells dose is only one tenth of the BM dose, usually no more than $2x10^7$/kg, for an adult.

Another relevant component of the graft that marks the difference between CB and BM is T cells. These are considered to have a deleterious effect regarding the immune response of the graft against the recipient. The total dose of T cells (CD3+ cells) in CB units is less than a fifth of the amount in BM grafts. When comparing it to mobilized peripheral blood grafts, CB units have less than 2% of T cell dose. But while less hematopoietic SC in CB is a setback regarding HSCT, the scarceness of T cells is an advantage, with respect to the risk of graft versus host disease (GVHD) [Bensienger, Bittencourt].

The low number of SC in CB graft is masked by an excellent proliferative response. When these cells are in a dormant state and cytokines are introduced into their environment, they expand much better than hematopoietic SC of BM. This trait enables CB to produce full hematopoietic recovery of BM in myeloablated recipients [Mayani, Lewis].

The naïve nature of the immune system's cells in CB is a different issue.

The lymphocytes in CB grafts have a more tolerant nature [Nitsche, Risdon$_{a,b}$ Roncarolo, Cohen, Garderet]. Other components of the system, such as dendritic and Natural Killer cells also have different properties, when compared to BM or adult peripheral blood [Leung, Kedereit, Sorg, Canque, Liu, Dalle]. Because of this, CB allows greater human leukocyte antigen (HLA) disparities in transplantations, with less rejection and lower rates of GVHD.

**Cord blood transplantation, clinical experience**

Reports on a series of CBT started to appear at the beginning of the end of the 1990s and at the beginning of the third millennium. These were based mostly on the American and the European registries, with some reports from Japanese and other institutes. Table 1 & 2 summarizes the largest clinical trials of CBT using unrelated donors [Rubinstein, Gluckman$_b$, Wagner, Michel,

Locatelli$_a$, Laughlin$_{a,b}$, Rocha, Takahashi$_a$, Long]. A few important concepts could be built upon results from these works. First was the notion that CB, with its limited nucleated cells dose, can produce full hematopoietic reconstitution after myeloablative conditioning. Secondly, the median time of myeloid recovery in CBT ranged from 22 to 33 days. This is a far longer period than the time in bone marrow transplantation (BMT) experience. When BM aplasia is prolonged, morbidity and mortality rates rise. The third notion was that despite the existence of a significant proportion of HLA disparity between donors and recipients, rejection and GVHD rates were surprisingly low.

So these trials proved that CB is a legitimate source for HSCT, with problematic engraftment kinetics, but less restriction to HLA matching when compared to BM.

Since each placenta contains a limited volume of blood, it follows that there is also a limited amount of nucleated cells per unit. The correlation between nucleated cell dose and transplantation outcomes was evaluated. A positive impact of cell dose on time to engraftment, and hence the overall survival, has been demonstrated in both pediatric and adult series. It is probably agreed that the minimal acceptable threshold of nucleated cells dose should be $1.5x10^7$ nucleated cells/kg, but an association between dose of $3.7x10^7$ nucleated cells/kg and more and faster time to neutrophil engraftment was suggested by the Eurocord [Gluckman$_b$, Arcese]. The New York Blood Group reported that $2.5x10^7$ nucleated cells/kg is the minimal threshold for transplantation [Rubinstein].

Historically CD34+ cells counts were not part of the tests done routinely on CB units. But it is reasonable to assume that it might be so in the near future. Counting nucleated cells involves many cells that do not contribute to the engraftment potential. And indeed, Wagner et al has shown a correlation between CD34+ dose of $1.7x10^5$cell/kg and higher to rapid neutrophil engraftment and probability of engraftment [Wagner].

**Related donor transplants**

Although the first CBT was done from a sibling donor, related donors transplants are used less frequently in this setup. For the cure of malignant diseases CB from a sibling could be used if there is a perfect timing of a birth in the family, or a if a CB unit had been cryopreserved earlier, either by chance or by intention. In non-malignant disease there is usually more time. Families that are aware of CB as a source for transplantation might act on time when births are due.

Several reports of large series of trials have been published. These series have demonstrated that CB is a valid therapeutic option as a source for pediatric transplantation for malignant and non-malignant diseases. The probability for survival at 1 year was 0.63 (95% CI: 0.57-0.69) in the Eurocord study, and 0.61 (95% CI: 0.81-0.49) at 2 years in the ICBTR study [Rubinstein, Gluckman$_c$].

The largest of the series is a joint European and American work that compared 113 related donor CBT in children with 2052 cases of related donor BMT. Neutrophils engraftment in the CB group occurred at a median time of 26 days, compared to 18 in the BM group. Probability of myeloid recovery at day 60 was 0.89 and 0.98 in the CB and BM respectively. Children who received CB had a significantly lower risk of both AGVHD and CGVHD than those who were transplanted from BM (relative risk 0.41; p=0.001 and relative risk 0.35; p=0.02, respectively). Overall survival at 3 years was 0.64 for the CB and 0.66 for the BM group. This study demonstrated the role of related donor CBT for malignant diseases in children [Rocha$_b$]. Related donor CBT for non malignant diseases will be discussed in the non malignant section.

**Comparison to bone marrow**

No randomized trials had been conducted to compare CB with

**Table 1. Umbilical cord blood transplantations - children**

| EFS % (y) | OS % (y) | TRM % (y) | Rel. % (y) | AGVHD (III-IV)/ Chronic % | Hematopoietic recovery | | | HLA loci disparity, % | | | | Median cell dose NC $x10^7$/kg/ CD34+ $x10^5$/kg | Median age in years | n | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Neu rec. % | Median day ANC≥500/ PLT ≥50000 | | | | | | | | | |
| EFS, (OS-NA) Gen. 48 M 27 Aq. 29 (2) | | 56.8 (1) | 56.8 | 24/31 | 92.7 | 23/75 | MU | ≥3 | 2 | 1 | 0 | ≥10=15% 5-9.9=22% 2.5-4.9=34% <2.5=29%/ NA | <2=20% 2-5=22% 6-11=23% 12-18=14% >18=21% | 861 | Rubinstein |
| | | | | | | 28/94 | MM | 7 | 48 | 39 | 6 | | | | |
| AA 21 Gen. 51 M 36 | | NA | | 39/NA | 82 | 29/ NA | | 83 | | | 17 | 5.6 (0.8-60)/ 1.9 (0.6-78) | 5.0 (0.2-15) | 291 | Gluckman$_b$ |
| NA | 47 (2) | 30 (1) | 37 (2) | 11/9 | 88 | 23/86 | | 2 | 41 | 43 | 14 | 2.8 (0.4-39.1)/ 3.1 (0.7-57) | 7.4 (0.2-57) | 102 | Wagner |
| 42 (2) | 45 (2) | 20 (2) | 29 (2) | 35**/15 | 78 | NA | | 13 | 33 | 46 | 8 | 4.4 (0.4-36)/ 1.4 (NA) | 6.0 (0.3-16) | 95 | Michel |
| 30 | NA | 52 | 40 | 23/12 | 79 | 33/85* | | 30 | NA | 52 | 40 | 5.0 (1.5-46.5)/ 1.8 (0.1-78) | 5.5 (1.7-14) | 60 | Locatelli$_a$ |

**Table 2. Umbilical cord blood transplantations - adults**

| EFS % (y) | OS % (y) | TRM % (y) | Rel. % (y) | AGVHD (III-IV)/ Chronic % | Hematopoietic recovery | | | HLA loci disparity,% | | | | Median cell dose | | Median age in years | Pts # | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Neu rec. % | Median day | | ≥3 | 2 | 1 | 0 | CD34+ $x10^5$/kg | NC $x10^7$/ kg | | | |
| | | | | | | PLT ≥ 50.000 | ANC ≥500 | | | | | | | | | |
| 20 (3.3) | 63 (5) | 22 (3.3) | 40/ 50 | 17 (5) | ~65 | 60 | 27 | 0 | 77 | 23 | 0 | NA | 2.2 (1-6.5) | NA | 150 | Laughlin$_a$ |
| 21 (1) | 54 | 27 (1) | 38/ NA | NA | 81 | NA | 32 | 94 | | | 6 | 0.8 (0.01-8.9) | 2.2 (1.2-7.3) | 26 (15-53) | 108 | Gluckman$_b$ |
| 33 (2) | 44 (2) | 36 (2) | 26/ 30 | 23 | 75 | NA | 26 | 4 | 39 | 51 | 6 | NA | 2.3 (0.9-6) | 24.5 (15-55) | 98 | Rocha$_a$ |
| 26 (3.3) | 51 | 28 (3.3) | 20/ 36 | 5.9 (1) | 90 | 99 | 27 | 17 | 54 | 26 | 3 | 1.2 (0.2-16.7) | 2.1 (1-6.3) | 31 (18-58) | 68 | Laughlin$_b$ |
| 74 (2) | 9 (1) | NA | 7/ 77 | 16 (2) | 88 | 48 | 22 | 25 | 54 | 21 | 0 | 0.9 (0.2-9.0) | 2.47 (1.1-5.29) | 36 (16-53) | 68 | Takahashi$_a$ |
| 15 (3) | 50 | 19 (3) | 16/ 32 | 15.7 | 71 | 84 | 26 | 5 | 77 | 14 | 4 | 1.37 (0.02-12.4) | 2.12 (1.1-4.4) | 31 (18-58) | 57 | Long |

BM grafts. Few retrospective reports have been published. As for children, it was shown by Eapen that 503 cases of matched CBT had better 5 DFS than 116 matched unrelated donor (8/8) BMT. Even the 5/6 matched CBT had comparable results with the BM group. An important factor was the cell dose. The group that received more than $3x10^7$ nucleated cell/kg had better DFS and OS [Eapen]. It was Rocha and Gluckman who assessed leukemia-free survival at 5 years after CBT or BMT in children. 503 children received CB—either matched or mismatched. The outcome of these transplantations was compared to BMT of 282 children. Allele-matched bone-marrow transplants had similar outcomes to transplants of umbilical cord blood mismatched for either one or two antigens. Higher survival rates were demonstrated after transplants of HLA-matched umbilical cord blood [Rocha$_c$].

Recent publications have managed to evoke hopes that even in adults CBT (matched, or 1-2 HLA antigens mismatched) is as good as matched unrelated donor BMT. The reports of Laughlin, Rocha and Takahashi in late 2004 compared a large series of adult patients who received unrelated CB or BM. Outcomes of CBT were similar, and in certain aspects superior, to unrelated donor mismatched BMT.

Laughlin found that patients receiving mismatched CB had similar treatment-related mortality, treatment failure, relapse and overall mortality rates, to those received mismatched BM. Rocha compared matched unrelated donor of BM with CB. He found no differences in treatment-related mortality rates, relapse and leukemia-free survival rates between them. These results may refine the accepted approach for unrelated donor search. Many believe that a search for a BM donor and a CB unit should generally be started simultaneously and CB (matched or mismatched in up to 2 HLA antigens) should be preferred if matched BM donor can not be found within a reasonable period of time [Rocha$_a$, Laughlin$_c$, Takahashi$_a$]. In late 2006 Takahashi et al published the first report of adult transplantation with CB as a first option for non related donor graft. The Japanese group transplanted 100 adults with hematological malignancies with CB, if they had no matched related donor. Results of the CBT were compared to matched related BM or peripheral SC transplantations. The outcome was similar in all groups. Whether this interesting approach is feasible in all cases of patients with no matched related donor, relies upon further reports from other ethnic groups [Takahashi$_b$].

**CBT for non malignant diseases**

HSCT can offer the only true chance for cure in many non-malignant diseases. CB offers some unique advantages in the area of transplantations for non malignant diseases. Many of these patients are children. This makes nucleated cells doses satisfactory in most of the cases. Moreover, rareness of GVHD tempts the preference of CB, especially in an unrelated donor setup. As opposed to HSCT for malignant disease, there is no presumed benefit from the Graft Versus Leukemia effect of GVHD. On the other hand, CB is a less attractive option for transplantation for bone marrow failure syndromes. There are high rates of graft rejection in HSCT in these diseases. When adding the negative impact of CB's tendency for delayed engraftment, it is regarded by some as a problematic solution for such patients. This was demonstrated in the work of Rocha et al. In a related donor setting, and definitely with unrelated donors, for bone marrow failure syndrome patients, it was clear that engraftment, and therefore event free survival (EFS) rates are not acceptable. The probability of myeloid engraftment at day 60 was not more than 67% for patients that were given related donor grafts, and it was 36% in unrelated donor-CBT. Only 33% of the Fanconi anemia patients engrafted [Aker]. Better results were reported by the European group when they summarized unrelated CBT for Fanconi patients. Although only 12 of the 93 cases were HLA identical; 60% of the patients engrafted by day 60. A positive impact of Fludarabine

based regimens, cell dose, and CMV negative recipients was seen [Gluckman$_d$].

Some limited experience was gained by us with a few bone marrow failure syndromes, namely Fanconi anemia. We observed high rates of event free survival (EFS), especially in children who received a matched family donor transplant [Goldstein].

In one case we used a novel strategy of pre-implantation genetic diagnosis for one of the patients. This method, which is based on CBT, could pave the way for many malignant and non-malignant diseases [Bielorai].

Although the role of HSCT for Thalassemia in the era of newer iron chelating agents is yet to be determined, this strategy is still being practiced widely in an attempt to cure this hemoglobinopathy. Locatelli et al reported results of related CBT in 44 children with hemoglobinopathies (Thalassemia and Sickle Cell Disease), and showed that this procedure is feasible. High rates of engraftment (89% at day 60) and EFS (79% for Thalassemia and 90% for Sickle Cell Disease) were achieved [Locatelli$_b$].

As for CBT in inborn errors of metabolism, Staba et al reported impressive results in children with Hurler syndrome who were given unrelated donors CB grafts. Even though 19 of the 20 patients received mismatched grafts, high rates of engraftment were reported (at 2.4 years follow-up, 85%). This was probably due to the relatively high nucleated cells doses (median of $10.5x10^7$ nucleated cells/kg). The disease itself was cured, as could be seen in all 17 patients who were alive, and had normal peripheral-blood α-L-Iduronidase activity [Staba]. Recently a report of a case of a child who was cured of Wolman disease by a CBT was published [Stein].

CBT for the cure of Sickle Cell Anemia was reported recently by a French group. Importantly the authors noticed that after a 6 year follow up the group of patients that received a CB graft did not develop the main contributing factor for the morbidity, GVHD [Bernaudin].

**Investigational approaches in cord blood**

Most patients needing HSCT are adults. For these heavier patients CB is a problematic solution because of the relatively low cell dose. Various strategies are being attempted in order to lower the toxicity of the conditioning regimen. This could be achieved either by lowering its intensity, or by hastening engraftment.

**Reduced intensity conditioning**

The practice of HSCT with reduced intensity conditioning (RIC) has emerged in the adult population. These older patients usually have pre-existing morbidities.

By reducing the intensity of the preparative regimen it has been shown that treatment-related morbidity and mortality rates could be lowered. The concept behind this is based on the assumption that in certain cases the immunological impact of the graft is more important than the ablative power of the conditioning regimen.

Experience with transplantations using RIC, though follow up time is still short, have shown encouraging results. Patients who benefit the most from RIC are those with diseases of a more indolent nature.

Few studies of RIC-CBT in adult and pediatric patients have been published. The major conclusion that could be drawn from these series is that RIC is feasible in CBT. Graft rejection happened mainly in cases in which the accumulative chemotherapy dose experienced by the recipients prior to the transplantation itself was low. Though survival rates are low, it must be emphasized that most studies included mainly high risk, heavily treated patients. GVHD rates correlated with unrelated donor BMT. Another encouraging finding is the lower than expected rates of treatment-related mortality at 100 days post-transplantation. Because of the small number of patients, and diversity of methods, conclusions regarding the optimal RIC conditioning regimen, or the GVHD prophylaxis, can not be drawn at this point. Even if it is definitely too early to recruit patients for RIC-CBT outside clinical trials for selected patients, these protocols could offer an alternative for selected patients [Miyakoshi, Del Toro, Chao$_{a,b}$, Bradley, Barker$_a$, Ballen$_a$, Yuji].

**Engraftment hastening**

The idea of shortening the period from transplantation to myeloid recovery is at the basis of many strategies. Some have shown preliminary encouraging results in the laboratory, in animal models, and even in clinical trials.

**Transplantations with double cord blood units**

Many recipients receive more than one partially matched CB units where the cell dose in each is not sufficient. In many cases the sum of these units provides an adequate number of SC. It has been shown in animal models that two CB units provide high rates of engraftment [Nauta]. Some studies have used this strategy for high risk adult patients who received two mismatched CB units. Many believe that this strategy could pave the way for lowering treatment-related mortality rates in CBT. In most of these trials two encouraging facts were observed: stable mixed chimerism, and no mutual rejection of mismatched units [Barker$_{b,c}$, De Lima$_a$, Gryn, Ballen$_b$,]. Brunstein et al have shown that by using a non-myeloablative regimen for CBT in adults, the OS of the group that received 2 units was higher than the patients who received 1 unit. In this study 92% of the patients achieved neutrophil recovery, at a median time of 12 days [Brunstein]. Interestingly, sustained hematopoiesis after double CBT is usually derived from a single donor. The relative percent viability, the infused number of NC and $CD34^+$ cell doses, and the donor–recipient HLA-disparity are not helpful in predicting which of the two CB units will predominate. Although early data suggested that the dominant unit had a higher median infused $CD3^+$ cell dose, this observation has not persisted with investigation of a larger cohort of patients. Order of infusion, location of HLA mismatch, ABO blood group and/or sex mismatch also did not have a predictive effect on engraftment.

Double CBT can potentially produce a better graft versus leukemia effect. This was demonstrated in a study of the University of Minnesota. They compared leukemia patients who received 2 units of CB to those who received a single unit. The group who received the double CBT had a lower risk for relapse. It is still

not known if the relatively high degree of HLA mismatch in this setting is responsible. It might also be a consequence of non-HLA disparity, such as KIR mismatch, between the CB units and the recipient, or between themselves [Verneris].

Double unit transplantation has become a major breakthrough in the field of CB during the last 2 years. Several 2 arms protocols for using double units are on their way. Whether these expectations are justified depends on preliminary results of these trials.

**Co transplantation with a Haploidentical donor**

Relaying on the assumption that almost every patient has a donor, namely a parent that has a similar HLA type of one of his haplotypes, Magro et al have succeeded in transplanting CB together with a Haploidentical graft. They succeeded in inducing a rapid engraftment via a BM transplant. By administering only a small dose of Haploidentical SC, the Spanish team managed to induce a temporary engraftment only. These cells were rejected later, due to their low dose and significant HLA disparity, allowing engraftment of the CB graft. 69% of these high risk patients survived at 4 years [Magro].

**Intra-osseous transplantation**

One of the obstacles to a short period of engraftment is the possibility that the homing process is influenced by anatomical barriers. It has been suggested that intra BM injection of the graft could induce a rapid engraftment. This has been shown to be feasible, and has improved engraftment kinetics in BMT in adults [Hagglund]. Time will tell if intra osseous transplantation could shorten the way for CB's SC into the BM, and therefore improve time to engraftment, as has been shown in animal models [Yahata, Wang].

**Ex vivo expansion of hematopoietic stem cells**

In vitro studies had shown that SC proliferate with the addition of cytokines. But uncontrolled expansion is not biologically satisfactory, since maturation and differentiation of SC occur in these conditions. The SC proliferate and become committed to specific hematopoietic cell lines. Such cells lack what is known as "long term population ability." The optimal composition of the cytokine-rich media of the ex vivo expansion process is an important challenge for researchers to face. It has been demonstrated by Shpall et al that co transplantation of ex vivo expanded and non-manipulated grafts are feasible. But in spite of this, improvements in engraftment kinetics, are yet to be achieved [Bruno, Piacibello, Kohler, Shpall$_{a,b}$ Jaroscak, Pecora].

Different attitudes have been taken in order to refine the expansion process, namely: co-culturing with different cells as feeder layers [Zhang, Chute], selection of SC for the expansion [Forraz], and multiplying the proliferative potential by performing a two step harvesting technique [McNiece$_{a}$, Pick]. None of these strategies have yet been introduced into clinical trials.

A somewhat more promising field is interference with the differentiation of expanded SC. Reports have been published recently regarding ex vivo expansion with copper chelator, Tetraethylenepentamine (TEPA), which enhances the long term populating ability of the CB cells. Following large scale experiments, this appealing approach has been introduced into the clinic in phase I trials. Preliminary encouraging results of this trial with no significant toxicity were presented recently [Peled$_{a,b,c}$, Grynspan, Shpall $_{c}$]. A Phase II multi center study has just started and the first 3 adult patients with hematological malignancies have already been recruited (De Lima$_{b}$). The same concept was behind the experiment held by Nolta et al, when they co-cultured primitive CB's SC (CD34+ CD38-) together with a feeder layer of immortalized murine stromal cell-line AFT024. This method has yielded high rates of myeloid and lymphoid engraftment in a NOD/SCID mouse xenograft model [Nolta]. Other molecules that play major roles in the differentiation of hematopoietic cells, and might be used in the future for ex vivo expansion of CB are Gfi-1 and some of the Notch ligand protein family [Lauret$_{a,b}$ Hock].

Novel methods have been studied recently with the use of epigenetic factors. Silencing of genes could be a consequence of methylation of their promoters or deacetylation of histones. By trying to inhibit these processes, reactivation of some genes could augment the hematopoietic SC's self renewal potential. Recent publications have shown some success in the in vitro repopulating potential of CB when using histone deacetylase inhibitors, such as Valproic acid [Milhem, De Felice]. This strategy is the basis of a clinical study which has recruited the first patients (personal communications).

**Cord Blood, Umbilical cord, and Mesenchymal Stem Cells**

As their unique qualities are revealed, the interest in mesenchymal stem cells (MSCs) is growing continuously. These cells are non-hematopoietic stromal cells that are capable of differentiating into, and contribute to the regeneration of, mesenchymal tissues, but possibly also to other tissue lineages. They have an in vitro expansion ability while their growth and differentiation potential is maintained. Currently it is expected that they could be used for tissue repair and regenerative medicine. MSCs have shown that they can modulate immune response both in vitro and in vivo. Preliminary studies are on their way for using MSCs as an anti GVHD prophylaxis. It was doubted that these cells could also play a roll in treating GVHD. It has also been postulated that these cells could be used for other immune mediated diseases. MSCs are used as a growing medium for ex vivo expansion of other cells [McNiece$_{b}$, Robinson]. Le Blanc et al showed that MSCs could be transfused in parallel to HSC grafts and demonstrated fast engraftment [Le Blanc]. Finally, MSCs are considered to be candidates as a vector for gene therapy.

Until recently only BM and adipose tissue were considered as a source for MSC. In the last few years it had been shown that CB contains MSC [Lee]. MSC from other sources has been demonstrated to have suppressive effect on T cells [Li]. Few studies have focused on the different properties of MSC originating from CB. Their tendency to differentiate into specific tissue, their genomic expression, and proliferative response, are all different from BM or adipose tissue MSC [Chang, Kern].

When considering the expulsion of the placenta at the end of delivery as a waste of a precious source of SCs, it is not only the CB itself that should be regarded as such. The Wharton jelly in the umbilical cord has also been found to be a source for MSCs [Friedman, Secco].

**Cord blood uses in other fields**

Gene transfer is an exciting new field in which CB could serve as a vector for correcting inborn genetic errors, or replace infected hematopoietic SC, such as in the case of HIV. Its availability, proliferative response, and engraftment potential, make it an appealing candidate for these uses [Ikeda]. Clinical trials of gene transfer to Adenosine Deaminase deficient Severe Combined Immunodeficiency children relied on BM and CB as a hematopoietic SC source. This method faced some obstacles that continue to prevent it from curing these patients [Kohn, Aiuti].

Another—to date only investigational—field is the potential non-hematopoietic use of hematopoietic SC. In recent years much interest has been focused on the ability of hematopoietic SC to differentiate into (or as some claim, to fuse with) cells of other tissues. It was demonstrated that cells with pluripotent differentiation potential could be found in CB [Pesce, Newsome, Goodwin]. CB has been suggested to have a role in improving performance of rats who were subjected to brain infarct [Vendrame]. CB is also considered by some to be a source of SC for regeneration of ischemic heart tissue by differentiation processes or neoangiogenesis [Ma]. It is too early to define whether SC's plasticity might have clinical benefits in repairing injured tissues, but this application is at the center of great interest and controversy.

**Discussion**

CB has become a legitimate source, not only for HSC for transplantations, but also for other uses. The experience gained in the last twenty years of work with CBT has shown us its advantages, as well as its setbacks.

Unlike BM donations, CB's collection is easier and poses no danger to the donor. In CB banks there is a greater proportion of ethnic minorities than in BM registries. It also has greater availability in an unrelated donor HSCT due to its shorter donor search time. Lesser risk for transmission of infectious agents in the transplantation process is another benefit of CB**.** There is no doubt that fewer HLA restrictions in unrelated transplantations is its main advantage. This fact allows successful transplantations with acceptable rates of graft failure and GVHD.

On the other hand, there is a slim potential for disease transmission, namely genetic, in CBT setup. In CBT there is almost no option for a second transplantation, or any boost of cells. A troubling disadvantage of CB is its low number of hematopoietic SC in each unit. This has proven to be a crucial point that has a direct relationship to relatively high rates of treatment-related mortality rates in CBT. This point is further emphasized within the setting of adult transplantations.

From the data collected in several series of CBT for both malignant and non malignant diseases it appears that CB can be used as a SC source in several settings.

The most urgent need for SC is transplantation for malignant diseases from unrelated donors. It is an acceptable approach to search first for BM donor. If a 5/6 or better HLA match can not be found, or progressive disease status does not allow completion of the search, then a CBT of 4/6 HLA match or better should be performed. This of course depends on a minimal cell dose of $2x10^7$ nucleated cells/kg per CB unit. Cell dose has greater relevancy in adult transplantation setup. Skepticism about the possibility of CBT in heavier patients might fade as newer strategies could overcome SC scarceness of nucleated cells in CB. At this stage the most appealing strategy is the double unit CBT. By receiving 2 CB units many adults could be transplanted with a reasonable time to engraftment. Time will tell if other methods could offer a solution for a better outcome in CBT for adults.

Impressive progress is constantly being achieved in the field of CBT. CB is still considered a second best choice for HSCT, but as newer reports are being published it is not so obvious whether it could not be preferred over BM. Interesting data in children showed that a perfect match (6/6) of CB could be the best choice. If larger studies can confirm this, we might see CB becoming the first option for transplantation in certain conditions.

For non malignant disease CB is a very good option, especially for the smaller patients. Caution should be practiced when using CB for bone marrow failure syndrome, though again it seems that larger units and better preparative regimens may overcome the tendency for graft failure.

Future uses of CB may not be just for HSCT. Time will tell if the fields of gene therapy and non hematopoietic injured tissues repair also benefit from the use of CB cells.

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# Пуповинная кровь: от побочного продукта родов до ценного источника жизнеспасающего лечения

**Гэл Гольдштейн, Амос Торен, Арнон Наглер**

**Расширенное резюме**

Обзор посвящен вопросам трансплантации гемопоэтических стволовых клеток из пуповинной крови (ГСК ПК), который ранее применялся в детской практике. Кратко перечислены процедуры сбора ГСК ПК во время родов, а также рутинные тесты оценки их качества (HLA-типирование, проверка инфекционных агентов). Сейчас в мире около 250000 доз ГСК ПК хранятся в 35 банках 21 страны. Этические проблемы с применением клеток ПК могут возникать при их длительном хранении. Указывается на противоречия, связанные с развитием частных банков пуповинной крови (по оценкам, в них хранятся ок.600000 доз ПК), ввиду неопределенности сроков гарантированного хранения стволовых клеток для возможной трансплантации. Свойства ПК как источника ГСК ограничены небольшим объемом образца и малым числом ГСК, обладающих высокой пролиферативной активностью, при меньшем содержании Т-клеток и их большей иммунологической толерантностью. Это дает возможность проводить пересадки, с меньшими ограничениями по HLA-совместимости, при меньшем риске отторжения и более низкой частоте РТПХ у больных.

Авторы обобщают клинический опыт ТГСК ПК в различных центрах, где показана высокая эффективность этого метода при более длительных сроках восстановления гемопоэза, чем трансплантации костного мозга. Минимально допустимой дозой ГСК ПК считается 1,5-2,5X107 миелокариоцитов на 1 кг массы тела больного. Описываются основные области применения ГСК ПК (родственная или неродственная трансплантация у детей при неопухолевых и злокачественных и заболеваниях). Подчеркивается нехватка сравнительных данных об эффективности ГСК из пуповинной крови и костного мозга.

Особое внимание уделяется ТГСК ПК при неопухолевых заболеваниях с аплазией костного мозга, где риск неприживления оказался недопустимо высоким. Описаны хорошие результаты ТГСК ПК при гемоглобинопатиях, мукополисахаридозах. При лечении взрослых больных посредством ТГСК ПК предлагаются немиелоаблативные режимы кондиционирования, хотя эффективность такого подхода пока неясна. Обсуждается возможность одновременной трансплантации двух и более доз ГСК от разных доноров, включая дозу ПК. Дискутируется вопрос о внутрикостном введении ГСК ПК, разрабатываются методы культивирования ГСК ПК в культуре, хотя темпы их размножения этих клеток пока недостаточны, а их способность к дифференцировке мало изучена. В качестве добавочного стимула предложено введение мезенхимных стволовых клеток совместно с ГСК ПК. В заключение описывается использование нормальных ГСК ПК для коррекции генетических дефектов у детей, а также их плюрипотентность для репарации дефектов других тканей (например, миокарда или сосудов).

**Ключевые слова:** пуповинная кровь, стволовые кроветворные клетки, заготовка, хранение, трансплантация, обзор