Alberta Heritage Foundation
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Cord blood transplantation

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David Hailey and Philip Jacobs

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This Health Technology Assessment Report has been prepared on the basis of available information of which the Foundation is aware from public literature and expert opinion, and attempts to be current to the date of publication. It has been externally reviewed. Additional information and comments relative to the Report are welcome, and should be sent to:

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Contents

Summary

- Cord blood transplantation (CBT) is an alternative to bone marrow transplantation (BMT) that has been used to reconstitute hematopoiesis in various hematological disorders. Most patients treated with CBT have been children.
- CBT has potential advantages over BMT because of the lower incidence and milder form of graft versus host disease. As a greater degree of HLA mismatch is possible, as compared to BMT, CBT is a potential treatment for many patients whose other management options are limited.
- Disadvantages of CBT compared with BMT are the slower rates of engraftment, the limit to the absolute cell dose and, possibly, the absolute number of stem cells available for a single transplant.
- Evidence of the efficacy and safety of CBT is still limited. Published reports on CBT as a treatment for various hematological disorders have been based upon uncontrolled studies, usually with small numbers of subjects.
- There are no standardized methods for cord blood banking and HLA typing. There are not yet adequate comparative studies on the efficacy and safety of CBT compared to other therapies for specific hematological diseases.
- If CBT is to become a widely available technology, there will be a need for prompt availability of suitable donors and for large cord blood banks. Costs of cord blood banks and their level of use will require careful consideration. All cord blood banks would need links to national and international cord blood transplant registries.
- A number of procedural matters require further study. These include standardization of methods of collection and cryopreservation, the number of stem cells required to reconstitute hematopoiesis, the best method for quantitative analysis of stem/progenitor cells and the use of granulocyte colony stimulating factor and erythropoietin.
- A number of ethical and legal issues require further discussion
- In the context of the Alberta health care system, CBT should be regarded as a promising, evolving technology for treatment of some types of pediatric patients.

Glossary

Allogeneic cells:	Cells obtained from a donor
ASCT:	Autologous stem cell transplantation
Autologous cells:	Cells obtained from the recipient
BMT:	Bone marrow transplantation
Cell dose:	Number of nucleated cells infused/kg
CFC:	Colony-forming cells
EFS:	Event free survival
GM-CSF:	Granulocyte-macrophage colony stimulating factor
GVHD:	Graft versus host disease
Hematopoiesis:	Normal formation and development of blood cells in bone marrow
HRT:	High resolution typing
ICBTR:	International Cord Blood Transplant Registry
LRT:	Low resolution typing
NC:	Nucleated cells
PBPSC:	Peripheral blood progenitor stem cells
UBMDR:	Unrelated Bone Marrow Donor Registry
WHO:	World Health Organization

Introduction

This assessment has been prepared in view of the interest at the University of Alberta Hospital in developing a cord blood banking facility in the province and to provide information to decision makers on the current status of cord blood transplantation. The principal purpose of the assessment was to review the evidence of efficacy of CBT. Cord blood banking requirements, potential impact of cord blood transplantation on the provincial and Canadian populations, and pertinent logistical considerations have also been considered. Sources of information and methodology used for the literature review are outlined in Appendix A. Economic aspects of CBT will be considered in a separate report.

CBT is an alternative technology to bone marrow transplantation (BMT). As a source of stem cells, it has been used to reconstitute hematopoiesis in patients, mainly children, with a variety of malignant and non-malignant hematological diseases following myeloablative therapy.

A wide range of diseases would potentially benefit from stem cell transplantation. Alternative treatment for each of these diseases varies. Certain inborn errors of metabolism and bone marrow failure syndromes like β-thalassemia and sickle cell anemia still lack a curative treatment through other approaches.

The physiologic homeostasis of the bone marrow is severely disturbed or completely fails in certain hematological disorders. These include high risk or recurrent hematological malignances, bone marrow failure syndromes and selected hereditary immunodeficiency states and metabolic disorders.

Hematopoiesis is the normal formation and development of blood cells in the bone marrow. As a result of hematopoiesis, the bone marrow is populated with mature cells and pluripotent progenitor cells (capable of further differentiation). The mature cells are continually replaced, under normal conditions, by differentiation of progenitor cells. Mature blood cells include erythrocytes, classes B and T lymphocytes, specialized myeloid cells (basophils, eosinophils, monocytes, neutrophils), and platelets.

Stem cells, which are considered a self-renewing population, give rise to progenitor cells, which are considered to have a limited life span. It has been estimated that stem cells occur in the bone marrow at a frequency of one per 100,000, based on the number of mononuclear cells (MNCs). The concentration of CD-34 + MNC fraction of the progenitor cells in the peripheral circulation is typically smaller than that in bone marrow. The concentration of CD-34+ cells in a sample of cord blood is roughly equivalent to that in adult bone marrow and significantly higher than the concentration in adult peripheral blood.

Approaches to stem cell transplantation

Autologous stem cell transplantation (ASCT)

Autologous and allogeneic stem cells from the peripheral blood and from bone marrow have been used for bone marrow transplantation to reconstitute hematopoiesis in various malignant and non-malignant hematological diseases. There are also possible clinical applications for gene therapies. (18)

Autologous bone marrow and peripheral blood stem cell transplantation in hematological malignances permit the use of higher doses of chemotherapy and radiotherapy than would have been possible without "rescuing" bone marrow function (22). High dose chemotherapy (with or without radiation therapy) is followed by hematopoietic stem cell support.

ASCT has been shown to be useful in the treatment of certain patients with lymphomas, leukemias, myeloma, breast cancer, testicular cancer, ovarian cancer, and selected other tumors (28, 32). Autotransplants of bone marrow and peripheral blood stem cells are increasingly used to support high-dose chemotherapy for solid tumors of childhood. As a result, individuals with these conditions now have a better prognosis especially in neuroblastoma and entities of small round cell tumor (9).

However, the long-term outcome of ASCT is affected by relapse of the underlying disease that represents the major cause of failure of this treatment. The contribution of re-infused tumor cells to relapse is suggested by several clinical studies and has been recently demonstrated by autografting genetically marked cells (25).

Allogeneic BMT

While recent years have seen increasing use of peripheral blood progenitor stem cells (PBPSC), BMT, remains the major technology in treatment of diseases which lead to failure of hematopoiesis.

Allogeneic bone marrow provides a large and suitable source of stem cells for hematopoietic reconstitution in bone marrow transplantation. Diseases that have been successfully treated with BMT are outlined in Appendix B. However, the limited availability of suitable marrow donors, and the risks of graft versus host disease (GVHD) and opportunistic infection have led to the exploration of other sources of hematopoietic progenitor stem cells for transplantation.

Cord blood as a source of stem and progenitor cells

Hematopoietic progenitor cells have been shown to be present not only in the bone marrow but also in peripheral and cord blood.

Cord blood contains high concentrations of progenitor cells which average 26,000/mL (1). This is a much higher concentration than that found in normal adult peripheral blood, and is similar to the concentration of progenitor cells mobilized by cytokines and/or chemotherapy.

There are also differences in the proliferative capacity between progenitor cell types in cord blood and in adult bone marrow. In fetal blood, about one-quarter of the colony-forming cells (CFCs) will be mixed-cell CFCs. In adult marrow, the proportion of mixed-cell CFCs is only 2%-3%. In addition, cord blood CFCs give rise to much larger colonies in vitro and there is a greater concentration of long-term culture-initiating cells in cord blood than in adult marrow (1). However, the cell dose available in cord blood is limited because of the low volume that can be collected.

The use of cord blood (umbilical or placental blood) as a source of transplantable hematopoietic stem cells was first suggested by Edward A. Boyse (University of Arizona, Tucson) in 1983, tested in an animal model in 1984, and used clinically to successfully treat a patient with Fanconi anemia in 1988 (37). Since then, a number of cord blood transplantations have been performed in several countries. There has, so far, been limited use of the technology in Canada (7).

CBT involves collection or harvesting of cord blood, cryopreservation in a cord blood bank, and subsequent retrieval and transplantation. Details of cord blood banking are given in Appendix C, and experience with CBT in Canada in Appendix D.

The cord blood transplantation procedure

Before transplantation of cord blood, confirmatory HLA typing of patient and cryo-preserved donor specimens are performed using standard serological techniques. All WHO-recognized specificities for HLA antigens are identified, and any additional specificity deemed necessary by the centre undertaking the transplantation.

Pre-transplant conditioning prior to cord blood infusion varies according to patient's disease and institutional practice. However, this generally consists of treating patients with high-dose chemotherapy, with or without total body irradiation.

Prophylaxis for acute GVHD also varies with the patient's disease and the institution. Prophylaxis generally consists of cyclosporine A alone or with one or more of a variety of combinations including methylprednisolone or an anti-T-cell antibody and methotrexate. Kurtzberg et al. (20) routinely used trimethoprim-sulfamethoxazole after transplantation as prophylaxis against Pneumocystis carinii.

When possible, stem cells from the patient's bone marrow are routinely cryopreserved for an eventual autologous bone marrow transplantation should there be a failure of cord blood engraftment (11, 30, 36). Donors are HLA matched or are mismatched by one or more HLA antigen loci. Recipients of cord blood are not necessarily ABO matched or sex matched.

To prepare the stored cord blood for use, the cord blood bag is placed in a warm water bath (about 38^0 C) with gentle agitation. Cord blood is thawed for 10-15 minutes until only a few crystals remain in the bag. Cord blood (usually fractionated) is then transplanted into the recipient by simple phlebotomy over 30 minutes to four hours.

In an alternative approach, after thawing of whole cord blood, dextran/albumin solution is added, centrifuged at 250g for 10 minutes at 10^0 C and the supernatant removed. The cell pellet is re-suspended in dextran/albumin and immediately infused into the patient (37).

Comparison of cord blood transplantation with bone marrow transplantation

From descriptions in the literature by a number of authors, the following points can be made in comparison of CBT with BMT (1, 11, 19-21, 34-37).

Advantages of CBT as compared to BMT

- Harvesting of cord blood is easy and, but for ethical issues, poses no problem to the donor.
- The length of donor search is shorter. Storage of cord blood in a cord blood bank reduces procurement time, potentially to one or two weeks, substantially less than the median 3.5 months (range, 1 month to 6 years) typically needed to find an unrelated bone marrow donor.
- Bone marrow transplant registries consist primarily of Caucasians, which limits the availability of potential donors for other ethnic groups. Wide range collection of cord blood, which can be readily performed, has the potential to improve availability of donors from all racial and ethnic groups.
- There is a decreased GVHD, particularly chronic GVHD. This decreased risk permits transplantation of cord blood with one or more HLA antigen-mismatches. Most studies have included some individuals who have been transplanted with cord blood with 3 or 4 HLA antigen mismatches.
- There is a lower risk of viral contamination, for example, with cytomegalovirus or Epstein-Barr virus.
- CBT has a significantly lower cost.

Limitations of CBT as compared to BMT

- Because of the significantly lower number of hematopoietic progenitor stem cells in cord blood compared with bone marrow, and the lower volume available, CBT appears to be most successful in patients weighing less than 30 kg and therefore mainly applicable to small children. Although adults have been successfully transplanted, it is not yet clear if this can be achieved routinely.
- There is only one unit of cord blood per transplantation.
- There is the possibility of transmission of a genetic disease not detected at the time of cryopreservation and quarantine of cord blood, but which becomes appearent as the donor matures.
- There is a possibility of maternal cell contamination. However, the incidence of maternal cell contamination is very low.

- Engraftment is slower than with stem cells derived from bone marrow or peripheral blood.

Possible explanations for a delayed engraftment rate with CBT as compared to BMT could relate to the infusion of smaller numbers of progenitor cells with CBT, or alternatively, to particular characteristics of the proliferative, self-renewing and differentiating capacity of cord blood cells (21).

Availability of cord blood for transplantation

The focus of CBT has been to provide benefits of hematopoietic stem cell transplantation to patients who do not have a histocompatible sibling donor.

Table 1 shows the ethnic profile of the Canadian bone marrow registry. As noted in various studies, bone marrow transplant registries are made up mainly of Caucasians. Comparison with data for the general Canadian population indicates that other ethnic groups have poorer representation in the registry.

Approximately 25% of patients suffering from diseases that would benefit from BMT will have a related donor, for 50% an unrelated donor will be located through the National or International Bone Marrow Transplant Registries at a median interval of 6 months, and for 25%, a donor will not be found.

According to the World Marrow Donor Association-Registry Annual Report, 3,032 marrow donors and 14 cord blood samples were donated to national and international patients in 1997 (31). Following this reasoning, about 1,000 patients will not have a transplant. This result is an under-estimate because, as shown in Table 1, these data refer mainly to Caucasians.

Some of this unmet demand for BMT might be met by CBT. Urgent cases might not survive long enough to benefit from BMT as there is a median 6-month time for a bone marrow donor search. Cord blood transplantation would also benefit such patients if there was an adequate cell dose (most likely to be achieved in children).

Given that BMT can only be undertaken for HLA identical or one HLA antigen mismatch recipients, cord blood that has been successfully undertaken on recipients with up to 3 HLA antigen mismatch would also be useful for those who cannot find adequate bone marrow donors.

Table 1: Ethnic profile of the Canadian bone marrow registry

Ethnic Origin	Number of donors	Percentage of UBMDR	Proportion in general Canadian population
Caucasian	128,392	74.1%	87.0%
Black	871	0.5%	2.0%
Oriental	8,128	4.7%	7.3%
Hispanic	595	0.3%	0.6%
Native	1,371	0.8%	2.8%
Other	7,823	4.5%	
Unknown	5,272	3.0%	
East Indian	2,405	1.4%	
Not tabulated	18,374	10.6%	
Total	173,231		

Source: Reference (5)

Evidence of efficacy

Definitions of outcome

In the studies reviewed for this report, hematopoietic recovery (engraftment) is defined in terms of time to a sustained absolute neutrophil count (ANC) of ≥ 500/μL and platelet count (APC) of ≥ 50,000 /μL (20, 35, 37). Platelet recovery is also defined in terms of time to a sustained APC of ≥ 20,000/μL (11, 14, 20). Failure of engraftment is defined as the absence of detectable engraftment at day 60, a second transplantation, or hematopoietic reconstitution with autologous cells (that is, the reappearance of cells with markers bearing the recipient's sex, ABO type, or HLA-antigen status).

Complete chimerism is defined as the presence of donor haematopoietic cells and mixed chimerism as the presence of both donor and recipient hematopoietic cells.

All studies have used rate of survival following CBT as the outcome measure. Survival was defined as time until death, and event free survival (for patients with malignant diseases) was defined as time to relapse or death, whichever occurred first (36).

Graft rejection was defined as engraftment followed by pancytopenia or GVHD without signs of myeloid engraftment. Acute GVHD was scored on the basis of standard criteria, classified as absent (grade 0), mild (grade 1), moderate (grade 2), moderately severe (grade 3), or severe (grade 4) and defined as that occurring within the first 100 days of cord blood transplantation. Chronic GVHD was

scored as limited or extensive based on organ involvement, and defined as that occurring after the first 100 days of transplantation.

Studies of efficacy

Most studies located with a sample size of greater than ten transplants, were retrospective and multicentre. Studies with less than ten patients are not elaborated here except for those by Shen et al. (30), who had the only study on multiple cord blood transfusion, and Issaragrisil et al. (14) who studied both BMT and CBT. No controlled studies of CBT were located.

Details of the six largest clinical studies are given in Table 2, Appendix E. In these, patients received either related or unrelated cord blood with HLA disparity of 0 to 3 antigens. The recent series reported by Rubinstein et al. (26) included three individuals with a 4 antigen mismatch. A patient receiving an HLA 4 antigen mismatch in the series reported by Gluckman et al. (11) showed no engraftment and had an early death. Some patients were included in more than one study.

In the retrospective study by Gluckman et al. (11), data were collected on 143 patients from 45 centres. Variables found to be associated with better survival were age of less than six years ($P< 0.001$), weight of less than 20 kg ($P< 0.001$), infusion of at least 37 million nucleated cells per kilogram ($P=0.04$), HLA identity with the donor ($P<0.001$), and cytomegalovirus-negative serologic status of the patient ($P<0.001$).

However, using the Cox model, only two of these factors were favorable for a better survival. These were, weight of less than 20 kg (relative risk, 0.24; 95% confidence interval, 0.11 to 0.52; $P < 0.001$) and cytomegalovirus-negative serologic status of the patient (relative risk, 0.41; 95% confidence interval, 0.18-0.90; $P = 0.02$).

Using the Kaplan-Meier estimate, the probability of an absolute neutrophil count of at least 500/mL by day 60 after transplantation was 79% in recipients of cord blood from related donors and 87% in recipients of cord blood from unrelated donors. The reason for recipients from unrelated donors having better results is unclear.

For platelet counts, the respective probabilities were 62% and 39%. Also, by the Kaplan-Meier estimate, survival at one year was 63% and 29% for recipients of cord blood from related and unrelated donors respectively, with a median duration of follow-up of 10 months (range, 1 to 30). These results suggest a correlation between parentage of cord blood (related or unrelated) and patient outcome. However, it should be noted that 60 of the 78 related recipients had no HLA disparity, while only 9 of the 65 unrelated recipients had no HLA disparity.

Overall survival at one year was 73% in recipients of HLA-matched cord blood and 33% in recipients of cord blood mismatched for one or more HLA antigens (P=0.006). The authors report that the number of HLA-mismatches between the donor and the recipient did not influence the survival rate. However, this could be due to the fact that the high-resolution molecular typing for class I and class II antigens was not used. Overall survival at one year for all 143 patients was 49%.

Most of the cases in the study reported by Gluckman et al. (11) were included in the consecutive series of 562 unrelated transplant recipients from 98 transplantation centres whose outcomes were reported by Rubinstein et al. (26) Outcomes were known at 100 days after transplantation for all patients and at one year for 94%.

Actuarial rates of engraftment were 81% at day 42 for neutrophils and 85% by day 180 for platelets. Grade III or IV GVHD occurred in 23% of patients and chronic GVHD in 25%. By one year, rate of relapse was 26%.

It is of interest that this group of recipients included a substantial proportion of older individuals (15% were aged 12 - 17 years and 18% were 18 or older). The oldest patient with successful engraftment was aged 58. Successful myeloid engraftment was associated with younger age, higher number of nucleated cells per kg body weight and absence of HLA mismatch.

By 100 days after transplantation, 261 patients had had transplanation-related events, including 218 deaths. Event-free survival correlated with diagnosis, age, number of leukocytes in the transplant, extent of HLA disparity and location of the transplantation centre.

The authors conclude that stored placental blood is a useful source of hematopoietic stem cells for patients who do not have a related histocompatible donor. The results indicate that the transplants regularly engraft, cause GVHD at a relatively low rate and produce survival rates similar to those for BMT using unrelated donors.

The largest study from a single centre was a prospective observational study of 25 patients with unrelated CBT (20). In this study, the number of nucleated cells infused per kilogram of the patient's body weight correlated with the rate of myeloid engraftment. The international cord blood transplant registry (ICBTR) found no such correlation in recipients of cord blood from related donors.

Both high and low resolution typing for HLA antigen disparity was done. However, no statistical difference was found with respect to end points or outcome measure.

Washing the cord blood unit with 10% dextran 40 and 5% human albumin before infusion seemed to accelerate myeloid engraftment but no observable effect was seen on platelet and red-cell engraftments. Primary graft failure occurred only

on high-risk patients who underwent transplantation during leukemic relapse, and severe acute or chronic GVHD was not observed.

Complete donor chimerism was achieved without total body irradiation in nine patients who were prepared with chemotherapy alone (20). This experience is relevant for transplantation in younger children, who are at especially high risk for treatment-related neurotoxicity. There was no correlation between the incidence or extent of GVHD and the degree of HLA disparity. The Kaplan-Meier estimate showed an overall 100-day survival rate of 64%, and an overall event-free survival of 48%.

A retrospective study of the first 44 related patients reported to the International Cord Blood Transplant Registry (ICBTR) was conducted by Wagner et al. (36). Time to neutrophil recovery was no different between those who received hematopoietic growth factor and those who did not. No correlation was found between cell dose infused and engraftment. According to the authors, this lack of correlation suggests that the number of progenitor cells in cord blood exceeds the threshold needed for engraftment.

With a median follow-up of 1.6 years, the probability of survival for recipients of HLA-identical or HLA-1 antigen mismatch was 72%. The probability of survival among the total group of patients was 62%. The probability of event free survival for patients with malignant and nonmalignant diseases was 46% (95% CI 0.22 to 0.69) and 78% (95% CI 0.59 to 0.97), respectively.

For patients with malignant diseases, the probability of relapse was 49% (95% CI 0.24 to 0.74). Graft failure occurred predominantly in patients treated for a nonmalignant disease. The authors noted that the etiology of graft failure is multifactorial, and may be related to cytoreductive therapy, immunosuppressive regimen, underlying disease and cell dose. Time of engraftment was not accelerated on patients who were given growth factors with respect to those who were not. The incidence of GVHD was low.

A retrospective overview of the clinical experience of 34 patients who had unrelated CBT as reported to the ICBTR was conducted by Wagner (35). No correlation was found between cell dose infused and engraftment, though subsequently, a correlation was found with a larger group of patients (Wagner, personal communication). Hematopoietic growth factors did not shorten time to neutrophil recovery. None of the patients with HLA identical donors developed grade 2-4 GVHD. Only two patients developed clinically significant GVHD. These recipients of HLA 3 antigen disparate grafts had grade 2 and grade 3 GVHD respectively.

Considering the fact that only three patients had HLA 3 antigen disparate grafts, this result might suggest a correlation between HLA disparity and GVHD. Data on deaths and survival rates were not given.

In a prospective study of 18 unrelated CBT patients by Wagner et al., (37). platelet recovery was markedly delayed as compared to that observed after unrelated donor BMT. There was no correlation between cell dose and engraftment. No transfusion reaction was observed in the eight patients receiving ABO incompatible graft.

Of considerable interest is that engraftment was observed in the largest recipient, weighing 78.8kg. As noted by the authors, there is no evidence that this could be routinely achieved.

Prophylactic use of hematopoietic growth factors correlated with neutrophil recovery. The median time to neutrophil recovery was 17 days (range, 16 to 24) for patients treated with growth factor beginning on the day of transplantation as compared to 26 days (range, 17 to 53) for patients not treated with growth factor before day 21. These results suggest a role of hematopoietic growth factor early after CBT. This observation contrasts with that reported for recipients of sibling donor cord blood (36).

Notably, only two patients developed grade 3 to 4 GVHD. This result seems in contrast to data shown in Table 2. However, only data on overall GVHD are documented there.

Shen et al. (30), conducted the only prospective study on the effects of unrelated, HLA-mismatch multiple CBT in four patients affected with advanced solid tumors. Despite multiple infusions of cord blood, only two patients had mild GVHD. Chimerism of sex chromosome was suggested in two of the four patients. Two patients had relapse of disease. One patient had an event-free survival, with a 6 months follow-up. No conclusions can be drawn on the risks and benefits of this approach.

Issaragrisil et al. (14) studied both bone marrow and cord blood stem cell transplantation for thalassemia. Six patients with thalassemia, with age from 1-7 years (median=5 years), had CBT. The number of mononuclear cells infused was 1.9-3.9x10^7/kg (median=2.9x10^7/kg. One patient died, five were alive and well.

Twenty-one patients had BMT. Outcome was favourable in those with hepatomegaly and splemomegaly <2 cm below the costal margin. Graft rejection is a problem in individuals with liver and spleen enlargement above this value. In this series, a higher dose of busulfan was used in seven patients with severe manifestations. One of these individuals died, the other six had complete engraftment and were alive and well.

Hematopoietic recovery, especially platelet recovery for CBT, was delayed as compared to BMT. The study lacked enough data for an adequate comparison of CBT vs. BMT.

A recent study by Kawano et al. (16) considered partially mismatched transplantation of 13 pediatric patients with allogeneic cells from a related donor.

Two individuals had early deaths. Nine of 11 showed signs of engraftment, there was subsequent rejection in four, two of whom had autologous recovery. Five patients were surviving free of disease at follow-up ranging from 476 to 937 days.

All studies had consensus on the following:

- Cord blood contains a sufficient number of hematopoietic stem cells and progenitor cells to engraft recipients with low body weight.
- Engraftment (platelet and neutrophil recovery) after CBT is slow compared with that following BMT.
- GVHD following CBT is mild and the incidence is relatively low.
- Cord blood is a safe source of hematopoietic stem cells for transplantation.

There was a lack of agreement between studies on a number of other points:

- A correlation was found between cell dose and engraftment by some studies (11, 20, 26) and not by others (35-37).
- A correlation was found between hematopoietic growth factors and time to neutrophil recovery by one study (37) and not by two others (35, 36).
- Two studies (11, 26) found that the incidence of GVHD increased with the degree of HLA disparity, while another (20) found no such correlation.
- Only the studies by Gluckman et al. and Rubinstein el al. found an association between HLA disparity and engraftment.

As observed by Kogler et al. (17), the risk of cord blood contamination decreases drastically as the cord blood collector (usually a nurse or midwife) gains experience. In this study, bacterial contamination of cord blood reduced from 18% to 1% as midwives gained experience. In all studies, cord blood used for CBT was hardly ever contaminated with bacteria.

The main post-transplant complications reported in all studies were relapse (in malignant diseases), graft rejection and GVHD. Relapse occurred mainly on patients with diseases at second or third remission ("high risk" patients).

Because pretransplant conditioning regimens varied, no conclusive statement can be made as regards its correlation with graft rejection. However, although the rate of engraftment was slow, all studies showed at least a 90% neutrophil and/or platelet recovery. GVHD was mild (Table 2).

Ethical issues

In this report ethical issues are presented following the approach by Burgio et Locatelli (4), considering the four fundamental and traditional principles of

bioethics, namely autonomy, nonmaleficence, beneficence and justice. Only studies with ethical issues relating particularly to CBT were considered.

Autonomy

As in all choices and decisions that concern the infant, with its intrinsic lack of autonomy, storage of cord blood for the purpose of CBT can only be validated by the informed consent of the parents (or guardians) (4, 10). McCullough et al. (23) propose that consent be obtained only from the mother. That of the father is not deemed necessary.

However, laboratory tests on the blood of the mother and on cord blood, and family medical history of both parents, especially of genetically transmitted disease are indispensable for a valid cord blood collection. Such information should normally be obtained only with consent of both parents.

The information made available to the parents should be explicit and objective, taking into account any implication, including the need for numerous diagnostic tests on the blood of the mother and on the cord blood before it is used. Consent should be obtained during prenatal care.

It might be argued that, because cord blood would normally be discarded, it could be considered unnecessary to obtain consent for collection. This would be violating human rights and would be unacceptable.

Nonmaleficence

Collection

Safety of the mother and infant is essential. Collection of cord blood obviously complies with nonmaleficence since no tissue useful to the donor is removed and no pain is caused. Early clamping of the umbilical cord at the time of delivery, a procedure practiced by some centres, results in collection of a larger volume of cord blood.

Detection of disease

Cord blood is not tested for presence of genetic disease. It would create a concern if testing of cord blood detects an incurable genetic disease. "The purpose of ethics in medicine is to reduce the likelihood of moral tragedy by subjecting dilemmas to systematic, rational analysis". A 1994 Institute of Medicine review recommends that minors not be tested for abnormal genes unless there is an effective curative or preventive treatment that must be instituted early in life (4).

Conception to produce a sample

A child conceived as a potential donor of cord blood to an ill sibling could feel at a future date like a "second class" child because he or she was conceived for the purpose of saving another child.

It is very difficult to separate the joy of having a child for extending a family from having a child in order to save an ill sibling. If the child were conceived just to save an ill sibling, HLA-typing in utero would tell if the cord blood would be suitable or not. There is a risk of abortion associated with this procedure, and hence the life of a healthy fetus may be jeopardized for another infant.

This scenario has already occurred in the USA, but in the context of an in vitro fertilization cycle where pre-implamentation HLA selection of the embryos allowed the parents to select the birth order of their subsequent children in favour of those whose cord blood would provide a suitable match for an older sibling in need. No abortion was involved, and an ethics committee found the decision to proceed reasonable, largely based on the fact the couple was prepared to transfer the remaining embryos at a future time (Cruickshank, personal communication).

Beneficence

Cord blood donation can be seen as an act of rewarding altruism and in any case a practice of solidarity. Cord blood, like any other human body part should never be commercialized. Payment to store cord blood for family purposes may be instituted as an "insurance policy" (6, 7). This, however, reduces the possibility of finding a donor and could be seen as defeating the purpose of cord blood banking itself, by excluding certain categories from a national cord blood transplant registry.

Cruickshank (personal communication) suggests this issue requires objective examination. Given adequate information about the chances of ever needing to use the cord blood for transplant within the family, should parents be denied the right to direct the outcome of their cord blood?

Another question that arises with CBB for family purposes is whether the cord blood owners, while refusing availability of their cord blood to others, should benefit from the national and international cord blood transplant registries.

Justice

The identity of the donor should be confidential. The donor must be followed-up to detect a genetic and infectious disease not found at birth, and strict anonymity between donor and recipient must be maintained (10, 23). At the University of Alberta, the donor identity is discarded after quarantine, that is six months after cryopreservation of cord blood. The identity of the donor is

discarded and cord blood validated only after a second blood test of the mother at this period proves negative for certain diseases, HIV in particular.

This way any negative response caused by a "window period" is avoided. This procedure eliminates the possibility of requesting a bone marrow donation in case of CBT failure. Nevertheless, extremely rare genetic diseases are unlikely to be revealed by the family history, and laboratory testing for them may be impractical or impossible, leading to the unwitting transmission of a genetic disease affecting hematopoietic cells. This could be a cause for law suits from the recipient or his/her family.

In the approach at the University of Alberta, parents not only accept an anonymous and free donation of cord blood but are also made to understand that the cord blood can be given for transplant purposes to an anonymous recipient. This implies that, if the donor has a hematological disorder for which a bone marrow transplant is needed, there is no guarantee that the autologous cord blood will be given back to the donor though all efforts will be made to trace the unit of cord blood.

If the unit of cord blood is traceable, it would be available for an autologous transplant. A point to consider is the impact through more frequent requests for unrelated bone marrow donations from infants. How would the recipient react to refusal of bone marrow donation, knowing the donor to be adequate? More importantly, how would the family of the infant donor react to such a request?

With advances in technology, certain genetic diseases not detectable at birth today might be detectable in the future. This poses the question of whether the infant's blood sample should be stored at the time of delivery or not.

Ethical issues are typically complex, involving social, cultural and religious belief. Addressing these issues may be tedious but also indispensable. As Burgio & Locatelli put it, "The day of the last bioethical discussion would also be the last day of civilized communal life." (4)

Discussion

Cord blood transplantation is an alternative to BMT that shows promise as a treatment for some types of patients, particularly children. CBT is being used as treatment for a range of diseases, with positive outcomes for patients for whom there is no alternative treatment.

However, while promising results have emerged, data on the safety and efficacy of CBT are still limited and the technology is in need of further validation.

The strength of evidence for benefit from CBT is limited. On the basis of a classification system that considers study design and conditions of scientific rigour, the strength of evidence would be regarded as fair to poor (15). There are still no well controlled, prospective studies of adequate power and follow-up.

The numbers of patients included in studies of CBT are still small. Most studies are retrospective with data obtained from the ICBTR and other transplant registries. Hence, data on most studies overlap, leading to some bias in conclusions.

There are no adequate comparative studies on the efficacy and safety of CBT compared to BMT. The differences in success rates are due in part to the lack of standardization of methods of HLA typing, limits of HLA donor mismatch, minimum cell dose required for transplantation and maximum weight of recipient to be transplanted. It is not evident that the use of growth factors enhances engraftment.

CBT is only one option for restoring hematopoieisis. In this rapidly developing field, the extent of any advantage of CBT over other approaches is still unclear. Russell (personal communication) points out that successful BMT with up to three antigens mismatched has been performed using bone marrow as a source of stem cells for many years in a few institutions. More recently, there has been much interest in the use of stem cells collected from the peripheral blood of donors. Collection of progenitor cells from the blood produces higher yields than from bone marrow thus compensating for losses during the necessary T-cell depletion procedures. Most children will have a parent who can act as a donor for this kind of transplant. Decisions on whether to refer individual patients for CBT will continue to require careful consideration by the specialists concerned.

The limited number of nucleated cells in cord blood raises concern about engraftment. All studies available for review showed slower engraftment of cord blood stem cells as compared to engraftment of stem cells derived from bone marrow. The number of nucleated cells infused per kilogram is a major factor in the recovery of neutrophil and platelet counts. Large prospective studies would be needed to determine an optimal number of cells needed for long-term engraftment.

An advantage of the technology is the lower incidence of GVHD in CBT compared with BMT, and the milder reactions for those who develop this condition. There seems to be no correlation between HLA disparity and GVHD. The rate of survival, particularly event free survival, which is the main outcome measure, has no correlation with any of the variables. CBT has the attraction of offering potentially useful treatment to patients for whom management options would otherwise be limited.

The question "Does the lower incidence of GVHD in CBT as compared to BMT, indicate a decrease in graft-versus-leukemia activity and thus an increase in the risk of leukemic relapse?" has yet to be answered.

Because the cell dose is an important factor for engraftment, controlled studies of the various methods of processing of cord blood are needed. From the literature, different approaches have been used in determination of cell counts, making

uniformity and interpretation difficult. As noted by Gluckman (10), standardization of methods of collection and cryopreservation of cord blood is needed. To be more specific, standarization is required in the techniques actually enumerating the mononuclear and CD 34+ cells. Once standarization of the evaluation of the cell components of the sample is achieved, then actual separation tecniques can be compared, based on the success of cell recovery. Such observations would naturally lead to a standard of quality expected in cord blood banking (Cruickshank, personal communication).

CBT offers an option for individuals, primarily children, who are unable to find a donor for BMT. Large cord blood banks would be needed to ensure adequate availability of the technology. The optimal size of such banks, their cost to the health system and their level of use, taking into account availability of other approaches to hematopoietic reconstitution, are matters that will require careful consideration.

Banking or transplantation of cord blood should be performed only in specialized centres with the necessary expertise and linked to recruitment of cord blood donors. Standard guidelines are needed for the cord blood banking system and the cord blood transplant registry. In the United States, the FDA and the Foundation for Accreditation for Haematopoietic Cell Therapy are coming forth with guidelines that would determine minimum standards for cord blood banking (Cruickshank, personal communication).

As indicated in this report, a number of ethical and legal issues need to be addressed. For example, there is not yet consensus as to who should give consent for cord blood donation. Rare genetic diseases are unlikely to be revealed by the family history, and laboratory testing for them may be impractical or impossible. The legal implications of this need to be considered.

Within the context of Alberta health care system, CBT should be regarded as a promising technology which is still developing. Any utilization should be linked to well-defined data collection protocols which include clinical outcome measures and appropriate patient selection criteria.

Appendix A: Methodology

Literature searches were conducted using the following electronic databases: Medline (1988-1998), EMBASE (1988-1998) and CancerLit (1995-1998). Search terms used included: 'stem cell', 'hematopoietic stem cell', 'hematopoietic stem cell transplantation', 'cell transplantation', 'umbilical cord', 'placenta', 'bone marrow transplantation', bone marrow', 'bone marrow cells', and 'cord blood banking'. These terms were used singly and in various combinations. Reference lists of retrieved articles were hand searched for studies that were missed by the electronic searches. All clinical studies on humans were considered for review.

Additional details on cord blood banking were obtained from University of Alberta Hospitals and the Canadian Red Cross Society.

Appendix B: Diseases that have been successfully treated with bone marrow transplantation

Bone marrow transplantation from HLA-identical sibling donors has been established as effective therapy for severe aplastic anemia (SAA), inducing hematopoietic reconstitution and long-term survival in 60-90% of patients (12, 29). Alternative therapeutic approaches to BMT for SAA are immunosuppressive therapy (IST) and antithymocyte globulin (ATG). A high rate of relapse with these approaches, make BMT first line therapy in pediatric SAA.

BMT remains the only form of curative treatment for patients with selected hereditary states (thalassemia, sickle cell anemia and Gunther's disease) who have an HLA-identical donor.

BMT is the treatment of choice for patients with primary immunodeficiencies (SCID, Wiscott-Aldrich Syndrome, chronic granulomatous disease) and metabolic diseases (i.e., osteopetrosis, Hurler's syndrome, adrenoleukodystrophy). Because BMT can only stabilize the clinical status of patients with metabolic diseases and will not reverse pre-existing organ damage, BMT for metabolic diseases should be done as soon as possible to maximize the quality of life of the transplant recipient.

However, the great majority of bone marrow transplants are performed on patients with neoplastic diseases. Chronic myelogenous leukemia (CML) accounts for 15% of all leukemias. BMT is the therapy of choice for younger patients (below 49 years) suffering from CML, and is the only proven cure for the disease. It is most successful if performed within the first year of diagnosis.

Long-term remission can be achieved in 50-70% of patients, if BMT is carried out during first remission of acute leukemia. When BMT is carried out later in the course of the disease, the disease free survival (DFS) for such patients decreases to the 10-40% range, depending on the stage of the disease and the overall performance status of such patients. Younger patients benefit more from this procedure.

Outcome of patients with acute nonlymphoblastic leukemia (ANL) following BMT in advanced or refractory disease is approximately 10% long-term DFS. For BMT in first relapse, patient outcome is 20-45% DFS, while patients in first remission have 50-70% DFS (13). Hence institution of early therapy greatly enhances patient prognosis.

DFS in advanced acute lymphoblastic leukemia (ALL) is 10-15%, depending on patient age. Pediatric patients have a better outcome than adults (2). Overall outcome in first relapse is 30-45%. DFS in first remission is 61% with a relapse rate of 10%. BMT for ALL patients with the Philadelphia chromosome resulted in an overall and DFS of approximately 40%, which is considerably better than that reported for chemotherapy.

An even more impressive result was obtained in 32 children with poor prognostic factors. The actuarial DFS was 84% at 5 years, with an actuarial relapse rate of 3.5% (3).

The timing of allogeneic BMT for patients with ALL remains an important question. For patients in second remission, prospective studies have demonstrated that marrow transplantation during second remission results in a DFS of 27-63%, with the more favorable results reported for children. The vast majority of patients are likely to be cured of their disease. But for high risk patients, there is controversy as to the routine use of BMT instead of chemotherapy for ALL cases. Early diagnosis is associated with better prognosis.

BMT is now established therapy and treatment of choice for the following diseases:

- SAA.
- Selected hereditary states (thalassemia, sickle cell anemia, Gunther's disease).
- Primary immunodeficiencies (i.e., SCID, Wiscott-Aldrich Syndrome, chronic granulomatous disease). It has had the greatest use in the Wiscott-Aldrich Syndrome.
- Metabolic diseases (i.e., osteopetrosis, Hurler's syndrome, adrenoleukodystrophy)
- Neoplastic hematological diseases (CML, acute leukemia, ANL, ALL), clinical trials for Hodgkin's disease, Non-Hodgkin's disease.

Appendix C: Cord blood banking

Collection

Beginning in 1993, the New York Blood Center initiated a program under the direction of Dr. Pablo Rubinstein to test the feasibility of a cord blood stem cell bank to collect, store, type and freeze up to 10,000 cord blood samples and make them available for transplantation (1).

Harvesting of cord blood is performed mainly by obstetricians or midwife nurses. Cord blood of healthy infants is collected after vaginal delivery. In a study conducted by Kogler et al. (17), the volume of cord blood obtained after cesarean section was on average 12% lower than that of cord blood samples from a normal vaginal delivery with the placenta in utero. However, due to the complexities involved most institutions do not collect cord blood after cesarean sections.

There are two methods of harvesting cord blood - while the placenta is still in utero or from the delivered placenta. The cord is doubly clamped about 5 cm from the umbilicus and transected between the clamps in 5-10 seconds after delivery while the placenta is still in utero.

Rubinstein et al. (27) collect cord blood from the delivered placenta rather than from the placenta in utero. They reason that blood collection in utero must be done at a time when the obstetrical staff is busy with mother and newborn and would be unable to perform the phlebotomy consistently. However, the volume of cord blood collected is not affected by the method of collection.

Seventy-five percent ethanol is used to disinfect the cord, and the puncture site is further disinfected with a povidone-iodine swab. Cord blood is harvested by veni-punture of the umbilical vein, and the blood is drained by gravity directly into a closed bag to reduce the risks of microbiological contamination. The closed container contains an anticoagulant.

Transportation

In Alberta, transportation of collected blood samples is by bus from locations close to Edmonton, and by air for collection from distant areas. Maximum time required for blood samples to get to cord blood bank for cryopreservation is generally between 24 and 36 hours. Minimum volume of cord blood required for processing in Alberta is 50 mL and volume of cord blood generally collected ranges between 40-200 mL (median 80 mL).

Quality control

Three mL of cord blood is removed under sterile conditions from the blood pack. Classes I and II HLA typing, ABO and rhesus grouping, and nucleated cell counts are performed both on the cord blood sample and on the maternal venous blood sample taken during phlebotomy for routine postpartum clinical tests.

HLA typing is done using serologic typing and if necessary, by polymerase chain reaction with sequence-specific oligonucleotides (PCR/SSO). Viral and infectious disease markers are performed only on the mother's sera. This includes screening for Anti-HIV1, Anti-HIV2, HIV1 Ag, Anti-HTLV, HbsAg, Anti-HCV, Anti-CMV, and syphilis. Markers of hematopoietic primitivity such as, CD-34+ and CFU assays are determined.

Processing

Because removal of erythrocytes from cord blood led to reduction of stem cells by 30%-50%, it was considered impractical to remove red blood cells from cord blood before cryopreservation. Cord blood was cryopreserved whole and subsequently used for CBT. This approach had two significant limitations: the risk of transfusion reactions (hemolysis) attributable to ABO blood group incompatibility between the donor and the recipient, especially if the recipient has high isoagglutinin titers, and the volume of cord blood stored. Reducing the volume of stored material may reduce the costs of storage and reduces the volume of DMSO transfused and, thus, should reduce the frequency and severity of post CBT reactions (toxicity) (23).

Because of the limitations associated with the transplantation of whole cord blood, cord blood is now fractionated by sedimentation of erythrocytes using one of various methods (24, 33, 38). A plasma extractor is used to extract the white blood cells into the transfer bag, which is to be stored in the cord blood bank. A 10 minute centrifugation separates most of the plasma remaining from the white blood cells.

Samples are then labeled. Each sample has a unique barcode. Cord blood and maternal blood have identical barcodes. The final label on the cord blood pack for storage contains the ABO and rhesus grouping, HLA typing, date of freezing and number of mononuclear cells.

Cryoprotection

DMSO at a concentration of 10% is used as cryoprotectant for storage of cord blood. A cyoprotectant allows the cells to shrink, hence protecting the integrity of stem cells by preventing intracellular ice formation. Intracellular ice (crystal-like) would cut and destroy mononuclear cells, thus destroying cord blood. The rate of addition of DMSO is very critical. Six mL of DMSO is added to mononuclear cells and the bag is placed on an agitator. Gentle agitation of the

bag ensures proper mixing of mononuclear cells and DMSO. The whole process takes about 15 minutes.

The final volume of cryopreserved cells, usually 25-30 mL, is noted on the bag. Quality control samples are also stored in three separate test tubes. The final cord blood sample contains about a million cells made up of mature cells and progenitor cells. The number of progenitor cells is not quantified.

Storage

Cord blood is placed in a cassette of metal plates and frozen by using the controlled rate freezing method to a temperature of -100^0C. The frozen bag is then transferred to the quarantine tank, and stored in nitrogen in the vapor phase at -175^0C.

Cord blood is quarantined for six months. If follow-up of mother and infant shows no evidence of infectious disease and no clinical genetic disease, then the cord blood is transferred to the main tank and stored in liquid nitrogen for five years at a temperature of -190^0C. At the University of Alberta, if no transmittable disease is detected, the identification of the donor is destroyed and deleted from every possible memory.

The quarantine tank will then be opened only when a recipient is detected. Under normal conditions, the test aliquot is thawed and tested, that is the cells are grown to assure existence of progenitor cells. This procedure takes 14 days. It is only after this period that the cord blood bank will give assurance that the cord blood is suitable, and the recipient will subsequently be prepared for transplantation. This procedure is eliminated when the request for cord blood is urgent i.e., the when the recipient is critically ill.

Transportation for transplantation

Cord blood for transplantation is transported in a dry shipper, that contains vapor nitrogen at a temperature of -175^0C. Cord blood can be preserved in the dry shipper for two weeks.

Transplant registries

In 1988 the World Marrow Donor Association (WMDA) was founded to facilitate international transplants and to contribute to high level standards worldwide. WMDA working groups have been established in areas such as donor registries, quality assurance, ethics, stem cells and establishment of guidelines.

Since the creation of the International Bone Marrow Transplant Registry (IBMTR), the possibility of finding an allogeneic unrelated donor has steadily increased. The chances of finding a donor for a Caucasian is considerably higher than for someone of a different ethnic group, since there are significantly fewer

volunteer donors in the latter. Today 37 bone marrow transplant registries are linked to IBMTR.

Cord blood registries collect information concerning the donor, and the HLA typing which are maintained in a centralized computer. This network is then linked to the existing registries for unrelated bone marrow searches. Eurocord, a group of physicians, is organized to standardize methods of collecting, testing, and cryopreserving cord blood from both related and unrelated donors, to study the properties of cord blood cells, and to manage a registry of cord blood transplantation performed in Europe (11). In Europe, NETCORD links three cord blood banks to its Bone Marrow Worldwide Registry. In the US, three cord blood banks which have received NIH funding for both banking and research are linked to a Bone Marrow Transplant Registry (7).

The International Cord Blood Transplant Registry was established in September, 1992 (36). This is aimed at facilitating international cooperation with other countries through the world marrow donor association and cord blood transplant registries. Searches for potential unrelated donor stem cell grafts are performed using the data bases of the various cord blood and bone marrow transplant registries.

Appendix D: Cord blood transplantation in Canada

Province of Alberta

Cord blood banks are either public or private. Public banks contain donated samples for use by the general population when necessary and may be partially funded by private donations and government grants. There is no direct financial cost to individuals who receive transplants. The Alberta cord blood bank (CBB) is a public bank. Private banks are for profit enterprises requiring an up-front processing charge as well as annual maintenance fees for the storage of the sample.

The Alberta CCB is the first public cord blood bank in Canada. It is dedicated to the provision of cord blood stem cells for transplantation of unrelated patients in treatment programs across Canada and in other countries.

In Alberta, consent to harvest cord blood is required from the mother, that of the father is not deemed necessary. Consent should be given early in the pregnancy or at the latest, one month prior to the due date. At present there is no recruitment of mothers for cord blood donation, due to limited funding.

Present freezing technologies allow storage of stem cells for a maximum of five years. The Alberta CBB intends to discard unused cord blood sample after five years of storage. Most of the stored samples will not be needed within this time frame.

It is estimated that with the storage of 50,000 cord blood stem cell samples, most Canadians needing transplantation in the unrelated setting will be able to find a suitable match. Cord blood stem cells appear to be very tolerant of their environment and are able to adapt, making it easy to find matches for transplantation among individuals who are not related to each other. For these reasons, the Alberta CBB does not emphasize self donations (Akabutu, personal communication).

Cord blood is banked at the Canadian Blood Services cord blood bank in Edmonton. To date, over 500 cord blood sample have been banked.

The first CBT in Canada was performed in Edmonton at the University of Alberta.

Other provinces

In Ontario, the Toronto Cord Blood Program (TCBP), supported through research funds from the Division of Reproductive Sciences, is an initiative of the Department of Obstetrics and Gynecology at the University of Toronto. (6,7).

The three downtown teaching hospitals particpating in this program are the Toronto Hospital, Mount Sinai Hospital and Women's College Hospital. The

TCBP has a fee for cord blood banking for family purposes, but will provide the service free of charge to expectant mothers who have a family member requiring immediate BMT and who have been referred to the program by an oncologist.

Samples have also been collected from patients delivering at other Ontario hospitals, but on a more limited scale. The priorities of this program are in the area of education and research. The program has researchers working in the areas of ex-vivo cell expansion, gene therapies, and collaboration with research into refining flow cytometry techniques to quantify the CD 34+ cells (Cruickshank, personal communication).

The program's first transplant took place in March 1997 (7). The patient, a 34-month-old child weighing 12.2 kg who had been suffering with beta Thalassemia Major since birth, received 6/6 HLA matched cord blood from a cousin. The patient engrafted and sustained no major complications. There were no available data on the patient's follow-up.

Lifebank offers expectant parents in Canada the opportunity to give their child "biological insurance" for the treatment of a variety of life-threatening hematological disorders, by registering their baby's cord blood, for exclusive use by the family (8).

The Canadian Red Cross Society[1] (CRC) has maintained a national databank of HLA-typed volunteer potential marrow donors known as the unrelated bone marrow donor registry (UBMDR). The UBMDR program created in 1988 operates as a network of CRC donor centres and laboratories that are linked to a search coordinating unit, the Canadian National Coordinating Centre (CNCC) which is subsequently linked to the International Bone Marrow Donor Registry.

CRC investigated options to create a national cord blood transplant registry that would subsequently be linked to the national and international bone marrow and cord blood transplant registries.

[1] Responsibility for these CRC activities now rests with Canadian Blood Services.

Appendix E: Efficacy of cord blood transplantation

The following table summarizes data from the from the six largest studies on CBT. Many patients are included in more than one study.

Table 2: Studies on the efficacy of cord blood transplantation

Study	Factor	Related Donors									Study Conclusions
		Sample Size	Graft Failure (%)	GVHD Grades					Death (%)	Relapse (%)	
				1	2	3	4	Chronic			
Wagner et al. (36). A retrospective study from 26 centres. October 1988 to November 1994. Data obtained from the ICBTR. N=44	Entire Group	44	7 (16)						16 (36)	8 (18)	- Hematopoietic growth factor did not shorten time to neutrophil recovery - No correlation was found between cell dose infused and engraftment - The incidence of grades 2-4 GVHD is extremely low
	Diagnosis Malignant Diseases	25	2 (8)								
	ALL	12	2(17)							3	
	AML	6								1	
	CML	4								2	
	Neuroblastoma	3								2	
	Non malignant diseases	19	5 (26)								
	Immunodeficiency	3									
	Inborn error	2	1 (50)								
	Bone marrow failure	14	4 (29)								
	HLA Disparity										
	0	34	4 (12)	27	1	-	-		9 (26)		
	1	4	1 (25)	2	-	-	-		1		
	2	1		1	-	-	-		1		
	3	5	2 (40)	1	1	1	-		5 (100)		
	Chimerism										
	Complete	27									
	Mixed	5									
	Not evaluated	3									
	Not tested	2									
	Growth factors										
	GM-CSF	8									
	G-CSF	17									

Table 2: Studies on the efficacy of cord blood transplantation (cont'd)

Study	Factor	Unrelated Donors									Study Conclusions
		Sample Size	Graft Failure (%)	GVHD Grades					Death (%)	Survival	
				1	2	3	4	Chronic			
Wagner et al. (37) A prospective study from two centres. July 1994 to December 1995 N=18	Entire Group	18	3 (17)	4	7	1	-		7 (39)	11	- Platelet recovery was markedly delayed as compared to that observed after unrelated donor BMT - There was no correlation between cell dose and engraftment - No transfusion reaction was observed in the 8 patients receiving ABO incompatible graft - The largest patient successfully transplanted weighed 78.8kg - Hematopoietic growth factors correlated with engraftment (data not shown) - Cord blood from unrelated donors is a safe source of transplantable hematopoietic stem cells for clinical transplantation
	Diagnoses										
	Malignant Disease	13	2 (15)	4	3	1	-		5 (38)	8	
	Non-Malignant Disease	5	1	-	4	-	-		2 (40)	3	
	Age										
	<6 years	11	1	2	6	-	-		2	9	
	6 - 15 years	5	1	2	1	1	-		3	2	
	>15 years	2	1	-	-	-	-		2	-	
	Weight										
	<20 kg	10	1	2	6	-	-		2	8	
	20 – 45 kg	4	-	1	-	1	-		2	2	
	>45 kg	4	2	1	1	-	-		3	1	
	Cell Dose										
	<20 million NC	2	2	1	1	-	-		1	1	
	20 – 40	6	-	2	1	1	-		3	3	
	>40	10	1	1	5	-	-		3	7	
	HLA Disparity										
	0	7	2	1	1	1	-		3	4	
	1	7	1	2	5	-	-		2	5	
	2	3	-	1	1	-	-		1	2	
	3	1	-	-	-	-	-		1	-	
	Chimerism										
	Complete	13	3	4	7	1	-		2*	11	
	Mixed	2	-	-	-	-	-		2	-	
	N.E.	3	-						3	-	
	CMV Status										
	Positive	9	1	3	2	-	-		4	5	
	Negative	9	2	1	5	1	-		3	6	

Table 2: Studies on the efficacy of cord blood transplantation (cont'd)

Study	Factor	Unrelated Donors									Study Conclusions
		Sample Size	Graft Failure (%)	GVHD Grades (%)					Deaths (%)	Survival (EFS)	
				1	2	3	4	Chronic			
Kurtzberg et al. (20) A prospective case series August 1993 to November 1995 N=25	Entire Group	25	2 (8)	6 (24)	9 (36)	2 (8)	-		7 (28)	12	- Cell dose infused correlated with the rate of myeloid engraftment - Myeloid engraftment was accelerated with' washed' units of cord blood - There was no correlation between the incidence or extent of GVHD and the degree of HLA disparity - Severe acute or chronic GVHD was not observed - The use of cord blood with an HLA disparity of 1-3 resulted in 100% donor chimerism, generally treatable GVHD, and immune reconstitution - Partially mismatched cord blood from unrelated donors is an alternative source of stem cells for hematopoietic reconstitution
	Malignant Diseases	19	2 (11)	5 (26)	6 (32)	1	-		6 (32)	7	
	Non-Malignant Diseases	6	-	1 (17)	3 (50)	1 (17)	-		1 (17)	5	
	Age										
	<6 years	12	-	4	5	-	-		3	7	
	6 - 15 years	11	2	1	4	1	-		4	3	
	>15 years	2	-	1	-	1	-		-	2	
	Weight										
	<20 kg	13	-	3	6	-	-		4	7	
	20 – 45 kg	10	2	2	3	1	-		3	4	
	>45 kg	2	-	1	-	1	-		-	1	
	Cell Dose										
	<20 million NC	6	2	1	2	-	-		1	1	
	20 – 40	11	-	1	3	2	-		5	5	
	>40	8	-	4	4	-	-		1	6	
	HLA Disparity (HRT)										
	0	1	-	-	-	-			-	1	
	1	9	1	5	-	1			2	5	
	2	11	-	1	6	1			4	4	
	3	4	1	-	3	-			1	2	
	HLA Disparity (LRT)										
	0	1	-	-	-	-	-		-	1	
	1	20	1	5	7	2	-		6	10	
	2	3	-	1	2	-	-		1	1	
	3	1	1	-	-	-	-		-	-	
	Chimerism										
	Complete	18								12	
	Mixed	-									
	ABO Compatible										
	Yes	19	1	3	9	1			6	9	
	No	6	1	3	-	1			1	3	
	Conventional Thawing Technique										
	Yes	3	1	-	1	2	-			1	
	No	22	1	6	8	-	-		7	11	

Table 2: Studies on the efficacy of cord blood transplantation (cont'd)

Study	Factor	Unrelated Donors									Study Conclusions
		Sample Size	Graft Failure (%)	GVHD Grades					Deaths	Survival	
				1	2	3	4	Chronic			
Wagner (35) A retrospective study from 18 centres. October 1988 to November 1993. Data obtained from ICBTR N=34	Entire group	34	4		1	1					- There is no correlation between cell dose and engraftment - There is delayed neutrophil and platelet recovery with respect to BMT - GVHD occurs with low frequency - Hematopoietic growth factors do not shorten time to neutrophil recovery - Cord blood contains a sufficient number of hematopoietic stem and progenitor cells to engraft small recipients < 40kg
	Diagnoses										
	Malignant disease	22	1								
	ALL	12	1								
	AML	4	-								
	CML	1	-								
	Juvenile CML	3	-								
	Neuroblastoma	2	-								
	Non-malignant disease	12	3								
	Fanconi anemia	5	1								
	Aplastic anemia	3	1								
	XLP	1	-								
	Hunter syndrome	1	1								
	Wiskott-Aldrich	1	-								
	ß-Thalassemia	1	-								
	Age										
	Median 5 years (range, 0.8 – 16 years)										
	Weight										
	Median 20.0 kg (range, 8.0 – 50.0 kg)										
	HLA Disparity										
	0	23			-	-	-				
	1	5									
	2	2									
	3	4			1	1					
	Cell Dose $4.0x10^7$/kg (range, $1.0\text{-}33.0x10^7$)										
	Growth factors	18									
	GM-CSF	8									
	G-CSF	9									
	Both	1									
	ABO-incompatible										
	Yes	12									
	No	22									

Table 2: Studies on the efficacy of cord blood transplantation (cont'd)

Study	Factor	Related Donors				Unrelated donors				Study conclusions
		Sample Size	Graft Failure	Death	Survival (%)	Sample Size	Graft Failure	Death	Survival (%)	
Gluckman et al. (11). A retrospective study from 45 centres. 1988 to 1996. Data obtained from Eurocord and 9 other centres. N=143	Entire Group	78	18	30	63	65	20	38	29	- Cell dose correlated with engraftment - HLA disparity increased the risk of engraftment - The incidence of GVHD increased with the number of HLA mismatches (only for related donors) - GVHD (data not shown) was not life threatening, and the incidence of chronic GVHD was low - Variables associated with better survival were: - Age < 6 years - Weight < 20kg - CMV negative recipient - Cord blood is an alternative source of hematopoietic stem cells for children and some adults with malignant and nonmalignant hematological diseases
	Diagnosis									
	Cancer	46	7 (4)	22	52	49	12 (7)	27	31	
	Bone marrow failure syndrome	17	3 (3)	6	65	9	1 (4)	8	0	
	Hemoglobinopathy	8	4	0	100	-	-	-	-	
	Inborn error	7	1 (1)	2	69	7	1 (2)	3	57	
	Age									
	<6 years	45	7	9	83	27	7	16	35	
	6 - 15 years	30	15	19	36	18	5	9	40	
	>15 years	3	1	2	33	20	8	13	16	
	Weight									
	<20 kg	40	6	7	82	24	5	13	38	
	20 – 45 kg	35	15	21	57	21	5	13	29	
	>45 kg	3	2	2	33	20	10	12	28	
	No. of nucleated cells infused/kg.									
	<37 million	38	14	16	57	36	15	23	22	
	>37 million	40	9	14	68	29	5	15	41	
	No of HLA mismatches									
	0	60	16	18	73	9	3	5	0	
	1	3	2	1	67	43	13	25	36	
	2	5	1	2	60	11	4	6	34	
	3	9	3	8	11	2	0	2	0	
	4	1	-	1	0	-	N.E.	-	-	
	Sex parity									
	Yes	41	10	12	73	30	10	17	29	
	No	36	12	17	53	32	9	18	37	
	CMV status									
	Negative	40	9	9	74	26	8	11	42	
	Positive	36	13	20	42	39	12	27	20	

Table 2: Studies on the efficacy of cord blood transplantation (cont'd)

Study	Factor		Sample Size	Graft Failure*		GVHD Grades (%)					Death % (100d)	Study Conclusions
				Sample Size	Events (%)	Sample Size	0 or 1	2	3 or 4	Chronic		
Rubinstein (26) 98 centres, August 1992 to January 1998	Entire Group		562	546	(31)	399	(54)	(24)	(23)	(25)	41	– successful engraftment associated with younger age, higher numbers of nucleated cells/body weight, absence of HLA mismatch. – GVHD did not correlate with HLA disparity. – In recipients with leukemia, relapse was associated with severity of GVHD, disease type and disease severity. – Placental blood is a useful source of allogeneic hematopoietic stem cells for bone marrow reconstitution.
	Diagnoses											
	Malignant Disease		383									
	Non-Malignant Disease		179									
	Age											
	<2 years		114	109	(19)		(59)	(28)	(13)			
	2-5 years		127	123	(30)		(54)	(25)	(21)			
	6 - 11 years		137	135	(29)							
	12 - 17 years		83	82	(48)		(48)	(18)	(34)			
	>18 years		102	97	(46)							
	Weight											
	<10 kg		77									
	10 – 19 kg		148									
	29 - 39 kg		152									
	40 - 59 kg		91									
	>60 kg		94									
	Cell Dose (million per kg)											
	<7 - 24		166									
	25 – 49		205									
	50 - 99		122									
	>100		69									
	HLA Disparity			553								
	0		40	36	(0)	34	(74)	(18)	(9)			
	1		218	211	(22)	156	(52)	(40)	(35)			
	2		261	257	(18)	207	(52)	(24)	(25)			
	3		37	39	(31)							
	4		3									
	Infection after transplantation	No				93	(57)	(30)	(13)			
		Yes				291	(53)	(22)	(25)			

* Kaplan-Meier estimation at Day 42

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