

. Review article

## Umbilical cord blood transplant in human\*

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### Abstract

Human cord and placental blood provides a rich source of hematopoietic stem cells. On the basis of the finding, umbilical cord blood stem cells have been used to reconstitute hematopoiesis in children with malignant and non malignant diseases after treatment with myeloablative doses of chemoradiotherapy. Early results show, that a single cord blood provides enough hematopoietic stem cells to provide short and long term engraftment and, that the incidence and severity of graft versus host disease has been low even in HLA mismatched transplants. These results are encouraging enough to embark on large scale banking of cord blood for purposes of future allogeneic and autologous stem cell transplantation, to promote studies on the unique properties of fetal and neonatal hematopoiesis, to study the immunological properties of cord blood cells and, to initiate investigations on gene transfer into human cord blood cells for future gene therapy trials. This review will briefly summarize the current knowledge on cord blood transplantation as well as the future development of research on this unique source of hematopoietic stem cells.

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### Clinical results of cord blood transplantation

The first cord blood transplantation was performed in 1988 in a patient who was affected by Fanconi's anemia [1]; the mother was pregnant, prenatal diagnosis and HLA typing was performed *in utero*. According to previously observed findings, showing that a single cord blood collected and cryopreserved at birth contained enough hematopoietic stem cells for long term engraftment, the patient was conditioned for transplant for Fanconi anemia according to ongoing protocols and received thawed cord blood cells from his HLA identical sibling. Eight years after transplantation, the child is doing well, and is apparently cured of his original disease. Since this first case, the number of cord blood collections and transplants has been rapidly increasing [2-6]. The donors were mostly HLA identical siblings, but some successes have been reported with siblings different for 1, 2 or 3 HLA antigens. In addition, cord blood banks have been developing worldwide, with an increasing number of unrelated cord blood transplants with HLA matched or partially matched donors.

A preliminary analysis of the International cord blood transplant registry shows that between October 1988 and September 1994, 50 patients aged 1.3 to 47.8 years with malignant (n : 30) and non malignant disorders (n : 20) received allogeneic umbilical cord blood for hematopoietic rescue after myeloablative therapy [7]. The graft was collected from 44 siblings, 34 HLA identical and 10 HLA mismatched for 1 to 3 antigens. Unrelated donors, matched in 1 case or mismatched in 5 cases, were also used. Median recipient weight was 20 kg; median number of nucleated cells:  $4.3 \times 10^7/\text{kg}$  (range  $0.8\text{-}33 \times 10^7/\text{kg}$ ); median number of CFU-GM:  $1.9 \times 10^4/\text{kg}$ . Median time to recovery was 22 days for neutrophils and 48 days for platelets. Neither engraftment of donor cells nor time to hematopoietic recovery correlated with number of nucleated cells or CFU-GM.

Engraftment was observed in 34 of 44 patients. Absence of engraftment can be explained either, by the poor status of the patient at time of transplant or, by an insufficient number of stem cells in the transplant. The addition of in-vivo growth factors did not modify the speed of engraftment. It was observed that the time to engraftment was delayed when it was compared to a similar group of bone marrow transplant patients, this could be due to the immaturity of the stem cells infused which could take more time to differentiate. Graft versus host disease was not observed in patients receiving an HLA identical sibling transplant and was limited in recipients of mismatched related or unrelated transplants. This decreased rate of GVH could be due to the young age of donor and recipient, to the absence of previous immunisation or activation of donor cells by infectious agents, to the low number of lymphocytes present in one cord blood, to the immunosuppressive effect of contaminating mother's cells, or to the immaturity of immunological functions at birth. It is known that cord blood is enriched with immature naive lymphoid cells, some of them might have a suppressive phenotype.

A recent survey, performed in Europe by the Eurocord transplant group, has collected 59 cases transplanted in 19 centers. Of these cases, 53 (90%) were aged < 15 years, and there was even distribution between the sexes (30 males and 29 females). Mean age was 8 years (0.2-28); mean weight was 27.8 kg (5.8-68). The most frequent diagnoses were malignant diseases (41 cases) with acute lymphoblastic leukemia (ALL) 20 (34%), followed by acute myeloid leukemia (AML) 8 (13.6%), chronic myeloid leukemia (CML) 6 (10%), myelodysplastic syndrome (MDS) 4 (7%), non Hodgkin lymphoma (NHL) 1 (1.7%) and neuroblastoma 2 (3.4%). Among the non-malignant diseases (18 cases), they were aplastic anemia in 4, Fanconi anemia in 7 and inborn error in 7 cases (leukocyte adhesion deficiency, bare lymphocyte syndrome, Gunther disease, Hurler syndrome and sickle cell anemia).

Most donors were HLA identical siblings (32; 54%), but 13 (22%) were HLA mismatched siblings, 1 (1.7%) was HLA matched but unrelated and 12 (20%) were both HLA mismatched and unrelated. In terms of ABO incompatibility, 24 cases were matched, 14 had a minor mismatch and 10 a major mismatch. All patients received various conditioning regimens including either TBI (26, 44%) or Myleran (21, 35%) with other chemotherapy and ATG in mismatched transplants (22, 37%). After transplant, 66% received growth factors, graft versus host prevention consisted of Cyclosporine alone (56%) or associated with methotrexate or prednisone. Mean values (ranges) of cells in the grafts were : mononuclear cells  $0.5 \times 10^8/\text{kg}$  (0.1-0.8); CFU-GM  $0.2 \times 10^5/\text{kg}$  (0.1-2.1); CD34  $0.5 \times 10^6/\text{kg}$  (0.1-5.4). Thirty-three (56%) of the patients survived the transplant, of whom 25 had (42%) had GVH  $\geq 2$ . There was no engraftment/rejection in 15 cases with 3 autologous reconstitution and 2 mixed chimerism. Eleven patients relapsed. Actuarial 2-year survival was 45%; it was 65% in HLA identical sibling transplants and 32% in mismatched transplants ( $p : 0.0058$ ). There was a significant association between non engraftment/rejection and the number of cells infused ( $p : 0.01$ ) but no association was found with HLA mismatch, use of hematopoietic growth factors, diagnosis or use of total body irradiation. This preliminary results show that HLA identical sibling cord blood transplants give results similar to bone marrow transplants with limited GVH and long term full hematopoietic and immunological reconstitution. Mismatched cord blood transplants are more difficult to analyze in this series because of the addition of several poor risk factors. These included advanced disease, a low number of cells and the degree of mismatch. Several unanswered questions remain; the minimum number of cells required for engraftment remains to be established, as does the role of contamination by maternal cells and the degree and severity of GVH compared to other sources of hematopoietic stem cells. Avoidance of selective reporting and agreement on prospective common protocols should give some answers on the indications of mismatched cord blood transplants or CD34+ mismatched family allogeneic peripheral blood stem cells.

### **Cord blood banking**

Following these preliminary encouraging data, several groups have been working for establishing criteria for collection, evaluation of the number of stem cells, volume reduction, purification, selection of CD34+ cells, screening for infectious and genetic diseases, freezing and thawing of cord blood for large scale banking [8, 9]. Most of the techniques used are not different from those used for adult peripheral blood or bone marrow hematopoietic stem cells. One of the problem is the possible loss of cord blood progenitors with any attempt at stem cell purification or concentration: different gradients of Ficoll or Percoll have been used with variable

success [10]. In ABO incompatible grafts, non red cell depleted cord blood has been used without any major side effect but, better method of selective red cell depletion have been investigated This is a very important question because if large frozen cord blood banks develop worldwide, there will be a space and economic advantage to reduce the cord blood volume cryopreserved from the usual 100 to 200 ml to 50 ml or less.

Another issue is the quantification of hematopoietic stem cells present in cord blood and the determination of the minimum number of cells required to transplant a child as well as an adult patient [11-14]. It is well known that the current methods of quantification by measuring the number of CD34+ cells or the number of CFU-GM are not standardized and cannot be compared from one laboratory to another one. Several authors measured the engraftment potential of cord blood stem cells, compared to peripheral blood or bone marrow stem cells, by limiting dilution analysis of long-term culture-initiating cells. The number of Long Term Colony Forming Cells (LTC-CFC) and CFU-GM per mononuclear cell from cord blood were 2.5-fold and 2-fold greater than those from peripheral blood cells respectively. Using a 2 stage long term culture system and limiting dilution techniques, scoring cobble stone areas of greater than 15 hematopoietic cells weekly for up to 15 weeks, Pettengell et al have shown that the incidence of putative stem cells in leukapheresis product and umbilical cord blood are at least comparable with that of bone marrow [15].

We have compared the number of cells infused in bone marrow, peripheral blood and cord blood, (Table 1) it shows the large variability of cells infused and the lack of predictability of the current measures for predicting the speed of engraftment.

	Cord blood n:59	pbscn:11	BM n:111
MNC 10 <sup>8</sup> /kg	0.5(0.1-9.8)	8.12(3.25-49.8)	2.3(0.5-7)
CD3 10 <sup>8</sup> /kg	ND	2.73(0.74-17.8)	ND
CFU-GM 10 <sup>4</sup> /kg	2(1-21)	47.5(14-390)	6.3(0.97-24.5)
CD34+ 10 <sup>6</sup> /kg	0.5(0.1-5.4)	7.14(3.15-39.7)	3.27(0.07-15.4)

**Table 1**  
Comparison of the number of cells infused according to different sources. Results from Saint Louis Hospital and Eurocord

Cord blood banking for autologous or allogeneic transplants has several advantages: easy access, indefinite storage, speed of donor search, viral safety, source of stem cells for expansion and gene therapy. For this reason, several banks have been established, in several countries, the most developed are in New-York, Milan, Dusseldorf and Paris. Ethical and legal issues have been debated [16, 17].

The number of cord blood samples necessary for providing cells for a large number of patients will depend on the results of transplants performed with full HLA matched or partially mismatched unrelated donors. These results will be available only in several years ; in order to have quicker answers, common prospective protocols must be applied to this type of transplants. Preliminary data in more than 100 patients transplanted with matched or 1 or 2 antigens mismatched unrelated donors indicate that the results seem as good as in related partially mismatched related bone marrow transplants. If these results are confirmed with more patients and more follow-up, cord blood banks containing a total of 20.000 to 50.000 samples should be sufficient to cover the needs.

In order to coordinate research and organisation of cord blood banking, several cooperative groups were formed in the USA and in Europe. The Eurocord transplant group was formed with the purpose of studying the properties of hematopoietic stem cells present in placental blood at birth for potential use in patients affected by hematological diseases. The potential uses of cord blood include familial and unrelated hematopoietic stem cell transplantation in adults, children and *in utero*. Gene therapy and immunotherapy are also possibilities.

Objectives of Eurocord are :

- standardization of the methods of collection, testing and cryopreservation of cord blood for banking and clinical use;
- study of the immune properties of cord blood;
- study of the hematopoietic progenitors present in cord blood;
- depository of cord blood cells, DNA and serum;
- European registry of cord blood transplants.

### Unique properties of cord blood hematopoietic stem cells

Among several advantages, various authors have shown that cord blood was enriched with CD34+ cells and mostly with the more immature compartment rh123 (rh=rhodamine) low, CD38- [18, 19]. Colonies obtained from this selected populations are larger, have a better replating capacity and grow longer in long term culture [20]. Contrary to what has been observed in adult bone marrow, LTCBC-IC and presumably CB cells, capable of *in vivo* engraftment, reside in the CD34+, HLA-DR+ Rh 123dull fraction of CB. Thy-1 CD34+ CD45RA lo CD71lo expression on primitive cord blood progenitors have the highest *in vitro* proliferative potential, this suggests that Thy-1 is involved in early stem cell development [21].

In contrast to adult bone marrow, purified progenitors obtained from umbilical cord blood undergo clonogenic maturation even in the absence of added growth factors [22]. Schibler et al conclude that production of growth factors occurs within culture dishes containing hematopoietic progenitors of umbilical cord origin, this autocrine or paracrine production of growth factors might explain some of their apparently unique features of *in vitro* growth [23].

Unseparated or purified hematopoietic stem cells from cord blood were transplanted to severe combined immunodeficient mice [24]. High levels of multilineage engraftment, including myeloid and lymphoid lineages were obtained with 80% of the donor samples as assessed by DNA analysis, fluorescence-activated cell sorting, and morphology. In contrast to previous and concurrent studies with adult bone marrow, treatment with human cytokines was not required to establish high-level human cell engraftment. There is growing evidence that the difference between adult and foetal hematopoiesis is not just a quantitative difference but, that there are ontogeny-related differences related to differences of cell signaling and growth receptors requirements. Another evidence has been provided by the study of telomeric length. The authors have shown that telomeric length decreases with age and they propose that the sequential loss of telomeric DNA, from the ends of human chromosomes with each somatic cell division, eventually reaches a critical point which triggers cellular senescence [25].

### Immunological properties of cord blood cells

Cord blood is enriched in immature lymphocytes with double positive CD3+ cells. Functional studies have given conflicting results [26, 27]. One major subset is CD3- belonging to NK subset. Using a monoclonal antibody, which recognizes human functional peripheral blood cytotoxic lymphocytes with either natural killer (CD3-) or cytotoxic T lymphocyte phenotype, it was shown that cord blood, in contrary to adult peripheral blood, contains exclusively cytotoxic natural killer cells and no cytotoxic T lymphocyte activity [28, 29].

### Gene transfer in cord blood

Retrovirus and adeno-associated virus 2 vectors have been transferred to purified and non purified cord blood hematopoietic stem cells. Both studies show that the efficiency of gene transfer in cord blood is higher than in bone marrow cells, even in the absence of added hematopoietic growth factors [30]. This property is very important for future studies of gene therapy in genetic or acquired disorders. A first attempt has been performed by D Kohn et al who introduced the ADA gene in cord blood cells of 3 children with ADA deficiency whose cord blood was collected at birth, transfected during a short culture and reinfused to the infants. The children have been treated with enzyme replacement and are doing well; at 23 months of age, it has been shown that the gene is expressed in about 1% of the bone marrow population, and that the expression increases with age suggesting that transfected cells might have a selective growth advantage [31].

In conclusion, research on cord blood has shown many interesting developments including transplantation, gene therapy and cell therapy including isolation and characterisation of primitive hematopoietic stem cells and manipulation of the immune system. Clinical results are encouraging, collection of clinical observations and elaboration of prospective protocols should improve quickly our knowledge in this field.

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