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Cord blood: a new source of hematopoietic stem cells

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A B S T R A C T

During the nearly 40 years elapsed since the first successful bone marrow transplantation, significant improvements have been registered in the field of allograft, also in connection with the use of cord blood as a source of hematopoietic stem cells. The number of cord blood transplants is increasing worldwide with the establishment of related and unrelated cord blood banks. The incidence of both acute and chronic graft-versus-host disease, the most important and life-threatening immune complications after transplantation, is significantly reduced with cord blood grafts and, in view of this fact, cord blood transplantation offers the opportunity of using HLA-disparate donors. This latter finding, together with the immediate availability of cryopreserved cells, is the main advantage for children who lack an HLA-identical sibling and who need transplantation from an unrelated donor. Moreover, in patients with hematological malignancies, the rate of relapse appears to be similar to that documented in bone marrow transplant recipients. The main limitation related to cord blood transplantation is represented by the number of cells available in a single unit which has so far restricted the use of cord blood as source of hematopoietic stem cells mainly to children. Approaches aimed at increasing the number of cells/hematopoietic progenitors infused, such as transplanting two units in the same patient or *ex vivo* expanding cord blood hematopoietic stem cells, could further increase in the future the number of cord blood transplantation performed every year.

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Allogeneic haematopoietic stem cell transplantation (HSCT) is a treatment largely employed for a number of hereditary and/or haematological conditions, both malignant and non-malignant in pediatric patients (see also Table 1 for details).[1] Nearly 40 years have elapsed since the first successful application of HSCT in 1968[2,3] and for the first 20 years (namely between 1968 and 1988), bone marrow (BM) was the only source of hematopoietic stem cells (HSC) employed for transplanting patients in need of an allograft.[1,4] In 1988, the first transplant with HSC deriving from placental blood of an HLA-identical, healthy sibling collected at the time of delivery was successfully performed in a patient affected by Fanconi's anemia.[5] Eighteen years later, this patient is doing well with full donor hematopoietic and lymphoid reconstitution.

After this first seminal case, the safety of placental blood collection for the newborn,[6] as well as the demonstration of cord blood transplantation (CBT) efficacy in several disorders,[7,8] have provided the rationale for starting large programs of collection, characterization, cryopreservation and storage of HSC from umbilical cord blood to be employed for transplantation even from a non-consanguineous donor. Indeed, since 1988, several hundred children have been cured by transplantation with cord blood cells.[7-12] The available evidence indicates that, as compared to bone marrow transplantation (BMT), CBT offers the clinical advantages of absence of risks to donors, reduced risk of transmitting infections, reduced incidence and severity of graft-versus-host disease (GVHD) and, for transplants from unrelated donors, immediate availability of cryopreserved cells, the median time-lapse for the donor search being only 3-4 weeks.[7-13]

Together with these clinical advantages, several biological studies have also demonstrated that, with respect to their BM counterparts, cord blood cells are enriched with *in vivo* long-term repopulating stem cells and, when compared *in vitro* with BM cells, they: (i) produce larger colonies; (ii) have higher recloning capacity; and (iii) have a better capability of engraftment into the NOD/SCID mouse model.[13-16] Likewise, vari-

Table 1. Main indications to allogeneic haematopoietic stem cell transplantation in childhood

Acute lymphoblastic leukaemia in I complete remission* in II complete remission in III or further complete remission	Severe combined immunedeficiencies (SCID) Immunodeficiency with hyper IgM
Acute myeloid leukaemia in I or further complete remission	Leukocyte adhesion deficiency
Chronic myeloid leukaemia	Omenn syndrome
Myelodysplastic syndromes	Wiskott-Aldrich syndrome
Hodgkin and non-Hodgkin lymphoma	Chediak-Higashi syndrome
Selected types of solid tumours**	X-linked lymphoproliferative disease
Severe aplastic anaemia	Kostmann syndrome
Fanconi anaemia	Chronic granulomatosis disease and other severe neutrophil function disorders (i.e. Schwachmann syndrome)
Dyskeratosis congenita	Life-threatening, hereditary platelet disorders (i.e. Glanzmann thromboasthenia, Bernard-Soulier syndrome)
Diamond-Blackfan anaemia	Haemophagocytic lymphohistiocytosis
Thalassaemia major	Selected types of mucopolysaccharidoses
Sickle cell disease	Selected types of peroxisomal and lysosomal disorders
Infantile malignant osteopetrosis	Selected types of life-threatening autoimmune disorders resistant to conventional treatments

**Patients at high risk of recurrence (i.e. t(9;22) o t(4;11); T-ALL with poor-prednisone response). **Stage IV Neuroblastoma, renal cell carcinoma, very high risk Ewing sarcoma.*

ous immunological properties and peculiarities of cord blood, which may contribute to the reduction of GVHD observed after CBT even when the donor is HLA-disparate with the recipient, have been identified.[17-21] In particular, it is now known that cord blood lymphocytes (CBL) are naive cells,[17-19] with low T-cell mediated cytotoxic capacity[20] and, *in vitro,* markedly reduced responsiveness to allogeneic stimuli in the secondary mixed lymphocyte reaction (MLR).[21]

In the past, the reported low incidence of GVHD after CBT has been hypothesized to also represent a major drawback in leukemia patients. In fact, since the role of allogeneic lymphocytes in the control and/or eradication of malignancy is well established, the absence or reduction of the component of graft-versus-leukemia (GVL) activity associated with GVHD could represent a theoretical concern in leukemia subjects given CBT. However, available data do not support the hypothesis that patients given CBT benefit from a lower GVL effect.[12,22] Innate cell-mediated immunity could likely represent the most important mechanism for controlling the re-growth of leukaemia blasts in CBT recipients. In fact, previously published studies indicate that cord blood LAK cells are able to lyse non-cultured fresh leukemia blasts[23] and that their activity towards cell lines, such as Daudi and YAC-1 cells, is greater than that of BM cells.[24] These findings support the hypothesis that aspecific cytotoxic activities can contribute to prevent leukemia relapse and that, notwithstanding a lower incidence of GVHD, patients given CBT may benefit from an efficacious, donor-derived GVL effect.

CBT from a related, HLA-compatible donor has been used for treating children with both malignancies and hereditary disorders, including hemoglobinopathies and immune deficiencies. In these patients, the reported results have been at least as good as those obtained in children given an HLA-identical sibling BMT.[5,7,25,26]

The absence of transplant-related mortality reported in a recent study in patients with thalassemia and sickle cell anemia provides stringent evidence that CBT from an HLA-compatible sibling may be an attractive option for couples who already have a child affected by an inherited disorder. The possibility of using cord blood HSC may also lead parents to program the birth of a child HLA identical with the prospective recipient, through the technique of pre-implantation genetic diagnosis, with the aim of saving the life of an affected brother/sister.[27] The ethical problems that such a program may raise are mainly inherent to the choice of giving birth to a human being exclusively as a "means" of saving another human being's life and they have been extensively discussed in the recent past.[6,28]

Compared with children transplanted from matched unrelated bone marrow donors, those given CBT from an unrelated donor have delayed hematopoietic recovery, experience a lower incidence of acute and chronic GvHD and, due to a higher risk of infectious complications, may be exposed to an increased risk of transplant-related mortality (TRM) in the early post-transplant period.[8-12,26]

The most relevant factor influencing the risk of TRM is the number of cells infused. In fact, the number of infused cells correlates inversely with the cumulative incidence of TRM, children given fewer cells per Kg/recipient body weight being at the highest risk of experiencing fatal complications.[8-12,26] This increased risk of fatal infections is mainly due to the slow neutrophil recovery, although also the lack of antigen-experienced (memory) T-cells, which are not transferred in CBT, may play a role. In fact, memory T cells significantly contribute to early immunological reconstitution of patients after unmanipulated allogeneic BM or peripheral blood stem cell transplantation. Engraftment and the kinetics of hematopoietic recovery are major concerns when the cord blood nucleated cells infused are less than 1.5-2.0 $x10^7$/Kg of recipient body weight. As a cord blood unit usually contains between $8x10^8$ and $1.5x10^9$ cells, it is not surprising that CBT is not routinely utilized in adolescents with a body weight of over 40-50 Kg. More recently, Wagner *et al.* showed that the infused $CD34^+$ cell dose may be a more potent indicator of prognosis than the nucleated cell dose.[29] They described a threshold of 1.7 $x10^5$ $CD34^+$ cells/kg and suggested that cord blood units containing less than this $CD34^+$ cell dose should be considered inadequate for routine use because of a very high risk for TRM.[29] These findings have to be interpreted in the context of HLA disparity. In fact, several studies have recently suggested that the impact of cell dose could be more significant when the donor/recipient HLA-incompatibility increases.[8-11,30] However, the real influence of HLA disparities on outcome of unrelated donor CBT is still controversial and not fully established. Approaches to increase the number of cells/hematopoietic progenitors infused, such as transplanting two units in the same patient or ex vivo expanding cord blood hematopoietic stem cells, are under development, with promising, initial results.[31,32]

Despite the increased risk of TRM in the early post-transplant period, the final outcome of children with leukemia given an unrelated donor CBT has been demonstrated to be comparable to that of patients given either an unmanipulated or a T-cell depleted BMT from an unrelated volunteer as the risk of leukemia recurrence is not increased and the cumulative incidence of chronic GVHD is lower after CBT.[22,33] Unrelated donor CBT has been documented to be a valuable option also for some inherited metabolic disorders, such as Krabbe's disease and Hurler syndrome.[33,34]

Due to the demonstration of efficacy in different clinical settings, unrelated donor CBT has become a widely employed therapy for many pediatric patients in urgent need of an allograft and umbilical cord blood banks have been established in many Western countries, with advantageous integration with volunteer BM donor registers. The current situation can be summarized as follows: although perfectly accurate and complete records are not available, it is estimated that, to date, at least 200,000 units of cord blood from a non-consanguineous donor have been successfully collected, characterized and cryopreserved and they have facilitated the realization of at least 3,000 transplant procedures. Thus, in view of these findings, there is no doubt that a new era in the field of HSCT was opened around 20 years ago by the pioneering work of Eliane Gluckman[4] and that cord blood, for many years considered only the way through which the foetus is nourished by the mother during the prenatal life, takes on a new meaning in life.

References

1. Locatelli F, Burgio GR. Transplant of haematopoietic stem cells in childhood: where we are and where we are going. Haematologica 1998; 83: 550-563.
2. Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA. Immunological reconstitution of sex linked lymphopenic immunological deficiency. Lancet 1968; 2: 1366-1369.
3. Bach FM, Albertini MJ, Joho P, Anderson JL, Bortin MM. Bone marrow transplantation in a patient with the Wiskott-Aldrich syndrome. Lancet 1968; 2: 1364-1366.
4. Gluckman E, Broxmeyer HE, Auerbach AD, et al. Hematopoietic reconstitution in a patient with Fanconi anemia by means of umbilical cord blood from a HLA identical sibling. N Engl J Med 1989; 321:1174-1178.
5. Burgio GR, Locatelli F. Programming a haematopoietic stem cell donor: the evolution of a project over time. Haematologica 2006 91: in press.
6. Bertolini F, Battaglia M, De Iulio C, Sirchia G. Placental blood collection: effects on newborns. Blood 1995; 85:3361-2.

7. Wagner JE, Kernan NA, Steinbuch M, Broxmeyer HE, Gluckman E. Allogeneic sibling umbilical cord blood transplantation in forty-four children with malignant and non-malignant disease. Lancet 1995; 346:214-19.
8. Gluckman E, Rocha V, Chammard A, et al. Outcome of cord blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. N Engl J Med 1997; 337:373-81.
9. Kurtzberg J, Laughlin M, Graham ML, et al. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med 1996; 335:157-66.
10. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors N Engl J Med 1998; 339:1565-77.
11. Wagner JE, Rosenthal J, Sweetman R et al. Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host-disease. Blood 1996; 88:795-802.
12. Locatelli F, Rocha V, Chastang C et al. on behalf of Eurocord-Cord Blood Transplant Group. Factors associated with outcome after cord blood transplantation in children with acute leukemia. Blood.1999; 93:3662-3671.
13. Barker JN, Krepski TP, DeFor T, Davies SM, Wagner JE, Weisdorf DJ. Searching for unrelated donor hematopoietic stem cell grafts: availability and speed of umbilical cord blood versus bone marrow. Biol Blood Marrow Transplant. 2002, 8:257-260.
14. Holyoake TL, Nicolini FE, Eaves CJ. Functional differences between transplantable human hematopoietic stem cells from fetal liver, cord blood and adult bone marrow. Exp Haematol 1999; 27: 1418-27.
15. Broxmeyer HE, Hangoc G, Cooper S, et al. Growth characteristics and expansion of human umbilical cord blood and estimation of its potential for transplantation in adults. Proc Natl Acad Sci USA 1992; 92: 4109-13.
16. Mayani H, Lansdorp PM Proliferation of individual hematopoietic progenitors purified from umbilical cord blood. Exp Haematol 1995; 23: 1453-62.
17. Jacoby DR, Olding LB, Oldstone MBA. Immunological regulation of fetal-maternal balance. Adv Immunol 1984; 35:157-208.
18. Madrigal JA, Cohen SBA, Gluckman E, Charron DJ. Does cord blood transplantation results in lower graft-versus-host disease? It takes more than two to tango. Human Immunology 1997; 56:1-5.
19. Chalmers I, Janossy G, Contreras M, Navarrete C. Intracellular cytokine profile of cord and adult blood lymphocytes. Blood 1998; 92:11-18.
20. Moretta A, Locatelli F, Mingrat G, et al. Characterisation of CTL directed towards non-inherited maternal alloantigens in human cord blood. Bone Marrow Transplant 1999; 24:1161-1166.
21. Risdon G, Gaddy J, Horie M, Broxmeyer HE. Alloantigen priming induces a state of unresponsiveness in human umbilical cord blood T cells. Proc Natl Acad Sci USA 1995; 92:2413-17.
22. Rocha V, Cornish J, Sivers EL et al. Comparison of outcome of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. Blood. 2001;97:2962-2971.
23. Keever CA, Abu-Hajir M, Graf W, Mc Fadden P, Prichard P, O'Brein J, Flomenberg N. Characterization of the alloreactivity and anti-leukemia reactivity of cord blood mononuclear cells. Bone Marrow Transplant 1995; 15:407-19.
24. Gardiner CM, O'Meara A, Reen DJ. Differential cytotoxicity of cord-blood and bone marrow-derived natural killer cells. Blood 1998; 91:207-13.
25. Locatelli F, Rocha V, Reed W, et al. Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. Blood 2003; 101:2137-2143.
26. Gluckman E, Locatelli F. Umbilical cord blood transplants. Cur Op Haematol 2000; 7:353-357.
27. Grewal SS, Kahn JP, MacMillan ML, et al. Successful hematopoietic stem cell transplantation for Fanconi anemia from an unaffected HLA-genotype-identical sibling selected using preimplantation genetic diagnosis. Blood 2004; 103:1147-1151.
28. Burgio GR, Gluckman E, Locatelli F. Ethical reappraisal of 15 years of cord-blood transplantation. Lancet 2003; 361: 250-252.
29. Wagner JE, Barker JN, DeFor TE et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and non malignant diseases : influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood. 2002; 100:1611-1618.
30. Gluckman E, Rocha V, Arcese W, et al, on behalf of the Eurocord Group. Factors associated with outcomes of unrelated cord blood transplant: Guidelines for donor choice. Exp Hematol. 2004; 32:397-407.
31. Barker JN, Weisdorf DJ, DeFor TE, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood 2005; 105:1343-7.
32. Jaroscak J, Goltry K, Smith A, et al. Augmentation of umbilical cord blood (UCB) transplantation with ex vivo–expanded UCB cells: results of a phase 1 trial using the AastromReplicell System. Blood 2003; 101: 5061-5067.
33. Barker JN, Davies SM, DeFor T, Ramsay NK, Weisdorf DJ, Wagner JE. Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of HLA-matched unrelated donor bone marrow: results of a matched-pair analysis. Blood 2001;97:2957-2961.
34. Escolar ML, Poe MD, Provenzale JM, et al. Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. N Engl J Med 2005; 352:2069-2081.
35. Staba SL, Escolar ML, Poe MD, et al. Cord-blood transplants from unrelated donors in patients with Hurler's syndrome. N Engl J Med 2004; 350:1960-9.