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First Report of Autologous Cord Blood Transplantation in the Treatment of a Child With Leukemia

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ABSTRACT

We present the case of a 3-year-old girl with acute lymphoblastic leukemia who developed isolated central nervous system relapse while receiving chemotherapy 10 months after diagnosis. The child achieved a second remission on retreatment with systemic and intrathecal chemotherapy. She then underwent myeloablative chemotherapy and radiation therapy followed by infusion of her own umbilical cord blood, which the parents had saved after her delivery. She is now doing well and is in complete remission 20 months after cord blood transplantation. In this first report of autologous cord blood transplantation for treatment of childhood leukemia, we discuss the safety and feasibility of this procedure as well as some of the uncertainties surrounding autologous cord blood collection and usage.

DESPITE THE IMPROVEMENT of survival in childhood acute lymphoblastic leukemia (ALL), relapse in the central nervous system (CNS) remains a challenging problem. Early CNS relapse carries a high risk of additional relapses, especially in the bone marrow, indicating the need for intensive systemic therapy, which may include hematopoietic stem cell (HSC) transplantation. Given its wide availability, easy collection, and rich and potent HSC content, umbilical cord blood (UCB) has been identified as a good source for HSC transplantation. Allogeneic UCB, mostly from UCB banks, has been widely used as a source of HSCs to provide transplants for children with relapsed leukemia. The use of autologous cord blood (when available) for HSC transplantation is a challenging and controversial matter. In addition to the rare availability of autologous cord blood, there is a concern that it may contain the leukemic clone that progressed to cause the child's leukemia. In this report we describe a case of autologous cord blood transplantation for treatment of relapsed ALL and discuss the feasibility and safety of such a procedure.

CASE REPORT

E.M. is a 6-year-old girl who was in a good state of health until she was 3 years of age, at which time she presented with excessive bruising and splenomegaly. A complete blood count revealed white blood cells at 98 ×

10^9/L, hemoglobin at 100 g/L, and platelets at 15 × 10^9/L. Bone marrow examination showed 94% lymphoblasts with L-1 morphology. The lymphoblasts expressed CD45, CD10, CD19, CD20, HLA-DR, and terminal deoxynucleotidyl transferase, which is diagnostic for B-precursor ALL. Cytogenetic analysis of the bone marrow was normal, and fluorescent in situ hybridization for TEL-AML1 translocation was negative. A cerebrospinal fluid cell count and cytological examination were normal, with no evidence of leukemia. The patient was treated with a high-risk leukemia protocol because of the presence of a very high white cell count. She achieved complete remission (CR) after 4 weeks of induction therapy consisting of prednisone, vincristine, daunorubicin, L-asparaginase, and intrathecal methotrexate. Consolidation therapy consisted of 6 cycles of

intermediate-dose methotrexate (1 g/m² per cycle) in addition to 6-mercaptopurine, dexamethasone, vincristine, daunorubicin, L-asparaginase, cyclophosphamide, cytosine arabinoside, 6-thioguanine, and intrathecal methotrexate. This was followed by continuation chemotherapy, which consisted of methotrexate, 6-mercaptopurine, vincristine, dexamethasone, and intrathecal methotrexate.

On week 44 of therapy, a routine lumbar puncture showed 35×10^6 nucleated cells per L without red blood cells. Lymphoblasts were unequivocally present on cytospin. Bone marrow examination was normal, and a diagnosis of isolated CNS relapse was established. The patient achieved a second remission after using reinduction therapy with weekly triple intrathecal chemotherapy (methotrexate, hydrocortisone, and cytosine arabinoside). Because of the early relapse, consideration was given to allogeneic HSC transplantation. With the lack of an HLA-matched family member, a decision was made to use the autologous cord blood that the patient's parents had electively collected and stored at a commercial laboratory after delivery. After 3 more cycles of consolidation chemotherapy, informed consent was given by the family, and the patient underwent autologous cord blood transplantation (CBT) while in second CR. The conditioning regimen consisted of total-body irradiation (1200 cGy delivered by 200 cGy twice daily) and a cranial radiation boost of 1200 cGy, followed by high-dose etoposide and cyclophosphamide.

Cord blood had been collected by the attending obstetrician in a community hospital following written guidelines. The physician had not had any specific training for cord blood collection and did not report any difficulties or complications in collecting the cord blood. Written informed consent was given by the mother for obtaining medical history, infectious disease testing, and collection of UCB. Cord blood was collected during an uncomplicated vaginal delivery after the umbilical cord was clamped and cut and before the placenta was delivered using a Baxter blood-collection bag containing 35 mL of citrate-phosphate-dextrose anticoagulant. The bag was kept at air-conditioned room temperature, with no temperature recording while being transported to the processing laboratory. It arrived 21 hours after delivery and was processed immediately. The processed cord blood was placed into a freezer 23.5 hours after birth.

Processing of cord blood was performed by using an aseptic technique under a sterile hood. Chilled dimethyl sulfoxide (Cryoserv; Research Industries, Salt Lake City, UT) in 0.8% sodium chloride was added gently to the citrate-phosphate-dextrose/cord blood while rotating the collection bag to ensure mixing to a final concentration of 10%. Aliquots were taken and placed in cryotubes for future testing. The remaining cord blood was transferred to 3 cryobags, put into aluminum cartridges, and then placed in a freezer at −80°C for 6 hours and

transferred to quarantine tank. Cord blood was stored in vapor phase of the liquid-nitrogen tank for quarantine until infectious-disease test (bacterial and fungal cultures) results were negative. Cord blood was then transferred to the permanent storage in liquid nitrogen, where the temperature was permanently controlled and recorded.

Cord blood was processed and stored by CorCell Inc, a private bank that was licensed by the states of New York and New Jersey at the time of processing. The parents were charged a collection-and-processing fee of $995 in addition to an $85 annual storage fee until the UCB was shipped. CorCell Inc chose not to charge any fees for the additional testing, medical professional expenses, and courier expenses.

Because of the possibility that the patient's leukemia clone may have been present in the cord blood, molecular testing was performed for the detection of the leukemia clone in the cord blood. Given the lack of any specific chromosomal markers in this child's leukemia cells, leukemia cells from the initial bone marrow were confirmed to have clonal immunoglobulin heavy chain (IgH) receptor gene and cross lineage T-γ Jg receptor gene loci rearrangements. The cord blood, however, showed no evidence of the same pattern of IgH and T-γ Jg gene rearrangements by polymerase chain reaction (PCR). The assay was performed by using consensus region primers with a sensitivity of 1% to 5%. For IgH PCR, primers were complementary to the $FRIII$ and JH regions. For T-γ PCR, 2 PCRs were performed by using consensus primers for V-$region$ genes with primers directed to either Jp or J-γ regions. Post-PCR analysis was performed by high-resolution capillary electrophoresis. Integrity of the DNA was assessed by amplification of a segment of the β-globin gene.

The total volume of stored UCB was 85 mL. It was stored as whole blood by controlled-rate freezing with 10% dimethyl sulfoxide. At the time of thawing of the cord blood, the number of nucleated cells was 979×10^6 (54×10^6/kg of the patient's weight at the time of infusion), and the number of CD 34^+ cells was 2.59×10^6 (1.4×10^5/kg). The recovery rate of nucleated cells and CD34$^+$ cells in UCB was 92%, and viability was 80% according to the trypan-blue method and 98% according to the fluorescence-activated cell sorter CD34$^+$-7AAD method. Colony-forming units were not tested because nucleated and CD34$^+$ cells are preferred measurements.

UCB was infused without complications, and engraftment occurred 15 days later. Institutional review board approval was not thought to be necessary for autologous CBT and was not sought in this case.

The girl's complete blood count had been completely normal up to 4 months post-CBT. The patient did very well after autologous CBT without serious infections, major complications, or graft-versus-host disease (GVHD). Bone marrow examination 1 year posttrans-

plant showed mild decreases in cellularity with otherwise normal hematopoiesis. The patient remains in CR 28 months after relapse and 24 months after CBT.

DISCUSSION

ALL is the most common malignancy in children and has a survival rate that now approaches 80%. The CNS is the second most common site of ALL relapse after bone marrow. Overall, 4-year event-free survival after CNS relapse is 71%, and it is better if the first CR was ≥18 months (83%) compared with first CR of <18 months (46%).[1] Reasons for CNS relapse include the sanctuary location of the CNS as well as resistance of the leukemia cells to chemotherapy. The role of the latter is highlighted by the fact that bone marrow relapse is the main form of treatment failure and additional relapses after isolated CNS relapse. After therapy for isolated CNS relapse, 50% of subsequent relapses occur in the bone marrow, compared with 14% in the CNS.[1] This underscores the need for intensive systemic therapy in addition to CNS therapy using intrathecal chemotherapy and radiotherapy in the treatment of patients with isolated CNS relapse, especially in those with short first CR. For these reasons, high-dose therapy followed by HSC transplantation may be indicated for some patients with early isolated CNS relapse.[2] Bordigoni et al[3] reported 77% disease-free survival after HSC transplantation in children who had CNS relapse while receiving chemotherapy. This is significantly better than reported survival rates after conventional chemotherapy and radiotherapy in this patient population.[1] However, any such improvement in survival rates with HSC transplantation needs to be evaluated in the context of the possible increase in acute mortality as well as short-term and long-term toxicities associated with HSC transplantation.

In our patient, we considered and discussed with the patient's family all treatment options including conventional chemoradiotherapy, alternative-donor transplantation, and autologous CBT. The patient's CNS relapse occurred very early (10 months into treatment) despite an intensive treatment program for high-risk A.L.L. This would indicate a very high risk of additional treatment failure and relapse, especially in the bone marrow, using conventional chemotherapy and radiotherapy. Myeloablative therapy followed by HSC transplantation would probably provide a better chance of survival. The decision to choose autologous CBT versus alternative-donor HSC transplantation was based on the assessment that the benefits of decreased transplant-related mortality and morbidity (especially the ~30% chance of GVHD) in autologous CBT outweighed the risks of possibly reinfusing the leukemia clone to the patient, and absence of graft-versus-leukemia effect. This impression was shared by the treating physicians and parents.

At the time of this writing, our patient was 24 months post-CBT and had been in second CR for 28 months.

Although a second relapse remains a possibility, it is unlikely. Ritchey et al[1] reported that most second relapses in patients who were treated for early CNS relapse occurred within 2 years. Only 4% of the second relapses occurred >28 months after the first one.

UCB was first used as a source of HSC transplantation in 1988.[4] Since then, UCB has increasingly become a popular alternative to bone marrow and peripheral stem cell transplantation, especially in children.[5] Advantages of UCB include abundant availability, easy collection, risk-free donation, reduced risk of blood-borne infection, immediate availability of stored units, and reduced risk of GVHD. In our patient, we had some concern that the leukemia clone may be present in the patient's UCB. Several studies have demonstrated the prenatal origin of childhood leukemia. Using PCR techniques, leukemia-specific fusion genes such as *TEL-AML1*, *MLL*, or immunoglobulin gene rearrangement were detected in the cord blood of a significant number of children who later developed ALL.[6–9] For our patient, we used *IgH* receptor gene and *T-γ JG* receptor gene loci rearrangements as molecular markers of the leukemia clone.[7,8] The negative rearrangement signal in UCB, although positive in the leukemic bone marrow, gave us some assurance that the cord blood did not contain the leukemia clone. Given the sensitivity limitation of our standard PCR testing, however, one cannot completely rule out the possibility that a low-abundance leukemia clone was present in the UCB. Although the presence of clonotypic immunoglobulin gene rearrangements in our patient's cord blood would have precluded us from using it for HSC transplantation, it is not clear what risk there would be of redeveloping leukemia as a result of infusing leukemia clone–contaminated UCB. The incidence of preleukemic clone is ~100 times that of the incidence of childhood leukemia.[10] This suggests that only 1 in 100 of infants with preleukemic clone in their cord blood eventually develop leukemia and that a second postnatal "trigger" would be necessary for the clinical development of childhood leukemia. Therefore, it is not certain whether infusion of leukemia clone–contaminated UCB would automatically result in the development of leukemia in the recipient. The role of "backtracking" the leukemic clone of the patient's UCB sample using more sensitive and specific surrogate molecular markers is currently of uncertain significance and requires additional investigation.

With the annual incidence rate of cancer in children estimated at 130 per 1 million, the chance of developing cancer during childhood is ~2 per 1000.[11] The majority of children who develop cancer are expected to survive using conventional front-line therapy without ever needing HSC transplantation. HSC transplantation is generally indicated in children with acute myeloblastic leukemia, very high-risk ALL, relapsed leukemia, relapsed lymphoma, and advanced neuroblastoma. Ap-

proximately one fourth of children with cancer would meet such criteria; therefore, the likelihood of any child requiring HSC transplantation for treatment of cancer is approximately 1 in 2000, not including other less common indications such as aplastic anemia. Federal legislation and funding are currently underway to create a national cord blood bank to store more UCB units to be used for allogeneic unrelated transplants. Several companies now have operations for private cord blood collection to be kept for the child and family as a "biological insurance" in case HSC transplant is ever needed in the future for the same child or other family members.

There are many ethical challenges and controversies surrounding the private banking and use of UCB. These challenges include issues related to consent, ownership, privacy, commercialism, and liability, in addition to economic and ethnic disparity.[12,13] There is also some concern that private UCB banking may reduce the number of units available for allogeneic CBT through cord blood registries. Currently in the United States, private UCB collection and storage is performed in <3% of all deliveries. Such a low rate would probably not have a significant impact on the number of UCB units available for allogeneic CBT, although this could change with the increased rate of private UCB collection.

Pediatricians, obstetricians, and pediatric hematologists are often asked by families and other colleagues about the benefits and value of private UCB collection. Our role in this is to explain the advantages, disadvantages, and uncertainties surrounding collection and future use of UCB. In addition to the few thousands of dollars in cost borne by the parents, there remain many unanswered questions regarding the viability of UCB cells after several years of freezing and storage. Studies have documented the sustained viability and engraftment potential of HSCs after 5 to 15 years of cryopreservation; therefore, an expiration date, if any, has not been established for cryopreserved HSCs.[14,15] Consideration also needs to be given to the possibility that a precancerous clone may be present in the cord blood of a child who later develops cancer.[16] These issues need to be weighed against the potential benefits of using UCB for HSC transplantation, as well as other potential uses in the field of regenerative medicine that may become a reality in the future.[17] When the time comes to consider the use of autologous cord blood in a patient, strong considerations need to be given to the safety of such a procedure compared with other alternatives such as related or unrelated allogeneic sources of HSCs.

CONCLUSIONS

To our knowledge, this is the first report of autologous CBT for the treatment of childhood leukemia. Autologous UCB transplantation has been reported in 1 patient with neuroblastoma and another with severe aplastic anemia.[18,19] With an increasing number of families opt-

ing for private UCB collection and storage, there will certainly be more cases of autologous UCB transplantation in the future, which may answer some of the questions regarding the value of autologous cord blood collection and the safety of its usage. The role of backtracking the leukemic clone to the patient's UCB sample using more sensitive and specific surrogate molecular markers is needed and requires additional study. The decision made by the parents of our patient to save the UCB may have increased the patient's chances of survival. It was not our intention in this report to advocate private UCB collection and its use but, rather, to report an isolated case and discuss some of the issues and uncertainties surrounding this procedure.

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