First report of donor cell derived acute leukemia as a complication of umbilical cord blood transplantation.

Short title for running head: Donor cell leukemia after cord blood transplant.

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Abstract

Donor cell leukemia is a rare complication after allogeneic hematopoietic stem cell transplantation. A twelve month old boy underwent unrelated donor umbilical cord blood transplant (UCBT) for refractory Langerhan’s cell histiocytosis. Forty months following transplant he developed acute myeloid leukemia. Cytogenetic and molecular analysis confirmed donor cell origin. The Cord Blood Bank (CBB) contacted the donor’s family and established that the child, now seven years old, was healthy. This represents the first reported case of donor cell leukemia following UCBT. This case illustrates that donor cell leukemia is a rare but real event after UCBT as with other stem cell sources and highlights the need for CBBs to maintain linkage data between donors and recipients.
Introduction

Donor cell leukemia (DCL) is a rare complication following allogeneic hematopoietic stem cell transplantation, with approximately 25 cases previously reported.\textsuperscript{1-7} Umbilical cord blood has emerged as an alternative source of hematopoietic stem cells over the past decade with more than 6000 umbilical cord blood transplants (UCBTs) performed worldwide.\textsuperscript{8} We report the first case of DCL following UCBT.

The etiology of DCL is unclear and the reported literature doesn’t suggest a common mechanism. Mechanisms proposed include occult leukemia in the donor, impaired immune surveillance, chemotherapy or radiation induced stromal abnormalities, inherent stromal abnormalities, transformation of donor cells by antigenic stimulation through host tissue and fusion of donor cells with residual leukemic cells resulting in oncogene transfection.\textsuperscript{1} The possibility of occult leukemia in the donor raises the question as to whether notification and investigation are warranted.

Study Design

This is a single case report with retrospective analysis of clinical and laboratory data. The Institutional Review Board of the University of Minnesota reviewed the protocols and informed consent documents relevant to the individual patient.
Case Report

A twelve month old male presented with a two month history of recurrent epistaxes, weight loss and a two week history of fever. Examination demonstrated hepatosplenomegaly, cervical lymphadenopathy and a petechial rash. Initial investigations revealed anemia, thrombocytopenia and hypoalbuminemia. A bone marrow biopsy demonstrated histiocytic infiltration with prominent hemophagocytosis. After lymph node biopsy demonstrated histiocytic infiltration and immunohistochemical staining was positive for CD1a, a diagnosis of Langerhan’s cell histiocytosis was made. Cytogenetic analyses of both the bone marrow and lymph node specimens revealed normal 46,XY male karyotypes with no evidence of a clonal chromosomal abnormality. There was no response to therapy with steroids, vinblastine and etoposide or salvage therapy with 2-chlorodeoxyadenosine. Five months after presentation, a two antigen mismatched, male donor UCBT was performed with a preparative regimen of busulfan, cyclophosphamide, etoposide and ATG. GVHD prophylaxis consisted of cyclosporine and short course steroids. The total nucleated cell dose was 5.6x10^7 per kg. Neutrophil engraftment occurred at day +43 although intermittent G-CSF therapy was required for nine months. Infectious complications included multiple episodes of bacteremia, parainfluenza III and adenoviral pneumonia and CMV pneumonitis and retinitis which necessitated eighteen months of ganciclovir and foscarnet. Cyclosporine was discontinued seven months post transplant with no evidence of GVHD. Molecular studies consistently demonstrated 100% donor engraftment on serial bone marrow examinations from day +21. The histiocytic infiltrate in the bone marrow resolved
slowly over the first twelve months. Eighteen months following transplant the patient was clinically well, had normal blood counts, a normal bone marrow examination and normal lymphocyte responses to a panel of mitogens. Cytogenetic analysis at that time revealed a normal 46,XY male karyotype in each of 20 metaphase cells examined. G band polymorphisms were consistent with these cells being of donor origin. Specifically, in previous studies of the recipient’s cells, both #22 chromosomes had short arms that were of similar size with short stalk (band 22p12) regions (Figure 1, Study #1). In contrast, in donor cells, the stalk region of one chromosome 22 was notably larger than the other and had more prominent terminal satellites (Figure 1, Study #2). This polymorphic difference could be reliably evaluated in each of the metaphase cells examined.

Forty months post UCBT the patient became thrombocytopenic (38x10^9/l) and bone marrow examination revealed 25% blasts with morphological features of acute myeloid leukemia (AML) with multilineage dysplasia. Immunophenotyping revealed a distinct population within the blast gate (weak CD45 expression) comprising 20% of nucleated cells. This gated population showed moderate to strong expression of CD34, HLA-DR, CD117, CD38, CD13 and CD33 consistent with neoplastic myeloblasts. Cytogenetic and molecular studies were undertaken to determine the cell of origin and for evaluation of a clonal chromosomal abnormality (Results, Figures 1-3). The patient failed to achieve a remission following attempted treatment with chemotherapy and a phase I agent, and died from infectious complications, ten months following the diagnosis of leukemia.
Methods

Cytogenetics: Cytogenetic evaluation involved G-banded analysis of twenty metaphase cells from unstimulated, cultured (24 hour) bone marrow aspirates. All numerical and structural chromosomal abnormalities were characterized according to the ISCN, 1995.10

Molecular Diagnostics: Genomic DNA was extracted from the bone marrow sample and amplified by PCR using a series of fluorescently labeled oligonucleotide primers specific for highly polymorphic genetic markers (VNTR) according to standard methods.11 Pre-transplant samples from the donor and recipient were previously analyzed for informative markers. The resulting products were analyzed on a Model 3100/Genescan system, (Applied Biosystems) from which the pre and post transplant specimens were compared.

Results

Nineteen of 20 metaphase cells examined comprised a clone characterized by a derivative chromosome 7, with its distal long arm at band 7q21.1 replaced by an extra copy of most of the long arm of a chromosome 1. Thus, this abnormality, designated as, , 46,XY,der(7)t(1;7)(q21.1;q22), results in monosomy for the region extending from 7q21.1 to the 7q telomere, and trisomy for the 1q region extending from 1q22 to the 1q telomere (Figure 2). Cytogenetic analysis of the patient’s karyotype pre and post UCBT demonstrated an informative G band polymorphism on the short arm of chromosome 22. The polymorphism of chromosome 22 showed the post-transplant cells to be of donor
origin, including the leukemic cells that harbored the derivative chromosome 7 (Figures 1-2). Molecular analysis demonstrated 100% donor cells (Figure 3).

Discussion

Cooley et al reviewed 18 published cases of DCL.¹ Subsequent to that report we identified 6 further reported cases.²⁻⁷ Analysis of these cases does not suggest a common mechanism and we can only speculate as to the etiology of this rare complication. Twenty one of 24 cases occurred following transplantation for leukemia but there have been cases reported after transplantation for non-malignant conditions.¹⁶ Deletion of 7q and gain of 1q, as seen in this case are well documented recurring abnormalities in both de-novo and therapy associated MDS and AML (t-MDS/AML).¹² Of the 24 cases of DCL previously described, three developed AML after antecedent MDS.¹⁴⁻⁷ Our patient had received only 600mg/m² of etoposide and 200mg/kg of cyclophosphamide, much less than doses usually associated with t-MDS/AML. The fact that the risk of t-MDS/AML is higher after autologous transplant than after conventional chemotherapy and radiation therapy¹³ suggests that the transplant process itself may potentiate t-MDS/AML, although the mechanism remains unclear. Furthermore, in contrast to the autologous setting, t-MDS/AML is very rare following allogeneic transplant¹⁴,¹⁵ Indeed, if chemotherapy or radiation induced stromal changes were important in the development of DCL one would expect a higher frequency than that observed following allogeneic transplant. It is of interest that patients with LCH have a higher risk of malignancy beyond that expected because of treatment with epipodophyllotoxins and that LCH has been associated with
myelodysplasia. This raises the possibility that particular hosts may have an intrinsic predisposition to the development of leukemia mediated by factors such as the marrow microenvironment or cytokine profiles that are not ameliorated by hematopoietic stem cell transplantation.

We were unable to identify any reported cases of a donor developing leukemia following the development of DCL. However, studies identifying clonotypic gene fusion sequences in the neonatal blood spots of children who subsequently developed leukemias characterized by MLL-AF4, TEL-AML1 and AML1-ETO translocations highlight the fact that transmission of leukemic or pre-leukemic cells from a neonatal donor could occur. Several of these children were older than 10 years at the time of leukemia diagnosis. This protracted postnatal latency would suggest that additional events or exposures are required for the development of leukemia. A follow up study which demonstrated that the TEL-AML1 translocation was detectable at frequencies consistent with clonal expansion in 6 of 567 cord blood samples screened is consistent with this hypothesis but also suggests that pre-leukemic cells may be infused more commonly than expected during UCBT. It has already been suggested that these data argue against the use of stored cord blood in an autologous setting for leukemia patients.

The possibility of an occult leukemia in the donor raises questions regarding the ethical responsibilities of the Cord Blood Bank (CBB) to the donor. In the case of adult donors, the National Marrow Donor Program inquires as to their health if they are notified of a case of DCL. No specific policy exists in the case of UCBT. In this case the parents of
the donor, now 7 years old, were contacted and asked whether their child had any health concerns and specifically if the child had been diagnosed with any blood disorders. The child remained well. However, such inquiries could create anxiety for the donor family and may not always be possible given the difficulties in maintaining current contact information. No stored sample of the cord blood unit was available to test for the presence of the cytogenetic clone. A decision was made not to request a blood sample from the child because of the lack of evidence of occult leukemia as a cause of this rare complication, the absence of any prophylactic treatment or validated screening strategy, and a desire not to create undue anxiety.

This case serves as a reminder that there are a number of ethical issues that are unique to UCBT. The recently released Institute of Medicine report on establishing a National Cord Blood Stem Cell Bank Program dedicates a chapter to these issues and highlights the fact that there is no uniformity in practices across different CBBs and transplant centers.22 The informed consent process is central to ensuring that donors’ understand issues regarding potential outcomes of their donation including the possibility that screening or additional testing could reveal unanticipated information about the mother’s or her child’s health which may otherwise have gone unnoticed. The most commonly cited examples include infectious or metabolic diseases but as this case illustrates there are other possibilities including malignancy. While in some situations this information may be beneficial, in others it has the potential to result in emotional, social and financial hardship. A recent study demonstrated that many donors do not fully comprehend the medical, legal and ethical issues despite having granted “informed consent”.23 These
ethical considerations are further complicated by the fact that, by necessity, consent to voluntary donation is granted by the parents on behalf of one of the major stakeholders, the neonatal donor. In the US and Europe, cord blood banks are required to maintain linkage data between each unit and the demographic details of the donors. This practice has been debated in terms of potential benefit to the recipient as it could facilitate removal of unsuitable units prior to UCBT but may cause potential harm to the donor as it may compromise donor privacy and raises the possibility of requests for second donations. This case illustrates that scenarios may arise where maintenance of linkage could potentially benefit the donor.

A further, related question is whether there is a need for active prospective follow up of donors. Currently policies regarding this differ between CBBs and such programs, which require significant resources, are not mandated by regulatory authorities. However, unrelated UBCT does not allow clinical or laboratory assessment of the donor immediately prior to transplant. A program of active prospective follow-up at six months instituted by the Milan CBB identified 5/2315 (0.2%) cords that needed to be discarded on the basis of post-natal history, although there were no cases of malignancy. CBBs rely upon the donor’s parents to contact the bank should their child develop a medical condition which would make the unit unsuitable for transplantation. However, in one study, almost 25% of women who had provided informed consent for cord blood donation did not know how to contact the CBB.
This first report of DCL after UCBT demonstrates that DCL is a risk following allogeneic transplantation irrespective of the graft source. Given the lack of evidence of occult leukemia in the donor as a cause of this rare complication and the absence of prophylactic treatment, we would argue that donor notification and investigation is not warranted. However, this case serves as a reminder of the ethical dilemmas that will undoubtedly be faced as the number of UCBTs performed increases and the need for standardization of regulations governing CBBs.
References


Figure Legends

Figure 1  Cytogenetic studies demonstrating donor origin of leukemic cells. Composites of the #22 chromosomes from pre-transplant (Study #1), 6 months post transplant when molecular studies demonstrated 100% donor chimerism (Study #2), and following diagnosis of AML (Study #3) illustrating informative G band polymorphism. One of the donor chromosome 22 has an enlarged short arm and satellite region, designated by the arrow. This polymorphism is seen in Study #2 and #3 suggesting donor origin of the leukemic cells. G band polymorphisms represent regions that differ between individuals but have no clinical significance. They are most commonly seen on acrocentric chromosomes such as chromosome 22.

Figure 2  Cytogenetic studies demonstrating clonal abnormality. Studies performed post transplant at diagnosis of AML revealed a clone characterized by a derivative chromosome 7 (marked by arrow). The chromosome 22 polymorphisms are consistent with donor origin of these cells (marked by bold arrow).

Figure 3  Assessment of donor chimerism. Electrophoretic profiles of informative VNTR PCR products of (A) the recipient pre transplant (allele size 836bp), (B) the donor product pre transplant (allele size 690bp) and (C) the recipient following the diagnosis of
leukemia demonstrating 100% donor cells. The donor and recipient were both homozygous for the respective alleles.
Figure 1

Study #1  Study #2  Study #3
Figure 2

46,XY,der(7)(q17;q22)
Figure 3

A. Recipient pre UCBT

B. Donor product

C. 40 months post UCBT
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