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Case report

Successful mismatched sibling cord blood transplant in Hb Bart's disease

X Zhou[1], SY Ha[1], GCF Chan[1], CW Luk[5], V Chan[2], B Hawkins[3], YH Lam[4], RHS Liang[2] and YL Lau[1]

Departments of [1]Pediatrics, [2]Medicine and [3]Pathology, [4]Obstetrics and Gynecology, The University of Hong Kong, Queen Mary Hospital; and [5]Department of Paediatrics, Queen Elizabeth Hospital, Hong Kong SAR, China

Summary:

A 20-month-old girl with Hb Bart's disease, who had survived neonatal complications, underwent HLA-DR antigen mismatched sibling cord blood transplantation successfully. Immune thrombocytopenia, which occurred around 2.5 months after transplant, responded to intravenous γ-globulin. The fetal hemoglobin level rose to a peak of 52.3% on day +69 post transplant and declined gradually during the following year. Ten percent of hemoglobin Bart's was detected 2 months after transplant and this reflects the α-thalassemia trait of the donor. *Bone Marrow Transplantation* (2001) **28,** 105–107.
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Alpha-thalassemia is the most common inherited disorder of hemoglobin synthesis in Southeast Asia and south China. The carrier rate in Hong Kong is 5% while the Southeast Asian type of deletion ($-^{SEA}$) accounts for 4.5% of the cases.[1] Homozygous patients with $-^{SEA}/-^{SEA}$ mutation (four gene deletions) have Hb Bart's hydrops fetalis syndrome, which usually leads to stillbirth or neonatal death. With improvement of neonatal care, a few babies have survived after birth.[2] Intrauterine transfusion also permits survival of some patients with this previously lethal condition. Survivors will require regular transfusion and iron chelation like patients with β-thalassemia major. Bone marrow transplantation may offer a cure for patients with transfusion-dependent thalassemia. Over the last decade, HLA-matched sibling cord blood has become a safe and effective alternative source of hematopoietic stem cells for transplantation.[3] We report a successful transplant in a female with Hb Bart's disease using HLA-DR antigen mismatched cord blood from her younger brother.

Correspondence: Dr SY Ha, Department of Pediatrics, Queen Mary Hospital, The University of Hong Kong, Hong Kong
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Case report

The patient is the first born child from the second marriage of her mother who had not attended any antenatal care and was not aware of her thalassemic carrier state. She was born at 24 weeks of gestation by spontaneous vaginal delivery with a birth weight of 1 kg. Apgar score was 2 at 1 min and 7 at 5 min. At birth she was noted to be anemic and mildly hydropic. A moderate degree of hepatosplenomegaly was present. She developed respiratory distress soon after birth and required assisted positive pressure ventilation. Despite ventilation however, she remained cyanotic. Subsequent investigations including 2-D echocardiogram confirmed that she had both patent ductus arteriosus (PDA) and persistent fetal circulation. Her hemoglobin level was 8.7 g/dl and the hematocrit was 0.34. She was given a blood transfusion after blood was sent for investigation. The hemoglobin pattern revealed 80.7% Hb Bart's ($\gamma 4$) and 19.3% Hb Portland ($\zeta_2\gamma_2$), with complete absence of hemoglobin A ($\alpha_2\beta_2$), A2 ($\alpha_2\delta_2$) and F ($\alpha_2\gamma_2$). Very occasionally, Hb H inclusion bodies could be found on supravital staining. The findings were consistent with Hb Bart's disease. Both parents were subsequently confirmed to be α-thalassemic carriers. Molecular testing showed that the child was homozygous for the $-^{SEA}$ deletion. PDA ligation was done at 2 weeks of age. Assisted ventilation was weaned off after 2 months. On follow-up, no definite neurological deficits or developmental delay could be observed. She was subsequently put on regular transfusion.

Two months after the birth of the index patient, her mother became pregnant again. She again did not attend antenatal care until 20 weeks of gestation. Ultrasound examination of the fetus did not show cardiac or placental enlargements or hydrops fetalis. These findings indicated that the fetus was not affected by Hb Bart's disease.[4] The family was referred to the pediatrician by the obstetrician for the possibility of cord blood transplantation. Cord blood was harvested from the placenta after the birth of her younger brother and subsequently cryopreserved with DMSO without volume reduction. Although the cord blood volume was small (30 ml), the total nucleated cell count was satisfactory at 3.74×10^8. HLA typing on the cord blood and later on the younger brother confirmed mismatch at one HLA-DR antigen (A 2,11; B 55,46; DRB_1 04,08 in

the patient. A 2,11; B 55,46; DRB_1 04,09 in the donor). The mother's HLA type is homozygous for locus A and B (A2; B46; DRB_1 08,09). Therefore the patient and the donor have inherited different haplotypes from the mother, but the same haplotype from the father. The parents were counseled about the possible increased risks of graft failure and GVHD. We then decided to proceed with transplant using the mismatched cord blood.

The transplant was performed when the female was 20 months old, ie 8 months after birth of her brother. Desferrioxamine had not been commenced. She had no hepatomegaly at the time of transplant. Histology of a biopsied liver specimen showed grade II iron overload, but no periportal fibrosis. Her serum ferritin was 2100 pmol/l. Overall assessment showed that she belonged to Lucarelli's class 1. As she only weighed 7.79 kg, the nucleated cell dose was 4.8×10^7/kg, and CFU-GM dose was 2.2×10^4/kg. The younger brother was later confirmed to have α-thalassemia trait. Both the patient and the donor were negative for HIV, HCV, CMV and HBsAg on serological studies. The blood group of the recipient is B+, while that of the donor is O+.

The preparative regimen consisted of busulphan (BU) 5 mg/kg/day (day −9 to −6), cyclophosphamide (CY) 50 mg/kg/day (day −5 to −2) and anti-thymocyte globulin (ATG) 30 mg/kg/day (day −4 to −2).[5] Graft-versus-host disease (GVHD) prophylaxis comprised cyclosporin A from day −1 with a short course of methotrexate (days +1, +3, +6, +11).

Neutropenic fever and erythematous maculopapular skin rash over the trunk and face developed on day +15. She was covered with amikicin and ceftazidime. With persistence of fever, antibiotics were switched to vancomycin and sulperazone 2 days later. Foscarnet and amphotericin B were later added on. Skin rash, which we considered either acute GVHD or serum sickness, became more extensive. She was treated with intravenous methylprednisolone (2 mg/kg/day for 5 days and then tapered over 14 days). Histology of the skin biopsy showed non-specific changes. Serum for HHV6 PCR was positive, but skin tissue for HHV6 PCR was negative.[6] Blood culture was also negative. Fever subsided 3 days after the commencement of steroids and the skin rash also gradually faded. Neutrophil engraftment (ANC $>0.5 \times 10^9$/l) occurred on day +26 with G-CSF augmentation. Platelet engraftment ($>20 \times 10^9$/l) occurred on day +38, and ($>50 \times 10^9$/l) on day +56. Bone marrow cytogenetics on day +30 showed 100% donor metaphases. Her last blood transfusion was on day +30. On day +65, 10% Hb Bart's was detected by Hb electrophoresis. Globin chain synthesis revealed $\alpha/\beta + \gamma$ ratio of 0.86. Hb Bart's fell rapidly to undetectable level in 3 weeks' time. HbF levels rose to 52.3% on day +69 after transplantation and slowly declined to 3.3% 9 months later (Figure 1).

At around 2.5 months post transplant, the platelet count gradually dropped from 100×10^9/l to 1×10^9/l over 3 weeks (Figure 1). Hemoglobin level and white cell count were stable. Although direct Coomb's test was weakly positive, reticulocyte count, serum bilirubin and lactate dehydrogenase remained unchanged, showing no evidence of active hemolysis. Clinically she did not have any bleeding tendency. There were no signs of intercurrent infection.

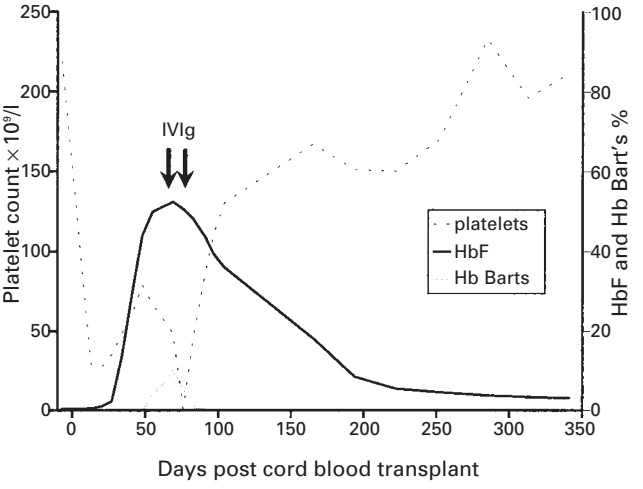

Figure 1 The trends of platelet count, fetal hemoglobin (HbF) and hemoglobin Bart's after cord blood transplantation. Thrombocytopenia remitted after the use of intravenous γ-immunoglobulin. HbF levels reached peak on day +69 post transplant and fell gradually afterwards.

In view of the possibility of graft rejection, cyclosporin A was stopped immediately. Bone marrow aspiration was repeated and it showed regenerating marrow with a reduction in the number of megakaryocytes which is an expected finding after transplant. Thrombotic thrombocytopenic purpura was excluded, as there was absence of fragmented red cells in the peripheral blood smear. We decided to try giving intravenous immunoglobulin while marrow karyotype was pending. After two doses of IVIG therapy (1 g/kg/day), platelet count rose to 49×10^9/l, but followed by transient mild anemia (Hb 9g/dl) which improved subsequently without further intervention. With the rapid improvement of platelet count after IVIG, the most probable cause was immune thrombocytopenia. There were no major complications afterwards. Karyotype at 1 year post transplant showed 100% donor metaphases. The patient is now 30 months after transplant and remains transfusion-independent.

Discussion

Despite the high prevalence of α-thalassemia carriers in Hong Kong, the birth of homozygous α-thalassemia has largely been prevented with the establishment of antenatal screening program and prenatal diagnostic service.[1] Our patient escaped the screening program because her mother failed to seek antenatal care. With immediate resuscitation and intensive support, she survived without major complications.

Transplantation of bone marrow from an HLA-identical sibling offers a good chance of cure in patients with thalassemia, particularly when performed early in life. In recent years, cord blood becomes a safe and effective alternative for it contains substantial numbers of hematopoietic stem cells and can be used for transplantation.[3] A substantial number of cord blood transplants have been successfully given to patients with β-thalassemia major in many centers including ours.[5] However, this is the only report describing

cord blood transplantation in Hb Bart's disease. The only reported case of successful transplant on this disease was an HLA-matched sibling bone marrow transplantation in a 21-month-old female.[7]

Furthermore, our case is a 1 HLA-DR antigen mismatched transplant. Marrow transplant using HLA-nonidentical siblings has not been advocated as a standard treatment for thalassemic patients because of the high risk of GVHD and graft failure.[8] It is possible that cord blood transplant may result in less severe GVHD. Successful engraftment in HLA mismatched cord blood transplant was reported in a thalassemic patient, but unfortunately the patient died of sepsis on day +300.[9] The other key issue for engraftment is cell dose. Despite the small volume of cord blood in our case, the nucleated cell dose was 4.8×10^7/kg, which is satisfactory according to the data from the Eurocord Transplant Group and the European Blood and Marrow Transplantation Group.[3] They reported that a nucleated dose of 37 million or more per kg of body weight was associated with high probability of engraftment. Despite the HLA disparity, our patient engrafted successfully and is a long-term survivor.

Based on our previous observation on patients who underwent cord blood transplant, we also examined her *in vivo* Hb switch after transplant.[5] In this patient, the HbF level rose to 52.3% at 10 weeks and gradually fell to 3.3% about 9 months later. In our previous report, an elevated HbF level was evidence of fetal erythropoiesis of transplanted cord blood in the host and could be a surrogate marker of successful engraftment. In addition, we noticed that 10% Hb Barts was detected 2 months post transplant. Since the donor is a α-thalassemia carrier and full chimerism was repeatedly demonstrated by karyotyping and FISH examination, the hemoglobin Bart's detected was likely to be derived from the donor.

This female had one episode of immune thrombocytopenia around 2.5 months after transplant. The diagnosis was made by exclusion of graft rejection and other causes such as thrombotic thrombocytopenia. Alloimmune or autoimmune thrombocytopenia or cytopenia have been observed after BMT.[10] The time of onset is variable. The clinical course could be mild, or refractory. Patients were often managed as immune thrombocytopenic purpura, while the response to treatment was variable. Occasionally, this complication could be fatal. Although our patient developed severe thrombocytopenia, she rapidly remitted after the use of intravenous γ-globulin.

In conclusion, we are seeing more neonates with Hb Bart's disease surviving with the advancement of neonatal care or intrauterine transfusion. For them, hematopoietic stem cell transplant offers the chance of cure similar to patients with β-thalassemia major. Cord blood transplant is an alternative to conventional marrow source of stem cells. To the best of our knowledge, this is the first reported case of successful 1-antigen mismatched sibling cord blood transplantation for a child with Hb Bart's disease.

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