Primary graft failure following unrelated cord blood transplantation with high-dose of CD34⁺ cells in the treatment of AML/MDS

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[Abstract] Objective To analysis the cause of primary graft failure of unrelated cord blood transplantation with high-dose of CD34⁺ cells in treatment of acute myelocytic leukemia (AML) /myelodysplastic syndrome (MDS). Methods A 4-year-old girl was diagnosed AML/MDS at the Department of Pediatric Hematology and Oncology of West China Second University Hospital of Sichuan University. She presented completely remission after induction and consolidation chemotherapy. She received unrelated partially human leukocyte antigen (HLA) -mismatched cord blood transplantation. We investigated the treatment outcomes of UCBT and associated complications. Results The patient suffered primary graft failure and then received secondary haploidentical hematopoietic stem cell transplantation (HSCT) from her mother. However, she suffered fatal multiresistant Acinetobacter spp septicemia. She died due to respiratory failure on 7 d after the second transplantation. Conclusions In this case, hematopoietic stem cells with high dose of CD34⁺ cells could not overcome the risk of primary graft failure and HLA disparity. The patient's primary graft failure was associated with platelet transfusion refractoriness and potent immunologic dysfunction, especially the anti-HLA donor specific antibodies before unrelated cord blood transplantation.

[Key words] Human leukocyte antigen; Mismatch; Cord blood transplantation; Hematopoietic stem cell transplantation; Donor-specific antibody

Umbilical cord blood transplantation (UCBT) has advantages in lower incidence and severity of graft-versus-host disease (GVHD). However, the hematopoietic recovery after UCBT is more delayed compared with bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSC). The successful rate of engraftment is associated with the higher number of nucleated cells infused and less loci of human leukocyte antigen (HLA) disparity. High-dose of nucleated cell in umbilical cord blood unit can remarkably decrease the treatment-related mortality (TRM) associated with HLA disparities. In this study, we reported a case of a 4-year-old girl developed primary graft failure following high-dose of CD34⁺ cells of unrelated UCBT. The role of cells dose infused and the immunity mechanism, such as anti-HLA antibody in the UCBT settings were discussed.

1 Clinical data

A 4-year-old girl was admitted to the Department of Pediatric Hematology and Oncology of West China Second University Hospital of Sichuan University due to fever and nose bleeding. Her initial peripheral cell count showed white blood cell(WBC) of 3.9 × 10⁹/L, hemoglobin (Hb) 78 g/L, and platelet 10 × 10⁹/L. Bone marrow film and biopsy test confirmed the diagnosis of myelodysplastic syndrome (MDS). She was initially received intermittent transfusion and orally folic acid for 3 months without clinical response and...
with irreversible anemia. She came to our department again with persistent fever, cough and nose bleeding.

Peripheral blood cell count showed WBC of 5.8 × 10⁹/L, with 21% blast cells, Hb 46 g/L and platelet 52 × 10⁹/L. The myeloblast cells in bone marrow were 35%. Flow cytometry results showed CD34 (+), HLA-DR (+), CD13 (+), CD7 (+), CD64dim (+), CD14dim (+), CD117 (+), CD19 (+), CD79a (-), CD10 (-), CD20 (-), MPO (-), CD41 (-), CD2 (-), and CD5 (-).

Then the girl was diagnosed acute myelocytic leukemia (AML) /MDS and initially treated with DAE (daunomycin, cytarabine and etoposide) for remission induction and achieved complete remission. Then she received consecutive chemotherapy for 7 months. Her bone marrow tests indicated a complete remission. During chemotherapy, she received intermittent transfusions of more than 20 units of packed red blood cell (pRBC) and 20 units of platelet. However, she became refractory to platelet transfusions during chemotherapy. After that, all blood products she transfused were irradiated and leukocyte-reduced.

The patient had no sibling and no fully matched unrelated donors were available. Therefore she received an unrelated donor cord blood transplant from 1-locus mismatched female donor after conventional conditioning regimens composed of busulfan (1.2 mg/kg every 6 h, 6-9 d pre-U CBT), cyclophosphamide (50 mg/kg every day, 2-5 d pre-U CBT), and ATG (3 mg/kg every day, 2-4 d pre-U CBT). HLA typing was shown in Table 1. The graft after thawing with 12.4 × 10⁷/kg mononuclear cells, consisting of 9.9 × 10⁵/kg CD34⁺ cells and 2.8 × 10⁵/kg colony forming unit granulocytes and macrophages (CFU-GM), were added back on operation day. Both the patient and the donor were cytomegalovirus (CMV) negative. GVHD prophylaxis consisted of cyclosporin, 1.25 mg/kg, given from 1 d pre-U CBT to 6 months post-U CBT. The dose of cyclosporin was adjusted to serum levels. Methotrexate (15 mg/m²) was given on 1 d post-U CBT, and 10 mg/m² on 3, 6, 11 d post-U CBT. Neutropenia prophylaxis was given by administration of granulocyte colony-stimulating factor (G-CSF) following 3 d post-U CBT. Septicemia prophylaxis during neutropenia was implemented by broad-spectrum antibiotics. Other supportive care was performed according to the guidelines for stem cell transplantation at our center.

**Results:** To prevent hemorrhages from important organs such as encephalic bleeding, she was received platelet transfusion almost every three days because of platelet transfusion refractory. Her peripheral WBC count was < 0.1 × 10⁹/L in week 4 post-U CBT. Because the extremely pancytopenia in the peripheral blood on 28 d post-U CBT, we choose bone marrow to perform the chimerism analysis. Unfortunately, the chimerism analysis by short tandem repeat-polymerase chain reaction (STR-PCR) showed no evidence of donor cells. Bone marrow smears demonstrated bone marrow failure state. Then we declared primary graft failure.

Therefore, we decided to undergo second transplantation with her mother as haploidentical donor (Table 1). The patient was transplanted with peripheral blood stem cell (PBSC) and bone marrow stem cells (BMSC) (PBSC + BMSC) in two consecutive days after conditioning with fludarabine (30 mg/m² every day, 2-6 d before the second transplantation) and ATG (3 mg/kg every day, 2-4 d before the second transplantation). The total nucleated cell dose was 9.28 × 10⁸/kg, consisting of 1.91 × 10⁸/kg CD34⁺ cells. GVHD prophylaxis consisted of cyclosporin, 1.25 mg/kg, given from 1 d before the second transplantation to 6 months after the second transplantation. Methotrexate (15 mg/m²) was given on 1 d after the second transplantation, and (10 mg/m²) on 3, 6, and 11 d after the second transplantation. Mycophenolate mofetil (20 mg/kg) was given from 6 d before the second transplantation to 2 months after the second transplantation. The standard criteria were used for grading of acute and chronic GVHD.

She subsequently developed progressive neuropina and threopina with a low-grade fever. Because she suffered pancytopenia almost 40 d after initial transplantation, the incidence of infection was very high.
though using septicaemia prophylactic regimen. PCR from the blood and bone marrow for CMV and EBV was negative. We implemented two sets of blood cultures and the blood samples were collected through the center venous cannula (CVC) and peripheral vein. Her blood culture was subsequently proved microbiologically documented infection (MDI) with multiresistant *Acinetobacter spp.* She was therefore treated with intravenous colistin which is the only sensitive antibiotics for multiresistant *Acinetobacter spp.* On this regimen her infection in skin puncture improved, her febrile episodes reduced. However, the patient developed serious dyspnea in association with irreversible hypoxemia due to pulmonary infection and suffered with diffuse pulmonary hemorrhage failed to respond to platelet transfusions. Despite specific antiviral as well as anti-fungal/anti-*Toxoplasma* therapy, the patient’s condition continued to decline and she died due to respiratory failure on 7 d after the second transplantation.

### 2 Discussions

Unrelated UCBT was associated with delayed neutrophil recovery and a higher incidence (10% - 30%) of engraftment failure as compared to BMT or PBSCT[1]. The major limitation to the wide use of UCBT for allogeneic transplantation has been the low absolute number of hematopoietic stem cell (HSC). However, pediatric patients have more advantages than adults. In the Eurocord analysis, an infused nucleated cell dose of > 1.7 x 10^7/kg was associated with more rapid neutrophil recovery[2]. In the context of pediatric patients, the recommended nucleated cell doses include 1.0 x 10^7/kg, 1.5 x 10^7/kg, and 2.0 x 10^7/kg[3,4]. A CD34+ cell dose of 1.7 x 10^5/kg had been established as the threshold dose for patients at the University of Minnesota[5]. If UCBT graft of 1-locus mismatched with high cell dose, that is > 3.0 x 10^7 nucleated cells per kg, the TRM was 29%. Otherwise, if UCBT graft of 1-locus mismatched with low cell dose, ≤ 3.0 x 10^7 nucleated cells per kg, TRM was 43%[6]. In this study, the girl with 1-locus mismatched received nucleated cell dose of 12.4 x 10^7/kg and CD34+ cell of 9.9 x 10^5/kg. We reviewed the corresponded references about the relationship between the dose of HSCs and graft failure after UCBT. Most researches proved that elevation higher cell dose of UCBT could overcome the risk of graft failure[2,5,9]. However, Urbano-Ispizua and his colleagues[10] reported that increasing dose may not necessarily be associated with increased benefit as noted in one retrospective series suggesting poorer survival with increasing HSC dose in matched related T cell-deplete allogeneic transplants. In that study, they observed that the higher infused HSC dose decreased the survival rate post T cell-deplete allo-PBSCT[10]. As for UCBT patients, the correlation between high nucleated cell dose and the successful rate of engraftment has not been established. Therefore, the best indicator of cell dose remains controversial.

Wolff[11] summarized the guideline of second transplantation after graft failure or relapse. They considered that graft failure without specific cause, especially with an adequate number of infused HSC, suggests a potent host immunologic mechanism of graft dysfunction. In our patient, the girl had platelet transfusion refractory and received amount of blood transfusions before undergoing UCBT. As we known,
platelets express HLA class I antigens. Patients who have had earlier exposure to allogeneic HLA class I antigens from prior transfusions are at increased risk for HLA alloimmunization, in which antibodies develop against HLA antigens. Patients with AML have a 10% to 15% risk of developing HLA allo-antibodies, with an associated increased risk of platelet transfusion refractoriness, during conventional induction chemotherapy\textsuperscript{[12-13]} . Hematopoietic stem cell transplantation (HSCT) recipients may become alloimmunized to Anti-HLA donor-specific antibodies (DSA) through pregnancy or amount of blood transfusions. Several studies have demonstrated that DSA have a significantly negative impact in outcome\textsuperscript{[14-16]} . Engraftment failure is observed at a rate of approximately 5% in unrelated donor HSCT, and DSA may increase the risk\textsuperscript{[17]}.

Ciurea et al\textsuperscript{[16]} detected 592 patients who received matched unrelated donor (MUD) stem cell transplantation. Graft failure occurred in 16 of 584 (2.7%) patients without anti-HLA antibody (Ab) compared with 3 of 8 (37.5%) patients with DSA ($P = 0.0014$). DSA were the only factor highly associated with graft failure. Spellman et al\textsuperscript{[18]} evaluated 37 graft failure in unrelated donor HSCT. Twenty-four percent of recipients possessed DSA against HLA-A, B, and/or DP, compared with only 1 (1%) of 78 engraftments ($P < 0.001$). Takashashi et al\textsuperscript{[14]} tested 386 patients underwent cord blood transplantation in Japan. Neutrophil and platelet recoveries were significantly affected in Ab-positive group compared with that in the Ab-negative group. Interestingly, neutrophil recovery was significantly affected in the Ab-positive group with the higher CD34\textsuperscript{+} cell dose ($> 0.85 \times 10^9$/kg) compared with the Ab-negative group. These findings maybe to some extend, explained that why our patient got graft failure with high dose of CD34\textsuperscript{+} cells after UCBT. The girl was allosensitized through amount of transfusion of blood products, which may increase the rate of DSA and the risk of graft failure. Unfortunately, we did not measure DSA in that unit of cord blood before undergoing UCBT.

In conclusion, high dose of CD34\textsuperscript{+} cells could not overcome the risk of primary graft failure and HLA disparity. The patient’s primary graft failure was associated with platelet transfusion refractoriness and potent immunologic dysfunction, especially the anti-HLA donor specific antibodies before unrelated cord blood transplantation.

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含高剂量 CD34⁺ 细胞的非血缘脐血移植治疗 AML/MDS 后原发植入失败

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【摘要】 目的 分析含高剂量 CD34⁺ 细胞的非血缘脐血移植治疗急性粒细胞白血病合并骨髓增生综合征（AML/MDS）后发生原发植入失败的原因。方法 1 例 4 岁女孩在四川大学华西第二医院儿科血液肿瘤科被诊断为 AML/MDS。患儿经诱导及巩固治疗获完全缓解后，行无血缘人类白细胞抗原（HLA）部分相合脐血移植。观察患儿术后造血重建及移植相关并发症情况。结果 患儿术后发生原发植入失败，再次进行血缘间的半相合造血干细胞移植，期间患多种耐药性病毒感染及败血症，于第 2 次移植后 7 d 死于呼吸衰竭。结论 含高剂量 CD34⁺ 细胞脐血造血干细胞移植并不能抵消 HLA 配型不合的缺陷。患儿原发植入失败可能与脐血移植前存在长期血小板输注无效及潜在免疫异常，尤其是移植后产生抗-HLA 供者特异性抗体有关。

【关键词】 人类白细胞抗原；配型；脐血移植；造血干细胞移植；供者特异性抗体
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