

# Unexpected unrelated umbilical cord blood stem cell engraft in two patients with severe aplastic anemia that received immunosuppressive treatment: A case report and literature review

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**Abstract.** Severe aplastic anemia (SAA) is a life-threatening bone marrow disorder. Bone marrow transplantation is the primary therapy for SAA; however, its efficacy is limited by numerous factors, including lack of histocompatible sibling donor, patient age and graft-versus-host-disease (GVHD) following transplantation. Immunosuppressive treatment (IST) is the first procedure developed for patients without a sibling donor. Our previous study reported that patients administered enhanced IST, in addition to a regime of unrelated umbilical cord blood (UCB) transfusion, exhibited higher efficiency and a reduced rate of relapse. Therefore, the present study reported the cases of 2 patients that received enhanced IST plus unrelated UCB transfusion. These patients exhibited complete hematological recovery with an increased rate of mixed chimerism and demonstrated no signs of GVHD or relapse during the 2-year follow-up period. Thus, enhanced immunosuppressive treatment (low-dose cyclophosphamide and antithymocyte globulin) combined with UCB transfusion may be an effective treatment for patients with SAA.

## Introduction

Aplastic anemia (AA) is a serious disorder characterized by pancytopenia and hypocellular bone marrow. Severe AA (SAA) is diagnosed when 2 of 3 blood parameters meet the following criteria: Absolute neutrophil count,  $<500/\text{ml}$ ; absolute reticulocyte count,  $<60,000/\text{ml}$ ; and platelet count,  $<20,000/\text{ml}$  (1). AA is a disease that may present at any age, and SAA is always fatal if untreated. Adolescent and young adult patients ( $\leq 30$  years old) with SAA may exhibit marrow failure, in contrast to older adult patients. Matched sibling hematopoietic stem cell transplantation (HSCT) is considered to be the primary treatment for patients with SAA (2). If a

matched sibling donor is not available, it is necessary to screen unrelated histocompatible donors from a bone marrow library. Umbilical cord blood (UCB) is an alternative hematopoietic stem cell source for patients with SAA (3); however, low cell dose, higher risk of rejection and delayed immune recovery limit its application. In clinical practice, immunosuppressive treatment (IST) has been used as the primary procedure for patients without a compatible donor; however, the relapse rate reported in previous studies is high (4). In a previous study (5), the present authors reported that the patients that received enhanced IST plus one regime of unrelated UCB transfusion exhibited higher efficiency and a reduced rate of relapse. In the present report, two patients with SAA were successfully treated with enhanced immunosuppressive treatment combined with unrelated UCB. Unexpectedly, the patients exhibited mixed chimerism following therapy. Chimerism studies were conducted on multiple occasions following the treatment. By analyzing short tandem repeat regions, chimerism was identified as 5.2% on day 16, which indicated low levels of donor T cell engraftment, and increasing to 74.6% on day 74. At 6 months after therapy, the chimerism level reached 75.2% and remained at 97.75% at 375 days after therapy.

## Case report

**Ethical approval and patient consent.** The therapy protocols of the present study were approved by the Ethics Committee of the General Hospital of Jinan Military (Jinan, China). Written consent was obtained from the patient or patient's legal guardian. The patients were screened for Fanconi anemia and paroxysmal nocturnal hemoglobinuria.

**Case 1.** A 10-year-old boy was diagnosed with SAA in September 2012 at the General Hospital of Jinan Military. The patient had no matched related donor for HSCT. The treatment regimen consisted of 3 mg/kg rabbit anti-thymocyte globulin (ATG) from day -5 to day -1, and 50 mg/kg/day cyclophosphamide (CTX) from day -3 to day -2. Cyclosporine A (CSA) was administered by intravenous infusion of 3 mg/kg/day (oral administration from day -1) to maintain a range of 150-250 ng/ml. Unrelated UCB was transfused on day 0, and a total of  $4.2 \times 10^7/\text{kg}$  nucleated cells and  $0.9 \times 10^5/\text{kg}$  CD34<sup>+</sup> cells were transfused. If the neutrophil count was  $<0.5 \times 10^9/\text{l}$ , then granulocyte-colony stimulating factor (G-CSF; 5 mg/kg) was administered; however,

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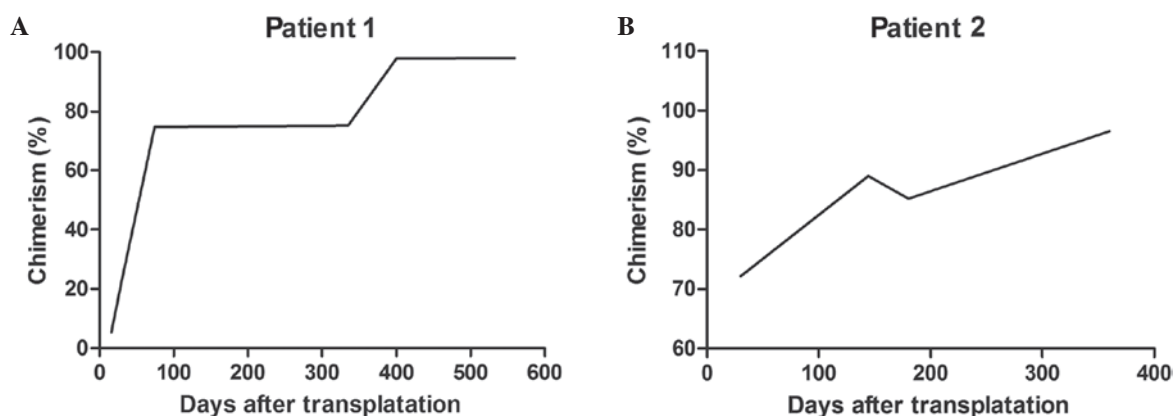


Figure 1. Chimerism of patients 1 and 2 following combined therapy.

the dose of G-CSF was gradually reduced if the neutrophil count was  $>1.5 \times 10^9/l$ . No significant toxicity was observed. Neutrophils reached  $0.5 \times 10^9/l$  on day 12 and platelet counts reached  $30 \times 10^9/l$  on day 27. The patient achieved complete response on day 74 and the cell phenotype of red blood cells was completely changed to the donor type at 4 months. Chimerism (through the analysis of short tandem repeat regions) was 5.2% on day 16, increasing to 74.6% on day 74. At 6 months after therapy, the chimerism reached 75.2% and remained at 97.75% at 375 days after therapy (Fig. 1). The latest follow-up occurred in May 2015. There was no indication of graft-versus-host disease (GVHD) or relapse, and chimerism was 100%.

**Case 2.** A 26-year-old female was diagnosed with SAA in June 2013 and treated following a similar protocol as for case 1. The patient was the only child in the family and had no matched related donor for HSCT. For unrelated UCB transfusion, a total of  $1.7 \times 10^7/kg$  nucleated cells and  $0.36 \times 10^5/kg$  CD34<sup>+</sup> cells were transfused. Neutrophils reached  $0.5 \times 10^9/l$  on day 20 and the platelet count reached  $30 \times 10^9/l$  on day 32. The patient achieved complete response on day 87. The cell phenotype of red blood cells was completely changed to the donor type at 5 months. In addition, chimerism was 72% on day 30, increasing to 89% on day 144. Due to Epstein-Barr viremia, the patient discontinued the CSA therapy on day 150, and the chimerism reduced to 85.2%. Chimerism reached 100% at 361 days after the therapy (Fig. 1B). During a 2-year follow-up period, the donor chimerism remained complete implant and normal blood cell counts were detected, while there was no indication of GVHD and no relapse.

## Discussion

Five decades ago, effective therapy for SAA was limited; however, advances in HSCT and IST have improved the survival rate of patients with SAA from 10-20% in the 1960s to 80-90% at present (6). Human leukocyte antigen (HLA)-matched HSCT is recommended as the primary therapy for young patients with SAA (7); however, the lack of matched donors, graft rejection, GVHD and poor immune reconstitution limit the success of HSCT. UCB is an alternative hematopoietic stem cell source for transplantation. Hemopoietic progenitor cells (HPCs) in UCB possess extensive proliferative capacity, and the quantity

of HPCs from a single UCB collection is associated with the success of bone marrow transplantation (BMT) (8). Related and unrelated UCB transplantation (UCBT) have been successfully used in the treatment of pediatric patients with various malignant or non-malignant diseases (9). Compared with BMT, UCBT is able to evidently reduce acute and chronic GVHD. However, graft rejection and poor immune reconstitution continue to limit the success rate of UCBT. A higher number of nucleated graft cells, certain conditioning regimens and the degree of mismatch between the graft and recipient are crucial for achieving improved engraftment following UCBT in patients with SAA, particularly in adult patients (3,10). In order to overcome the high engraftment/alloreactivity barrier in SAA, myeloablative chemotherapy or radiation therapy have been used to induce sufficient immunosuppression or clearance of reactive recipient T cell populations. Total lymphoid irradiation or total body irradiation are typically used in the regimen (11-13); however, the irradiation has been reported to affect the neuroendocrine system in children and their subsequent growth and development (14), in addition to increasing the risk of malignancies.

IST is used to treat patients that lack a histocompatible donor or elderly patients, and ATG combined with cyclosporine remains the standard procedure for IST (6). Hematological responses to transfusion independence appear in ~2/3 of patients; however, disease relapse ultimately occurs in 30-40% of patients (1,4). A 10-year follow-up study revealed that the response rate of SAA is 71% and the actual event-free survival is 58% in 44 treatment-naïve patients with SAA that received high-dose cyclophosphamide (15). The success of IST application is significantly limited by poor response, high relapse and clonal evolution. In a previous study, the present authors treated patients with enhanced immunosuppressive treatment (100 mg/kg CTX) and UCB transfusion as an adjuvant therapy (5). Patients rapidly achieved reconstitution of hematopoiesis, while the efficacy rate was 88% and the 3-year overall survival rate was 92% (5). However, there were no signs of implant, so the therapy was named intensive IST combined with UCB transfusion, rather than UBCT (16). Furthermore, in a previous study, CTX (200 mg/kg) and rabbit ATG (7.5 mg/kg/day for 4 days) were used as a conditioning regimen in a pediatric patient with SAA that was receiving UBCT (17). The patient's

neutrophil count reached  $0.5 \times 10^9/l$  on day 37, while red blood cell and platelet transfusion independence were reached on days 50 and 52, respectively. The patient demonstrated stable mixed chimerism at 18 months and full donor chimerism at 20 months after UCBT (17). The doses of CTX and ATG employed in the present cases were half of the previous dosages (18), and the adult patient received a reduced number of total nucleated cells and CD34<sup>+</sup> cells compared with a previous study (18). However, the neutrophils and platelet engraftment recovered faster compared with those in the previous study. Furthermore, the appearance of full donor chimerism occurred earlier and without relapse during the 2-year follow-up period. Though the treatment may induce a higher risk of rejection due to the unrelated and mismatched UBC, the engraftment in two patients in the present study was successful.

In conclusion, the modified protocol used in the present study, which involved no irradiation, resulted in mixed chimerism and rapidly achieved complete reconstitution of hemopoiesis. The present report suggested the feasibility and efficacy of enhanced IST combined with UCB for the treatment of patients with SAA. Thus, the improved therapy may be a viable therapeutic option for patients that lack a suitable HLA-matched donor and may enhance the response rate of IST.

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