Successful Selective CD34⁺ Cell Infusion after Late Graft Failure of Hematopoietic Stem Cell Transplantation in Two Cases of Severe Aplastic Anemia

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Matched sibling bone marrow transplantation (BMT) in severe aplastic anemia (SAA) has been known as the treatment of choice in children and young adults [1], although graft failure occurs in 5-25% of patients after transplantation [2]. Unlike primary graft failure which means no engraftment of donor cells, secondary graft failure is associated with loss of donor chimerism after engraftment [3] showing marrow hypoplasia with a requirement of frequent transfusions [4]. To overcome graft failure which leads to increased mortality and morbidity, second stem cell transplantation is suggested in several previous studies [3-5]. Cells of the same donor or other allogeneic donor have been used for the boost treatment, and moreover selective CD34⁺ cells are proposed to be a successful source of re-infusion in graft failure [6].

We report our experience of the selective CD34⁺ cell boost treatment in two aplastic anemia patients who had
late graft failure after matched sibling BMT.

**Case Reports**

1) Case 1

An 11-year-old girl underwent allogeneic BMT from her HLA (8/8) and ABO matched sister 11 months after the diagnosis of SAA. Fanconi anemia and other myelodysplastic syndromes were excluded. The conditioning therapy included cyclophosphamide (50 mg/kg) for 4 days and anti-thymocyte globulin (ATG) (2.5 mg/kg) for 3 days. Infused CD34+ cell dose was 1.4×10^6/kg of recipient. The engraftment, which is defined as the first day of three consecutive days of more than 0.5×10^9/L of absolute neutrophil count (ANC), was achieved on day +22 and on day +25 bone marrow (BM) biopsy showed hypocellular marrow with complete donor chimerism. The patient received granulocyte colony-stimulating factor from day +3 and cyclosporine and methotrexate were used for GVHD prophylaxis. She had no symptoms and signs of acute graft versus host disease (GVHD). On post-transplant day +88, her CBC showed decreased result of WBC 1,880/μL, hemoglobin 9.1 g/dL, and platelets 2.9×10^3/μL. Day +99 BM biopsy showed hypocellular marrow with complete donor chimerism without change. Since then, her CBC steadily decreased, she started to require RBC and platelet transfusions by 2 years after transplantation. Four years after BMT, her sister’s peripheral blood stem cells were collected for the treatment of late rejection. We used CliniMACS sorting device (Miltenyi Biotec) to select CD34+ cells at post-transplant 52.4 months, CD34+ cells of 1.83×10^6/kg were infused as boost therapy. No additional conditioning drugs or GVHD prophylaxis were used and no complications occurred during and after the infusion. Until now, she has been being followed up in the outpatient clinic without transfusion for 28 months (case 1) and 16 months (case 2). Their CBC results after the boost treatment show significant increase compared to the results before their boost treatment. In case 1, median WBC (2,490±959/μL vs. 5,149±1,072/μL, P<0.001), median hemoglobin (6.6±1.4 g/dL vs. 10.5±1.6 g/dL, P<0.001), and median platelets (3.52±1.27×10^3/μL vs. 8.28±3.63×10^3/μL) all increased. In case 2, median WBC (3,113±474/μL vs. 3,636±555/μL, P=0.01), median hemoglobin (8.5±1.4 g/dL vs. 11.5±2.0 g/dL, P<0.001), and median platelets (4.05±0.96×10^3/μL vs. 9.44±2.83×10^3/μL) showed similar results.

2) Case 2

A 15-year-old girl who had a history of idiopathic aplastic anemia and sibling BMT visited our hospital under the diagnosis of late graft rejection. She was diagnosed as aplastic anemia at the age of 13, and received BMT from HLA matched (8/8) but ABO and Rh mismatched brother, in another hospital. Conditioning regimen consists of cyclophosphamide (50 mg/kg) for 4 days, ATG (2.5 mg/kg) for 3 days and procarbazine (12.5 mg/kg) for 3 days was undergone. The CD34+ cells of 0.18×10^6/kg were infused and on day +16, ANC increased up to 0.5×10^9/L. Although the engraftment was successful the graft rejection was suspected after 11 months of the BMT. Laboratory results at the time of her first visit to our clinic were as follows: WBC 2,770/μL, hemoglobin 6.3 g/dL, and platelets 3.1×10^3/μL. She received regular check-ups in our outpatient clinic. During follow-up, her pancytopenia progressed and BM biopsy showed hypocellular marrow (cellularity 10-20%) with complete donor chimerism. Five years after BMT, a dose of 8,38×10^6 selective CD34+ cells per recipient body weight were administered without conditioning and GVHD prophylaxis. During the 1 year follow-up, she has shown recovery of cell counts with no complications or GVHD.

**Discussion**

In this report, patients of SAA showed successful engraftment after sibling BMT with full donor chimerism although sustained stabilized hematopoiesis was not successfully achieved during follow-up. We have chosen CD34+ cells boost treatment for rescue therapy, and two weeks after the boost infusion, patients’ CBC showed complete recovery. They have been being followed up in the outpatient clinic without transfusion for 28 months (case 1) and 16 months (case 2). Their CBC results after the boost treatment show significant increase compared to the results before their boost treatment. In case 1, median WBC (2,490±959/μL vs. 5,149±1,072/μL, P<0.001), median hemoglobin (6.6±1.4 g/dL vs. 10.5±1.6 g/dL, P<0.001), and median platelets (3.52±1.27×10^3/μL vs. 8.28±3.63×10^3/μL) all increased. In case 2, median WBC (3,113±474/μL vs. 3,636±555/μL, P=0.01), median hemoglobin (8.5±1.4 g/dL vs. 11.5±2.0 g/dL, P<0.001), and median platelets (4.05±0.96×10^3/μL vs. 9.44±2.83×10^3/μL) showed similar results.

Graft rejection is one of the most life threatening complications of BMT. Low stem cell dose, patients receiving transplantation from an HLA-mismatched, from an un-
related donor, T cell-depleted graft, viral or fungal infection after transplantation and GVHD are known factors that increase incidence of graft failure [5,6]. To prevent graft failure, large doses of stem cell infusion, matched donor transplantation, lesser transfusion to lower sensitization have been suggested although the cause of graft failure is still not well known and it is difficult to predict graft failure [4,7].

To overcome this condition, boost treatment or second hematopoietic stem cell transplantation is being performed. One of the option is reinfusion of stem cells from a same donor with or without preparative conditioning treatment or from an alternative donor. Nevertheless, prolonged pancytopenia and additional conditioning regimens increase complications such as infections and GVHD resulting in poor prognosis of graft failure [3-5].

Selective CD34+ cells collected from peripheral blood are one of a graft source for the patients. Positive stem cell selection was first developed for autologous transplantations in high risk patients of refractory and relapsing diseases. Along with the development of selection techniques and devices, it has been used for allogeneic transplantation in leukemia and myelodysplastic syndrome which have high risk factors of GVHD with or without graft failure [8]. This method was used to reduce the risk of GVHD, and similar results were observed in SAA patients, selective CD34+ cell infusion significantly decreased GVHD while slightly raised the risk of graft failure due to small amount of T cells [2,9-11]. To prevent graft failure with lesser risk of GVHD, highly selective and large numbers of CD34+ cell infusion has been shown to be a successful choice [2]. They were also useful for patients who had history of heavy transfusions before transplantation, for patients with unrelated, mismatched or haploidentical donor in SAA. Large numbers of selected stem cells are thought to accelerate immune reconstitution.

Trakhtman et al. [6] reported a SAA patient with graft dysfunction after matched sibling BMT who showed mixed chimerism (95% of donor cell) in BM study, this resulted in reversion to full donor-type chimerism after second infusion of CD34+ enriched peripheral blood cells. The patients experienced acute GVHD in spite of GVHD prophylaxis with mofetil mycophenolate and basiliximab. In this case, boost treatment was performed 3 months after BMT. Chung et al. [12] also reported similar result showing successful peripheral blood stem cell rescue for 7 aplastic anemia patients with late graft failure. Complete donor chimerism was maintained at the time (median 9.2 months from the initial transplantation) of boost treatment. Cyclosporine A was used for GVHD prophylaxis although 2 patients developed acute GVHD and 3 patients developed chronic GVHD, Larocca et al. [13] compared trilineage recovery and GVHD among three groups of patients with primary and secondary poor graft function following allogeneic stem cell transplantation. Among patients who received no further infusion, who received a boost of unmanipulated stem cells from original donor, and who received CD34+ selective stem cells, the third group showed significant better trilineage recovery (40% vs. 36% vs. 75%) and low incidence of acute GVHD (0% vs. 29% vs. 0%).

In our two cases, boost treatment using selective CD34+ cells in condition of secondary graft insufficiency showed successful results, BM was replaced with 100% donor cells and the boost treatment was performed 4 to 5 years after BMT as a result of late graft failure. The signs of graft failure were shown earlier after the transplantation (3 months and 11 months after, respectively) however boost treatment was done several years after as the need of transfusion increased. Since the patients showed complete donor chimerism, and CD34+ cell infusion showed lesser risk of acute GVHD [2,10,13], we performed rescue therapy without GVHD prophylaxis and they showed no complications. Due to the short duration of follow-up period, the development of chronic GVHD is not discussed here although it should be monitored since there is higher risk of extensive chronic GVHD following boost cell infusion [12,13].

Few studies have discussed the role of boost treatment of purified selective CD 34+ cells in late graft failure after sibling BMT in children with aplastic anemia. This report suggest that selective CD 34+ cell boost treatment, without further conditioning or GVHD prophylaxis, can be a promising curative treatment in late graft failure of SAA patients who previously underwent sibling BMT.
References

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