Cotransplantation of Allogeneic Mesenchymal and Hematopoietic Stem Cells in Children With Aplastic Anemia

abstract

We report here the preliminary results of allogeneic hematopoietic stem cell transplantation with mesenchymal stem cells (MSCs) for 6 cases of severe aplastic anemia. The patients ranged in age from 3 to 16 years, and the median time from diagnosis to transplantation was 32 months (range: 3–156 months). The conditioning regimens consisted of fludarabine, cyclophosphamide, and antithymocyte globulin with or without busulfan. Graft-versus-host disease (GvHD) was prevented by the administration of cyclosporine A, methotrexate, and mycophenolate mofetil, with or without anti-CD25 monoclonal antibody. The grafts were granulocyte colony–stimulating factor–mobilized bone marrow and peripheral blood from HLA antigen-haploidentical donors (3 cases) or peripheral blood only from unrelated HLA antigen-identical donors (3 cases). MSCs were intravenously injected at a median dose of $1.43 \times 10^6$/kg (range: $0.85–2.5 \times 10^6$/kg). The mean time for neutrophil and platelet recovery was 12.3 and 13.8 days, respectively. Acute GvHD grade I and II developed in 2 cases, and no chronic GvHD was documented. All patients were alive and transfusion independent at a median follow-up of 15 months (range: 6–29 months). Our report suggests that cotransplantation of allogeneic hematopoietic stem cells and MSCs might provide an opportunity for therapy for children with severe aplastic anemia. Pediatrics 2012;129:e1–e4
Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment option for patients with severe aplastic anemia (SAA), and it represents the first choice of treatment for most patients who have failed to improve with intensive immunosuppressive therapy (IST) and have an HLA antigen-identical sibling donor. However, allo-HSCT from unrelated donors is usually associated with inherent complications, including graft failure, graft-versus-host-disease (GvHD), and infections, especially in patients who have received hematopoietic grafts from an HLA antigen-mismatched donor. A previous report indicated that the incidence of graft failure after unrelated donor transplantation was ~14%, and the 10-year probabilities of overall survival were 57% for all 8 HLA-loci matched transplants and 39% for ≤1-loci mismatched transplants. The application of fludarabine and low-dose body irradiation in the preconditioning phase has improved overall survival; however, the incidence of graft failure (17%) was not obviously reduced. In some cases, an HLA antigen-matched unrelated donor is not available, and haploidentical hematopoietic stem cell transplantation (Haplo-HSCT) has become an optional choice. The protocols in Haplo-HSCT have dramatically improved over the last decade, and the outcome seems acceptable in the treatment of patients with hematopoietic malignancies. Nevertheless, Haplo-HSCT in patients with SAA has not been widely investigated in clinical trials probably because of the high risk of graft rejection.

Mesenchymal stem cells (MSCs) have been approved by the US Food and Drug Administration for use in a clinical Phase III trial for the management of steroid-resistant acute GvHD (aGvHD). In addition, it has been suggested that MSCs enhance hematopoietic engraftment in HLA antigen-identical HSCT in malignancies. Thus, we assessed the outcome of cotransplantation of MSCs and unrelated donor or haploidentical hematopoietic stem cells in 6 patients with SAA.

**CASE REPORT**

Six patients with a median age of 9.3 years (range: 5–16 years) were enrolled in this study from February 2009 to December 2010. The study was approved by the Ethics and Technological Committees of the General Hospital of the Air Force (Beijing, China). All patients had failed to improve with IST and were heavily transfused and transfusion dependent. The patients and donors had provided written informed consent for the protocol. Patient characteristics are shown in Table 1.

Three patients with haploidentical transplantation received a conditioning regimen consisting of fludarabine 30 mg/m² for 4 days, cyclophosphamide 50 mg/kg for 4 days, and busulfan 4 mg/kg for 2 days. The other patients with unrelated transplantation received fludarabine 30 mg/m² for 6 days and cyclophosphamide 60 mg/kg for 2 days. All patients received antithymocyte globulin or antilymphocyte globulin from day −4 to −1.

Umbilical cord and bone marrow MSCs were prepared, identified, and examined as previously reported by our group. The first patient received MSC from donor bone marrow, and the other patients received umbilical cord MSC. MSCs at a median dose of $1.43 \times 10^{10}$/kg (range: 0.85–2.5 \times 10^{10}$/kg) were intravenously infused before hematopoietic graft infusion. Three patients were infused with peripheral blood stem cell (PBSC) grafts from unrelated donors; the others were infused with PBSC and granulocyte colony-stimulating factor–mobilized bone marrow grafts from haploidentical parental donors as described previously by our group.

The aGvHD prophylaxis protocol included cyclosporine A, methotrexate, and mycophenolate mofetil as described in our previous report. In haploidentical transplantation, monoclonal antibody against human CD25 (basiliximab) was added. Supportive care was performed as previously described.

The characteristics of the hematopoietic grafts are shown in Table 1. The mean total nucleated cell numbers were 11.6 \times 10^{10}$/kg. No adverse effects occurred during MSC infusion. The hematopoietic reconstruction is shown in Table 2. All patients attained successful neutrophil and platelet recovery, and the mean time to neutrophil engraftment was 12.3 days (range: 11–18 days) and that of platelet recovery was 13.8 days (range: 11–22 days). Analysis of donor cell chimerism showed complete donor-originated hematopoietic reconstruction 1 month after transplantation. According to the grading system provided by the International Bone Marrow Transplant Registry, 2 patients developed aGvHD, including 1 patient (case 4) with grade I skin lesions.

**TABLE 1 Patient Characteristics**

<table>
<thead>
<tr>
<th>No.</th>
<th>Age, y/ Gender</th>
<th>Disease Course</th>
<th>Transfusion Before Transplantation, units</th>
<th>aGvHD Prophylaxis</th>
<th>Nucleated Cells, \times 10^8/kg</th>
<th>MSCs, \times 10^6/kg</th>
<th>Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RBC Plt</td>
<td>CAMMB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3/F</td>
<td>8 mo</td>
<td>15 28</td>
<td>CAMMB</td>
<td>1.91</td>
<td>11.75</td>
<td>1.25</td>
</tr>
<tr>
<td>2</td>
<td>10/M</td>
<td>3 mo</td>
<td>18 6</td>
<td>CAMMB</td>
<td>4.98</td>
<td>5.2</td>
<td>1.85</td>
</tr>
<tr>
<td>3</td>
<td>16/M</td>
<td>15 y</td>
<td>20 8</td>
<td>CAMMB</td>
<td>4.15</td>
<td>4.8</td>
<td>1.06</td>
</tr>
<tr>
<td>4</td>
<td>6/M</td>
<td>3 mo</td>
<td>18 10</td>
<td>CAMM</td>
<td>8.79</td>
<td>—</td>
<td>1.09</td>
</tr>
<tr>
<td>5</td>
<td>15/F</td>
<td>3 mo</td>
<td>18 6</td>
<td>CAMM</td>
<td>6.09</td>
<td>—</td>
<td>0.85</td>
</tr>
<tr>
<td>6</td>
<td>6/F</td>
<td>3 mo</td>
<td>54 27</td>
<td>CAMM</td>
<td>22</td>
<td>—</td>
<td>2.5</td>
</tr>
</tbody>
</table>

BM, bone marrow; CAMM, cyclosporine A + antithymocyte globulin + methotrexate + mycophenolate mofetil; CAMMB, CAMM + basiliximab; F, female; M, male; Pit, packed platelets; RBC, packed red blood cells; UD, HLA antigen-identical unrelated donor.
that were controlled with steroid therapy. One patient (case 2) developed aGvHD grade II (intestinal aGvHD grade I) and exhibited a good response to steroid treatment and administration of umbilical cord MSC at a dose of $1.5 \times 10^6$/kg. No chronic GvHD or symptomatic infections from bacteria, fungus, or cytomegalovirus were documented.

**DISCUSSION**

SAA is a peculiar situation in the settings of unrelated transplantation because the patients have usually received multiple blood transfusions before HSCT. Furthermore, it is generally considered that graft failure, severe GvHD, and delayed immune reconstruction are the intractable complications in Haplo-HSCT, and it could be expected that intractable complications in Haplo-HSCT may occur in the treatment of SAA, although Haplo-HSCT in SAA has not yet been intensively investigated.

MSCs can provide the supportive microenvironmental niche for hematopoietic stem cells. MSCs have low inherent immunogenicity, modulate/suppress immunologic responses through interactions with immune cells, and could migrate into the damaged tissues to contribute to the regeneration through their diverse biological properties. Therefore, MSCs have been used in autologous HSCT, allo-HSCT, and Haplo-HSCT to improve engraftment and prevent the occurrence of severe GvHD, by means of supporting hematopoiesis and inhibiting the allogeneic activity of immune cells from both hosts and donors. More recently, Liu et al. reported that transplanted bone marrow MSCs promote the recovery of megakaryocyte lineage in Haplo-HSCT in the treatment of patients with leukemia, although the incidence of aGvHD was comparable to that of the control group. Of note, the doses of MSCs were $3 \times 10^6$/kg, greatly lower than that of our study and those ($>1 \times 10^6$/kg) commonly reported otherwise. Consistently, the beneficial effect of transplanted MSCs on hematopoietic engraftment was also obvious in this study, although all the patients were heavily transfused as the average number of blood transfusions was 38 (range: 24–81 times). The mean time to neutrophil and platelet recovery was 12 and 14 days, respectively, whereas those times were 17 and 19 days in Haplo-HSCT in children with leukemia, as reported by our group. Furthermore, the incidence of aGvHD (2 of 6) and chronic GvHD (0 of 6) was also lower than those in the previous report (71.4% for aGvDH and 37.5% for chronic GvHD), although the same protocol for GvHD prophylaxis was used. The results here are consistent with those previously reported by other groups, supporting the application of MSCs in allo-HSCT and Haplo-HSCT in SAA.

Basiliximab is a chimeric antibody against human CD25 that has been used for the prevention of aGvHD in Haplo-HSCT in our institution and other centers. CD25 is a late marker for T-cell activation and a marker for regulatory T cells. Thus, basiliximab might remove the transplanted regulatory T cells that have been generally considered to play a critical role in the development of GvHD. Interestingly, a study of kidney transplantation demonstrated that basiliximab decreases the percentage of CD4+CD25+ T cells but fails to influence the percentage of CD4+FoxP3+ Treg cells. However, detailed investigations might be needed to ascertain the contribution of basiliximab in reducing the severity of aGvHD in Haplo-HSCT.

In conclusion, our data indicate that cotransplantation of MSCs promotes stable engraftment in haplo- or unrelated donor HSCT without severe aGvHD, although larger-scale, prospective, and randomized studies are required to confirm these benefits.

**REFERENCES**


An error occurred in this article by Wang et al, titled “Cotransplantation of Allogeneic Mesenchymal and Hematopoietic Stem Cells in Children With Aplastic Anemia” published in the June 2012 issue of *Pediatrics* (2012;129(6):e1612–e1615; originally published online May 7, 2012; doi:10.1542/peds.2011-2091). On page e1613, under the heading of Table 1 Patient Characteristics, lines 8–10, this reads: “in column Nucleated Cells, subcolumn BM, the listed numbers are 9.79, 6.09, and 22.” This should have read: “in column Nucleated Cells, the numbers 9.79, 6.09, and 22 should be sequentially listed in subcolumn PBSC.”

doi:10.1542/peds.2012-2593


An error occurred in this article by Maguire et al, titled “Estimating the Probability of Abusive Head Trauma: A Pooled Analysis” published in the September 2011 issue of *Pediatrics* (2011; 128:e550–e564; doi:10.1542/peds.2010-2949). On page number e553, in Table 2, on line 1, this reads: “Bechtel et al12; Ettaro et al13; Hettler and Greenes14; Hobbs et al19; Kemp et al15; Vinchon et al16.” This should have read: “Bechtel et al12; Ettaro et al13; Vinchon et al16; Hettler and Greenes14; Hobbs et al19; Kemp et al15.”

doi:10.1542/peds.2012-3300


Two errors occurred in this article by Rochow et al, titled “Misclassification of Newborns Due to Systematic Error in Plotting Birth Weight Percentile Values” published in the August 2012 issue of *Pediatrics* (2012;130(2):e347–e351; originally published online July 23, 2012; doi:10.1542/peds.2011-3884). On page e347, under the Abstract Results section, line 1, the copy reads: “Fourteen of the 16 identified publications contained the systematic error in plotting.” This should have read: “Twelve of the 16 identified publications contained the systematic error in plotting.”

On page e349, under the Results/Literature Search section, lines 5–6, the copy reads: “The plotting error was identified in 14 of these 16 publications.”
This should have read: “The plotting error was identified in 12 of these 16 publications.”

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