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Conditioning regimens

Fludarabine-based conditioning for allogeneic stem cell transplantation for multiply transfused patients with Fanconi's anemia

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Summary:

A fludarabine-based protocol (fludarabine (25 mg/m^2/day × 6 days), cyclophosphamide (10 mg/kg/day × 2 days) and ATG (ATGAM 10 mg/kg/day × 4 days)) was used in four multiply transfused Fanconi's anemia (FA) patients aged 5–15 years to reduce rejection during allogeneic bone marrow transplantation (BMT). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and mini methotrexate. The graft source was G-CSF-stimulated bone marrow or peripheral blood stem cells (PBSC) in two patients each. All patients engrafted with median time to ANC > 500/mm^3 being 14 days (range: 12–17) and unsupported platelet count > 20,000/mm^3 being 13 days (range: 11–18). One patient had secondary graft rejection on day 56 and expired on day 69 due to fungal pneumonia. One patient who developed acute myeloid leukemia on day 56 underwent successful induction with cytosine and daunorubicin followed by peripheral blood stem cell (PBSC) rescue on day 70 and is presently in remission with complete donor chimerism and grade I GVHD. At a median follow-up of 13 months (range: 4–21), three patients (75%) are well with complete donor chimerism. Addition of fludarabine to the conditioning regimen for BMT in FA can provide additional immunosuppression for engraftment without increasing toxicity.
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Allogeneic bone marrow transplantation (BMT) has produced excellent survival rates in patients with Fanconi's anemia (FA) using low-dose cyclophosphamide with or without radiation therapy.[1] Graft rejection rates of 30–60% occur in multiply transfused patients with aplastic anemia requiring intensification of immunosuppression.[2] Few case reports exist on the use of fludarabine-based protocols as conditioning regimens for patients with FA with encouraging results[3–8] and we describe our experience in multiply transfused FA patients.

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Patients and methods

Between 1999 and 2003, four male patients with FA underwent six antigen-matched sibling or family donor BMT at our center. The diagnosis of FA was confirmed by stress cytogenetics using mitomycin C and all had a bone marrow aspirate suggestive of aplastic anemia done 3–6 months prior to BMT. Standard karyotyping was not performed. Conditioning regimen consisted of fludarabine 25 mg/m^2/day (day −11 to −6); cyclophosphamide 10 mg/kg/day for 2 days (days −7 and −6), antithymocyte globulin (ATGAM, Pharmacia Upjohn, USA) 10 mg/kg/day for 4 days (days −5 to −2). Graft source was either G-CSF-stimulated bone marrow or G-CSF-stimulated peripheral blood stem cells (PBSC) in two patients each.

Supportive care

Patients were nursed in HEPA-filtered rooms with adequate sterile precautions and barrier nursing. G-CSF (5 μg/kg/day) was started on day +7 following the infusion of bone marrow or PBSC. Broad-spectrum antibiotics and antifungals were administered as per existing protocols for febrile neutropenia. Acyclovir and Septran were administered as CMV and Pneumocystis carinii prophylaxis, respectively. Ganciclovir was administered started as preemptive prophylaxis if the CMV polymerase chain reaction (PCR) became positive. Graft versus host disease (GVHD) prophylaxis consisted of cyclosporine (3 mg/kg/day i.v. starting day −1) and mini methotrexate (5 mg/m^2 days 1, 3 and 6). Acute GVHD was treated with either dexamethasone or methylprednisolone.

Results

There were a total of four patients (all male) with a median age of 10 years (range: 5–15). The median duration from diagnosis to BMT was 34.5 months (range: 20–60) and the median number of packed red cells transfused prior to BMT was 28 (range: 22–45).

Table 1 Patient characteristics, engraftment, GVHD and follow-up

	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*
Age/sex	15/M	12/M	8/M	5/M
Symptom duration	60	28	20	30
Prior transfusions	45	22	22	24
Prior treatment received	Stanazolol/prednisolone	Stanazolol/prednisolone	ALG/cyclosporine	Stanazolol/prednisolone
Source/cell dose	GCSF-BM	GCSF-PBSC	GCSF-BM	GCSF-PBSC
($\times 10^8$ TNC/kg)	4.69	6.1	16	12
ANC $>500/mm^3$	15	12	17	12
ANC $>1000/mm^3$	15	13	18	14
Platelet count $>20\,000/mm^3$	18	11	15	11
GVHD	Nil	Nil	Nil	Nil/grade I after PBSC rescue
Complications	Secondary graft failure day 50	Nil	Nil	AML day 56 – induction with cytosine/daunorubicin
Follow-up	Expired day 69	Well 21 months	Well 16 months	Well 4 months

Graft

The mean cell dose infused was 10.3×10^8 nucleated cells/kg (range: 4.6–16) for G-CSF-stimulated bone marrow, while it was 9.1×10^8 nucleated cells/kg (range: 6.1–12) for PBSC (Table 1).

Toxicity, engraftment and GVHD

Toxicity was minimal with only grade I mucositis seen in two patients. All patients engrafted with a time to ANC $>500/mm^3$ ranging between 12 and 17 days and ANC $>1000/mm^3$ ranging between 13 and 18 days. An unsupported platelet count $>20\,000/mm^3$ was achieved between 11 and 18 days. During the first 30 days, 1–2 packed cells and 10–12 platelet transfusions were given. Three patients received G-CSF to hasten engraftment for 2–5 days. None of the patients developed acute GVHD.

Hospital course following BMT

Patient No 1 developed secondary graft rejection on day 50 after BMT and expired on day 69 due to fungal pneumonia. Patient No 4 had a complete chimerism on day +21, but was found to have immature cells in the peripheral blood on day 56 with a mixed chimerism. Bone marrow aspirate revealed 54% blasts, suggestive of AML – M5 with cytogenetic analysis showing a complex karyotype {46-47 XY, add(2)(q37), add(3)(q26), dup(3)(q21q26), del(7)(q22), ins(12)(q13), del(20)(q11), +mar [cp20]}. He had induction chemotherapy with cytosine ($200\,mg/m^2$/day continuous infusion for 7 days) and daunorubicin ($45\,mg/m^2$/day for 3 days). The day 14 and day 28 marrows did not show any evidence of residual disease. He had a peripheral blood stem cell (PBSC) rescue using the original donor on day 70 (cell dose: 12.3×10^8 MNC/kg) and is presently in remission with complete donor chimerism 120 days post BMT (day 50 following DLI) with grade I gastrointestinal acute GVHD. Patient Nos. 2 and 3 had an uneventful hospital stay.

Survival and follow-up

At a median follow-up of 13 months (range 4–21), three patients (75%) are alive and well with complete donor chimerism.

Discussion

The unique sensitivity of FA cells to DNA crosslinking agents has led to the use of low-intensity conditioning regimens (low-dose cyclophosphamide with thoracoabdominal radiation (Cy/TBI) or low-dose cyclophosphamide (Cy) alone) during BMT to reduce toxicity.[9] These regimens, however, have not been immunosuppressive enough to reduce graft rejection in patients who are heavily transfused. Fludarabine-based protocols have the twin advantage of potent immunosuppression, thus reducing the incidence of graft rejection along with minimal regimen-related toxicity. No patient had any major toxicity associated with this conditioning regimen and initial engraftment was seen in all patients though one patient had a secondary graft rejection on day +50. Acute GVHD was not seen initially in any of our patients though one patient developed grade I GVHD following the PBSC rescue. The incidence of GVHD in our series is quite low compared to the incidence of 50–60% seen in patients transplanted using Cy/TBI or Cy alone regimens with the majority of them also developing chronic GVHD.[9] Approximately 16 patients with FA treated with fludarabine-based regimens have been described in the literature, and the incidence of regimen-related toxicity (RRT) has been minimal with nil to grade I–II GVHD[3–8] with all patients showing engraftment. Only Guardiola *et al* in their report of three patients, where fludarabine-based regimens were used for a second transplant, showed fatal grade IV GVHD in two of the three patients.[10] Fludarabine-based conditioning regimens have been used effectively with early stable engraftment and minimal toxicity in patients with poor risk for conventional myeloabalative therapy. An ideal conditioning regimen would be one that combines

adequate engraftment with minimal toxicity and fludarabine-based protocols seem to come close to this definition. However, longer follow-up is required to evaluate late rejection and long-term effects in children.

In conclusion, fludarabine-based conditioning regimens are associated with early engraftment with minimal toxicity in patients with FA who are multiply transfused and where rejection rates are expected to be higher.

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