Successful Unrelated Cord Blood Transplantation in Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia During Pulmonary Aspergillosis Treated by Anti-fungal Therapy, Granulocyte Colony-stimulating Factor-mobilized Granulocytes and Surgical Resection: Case Report

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A 3-year-old girl with Philadelphia chromosome positive acute lymphoblastic leukemia developed pulmonary aspergillosis during severe neutropenia by re-induction therapy. She was treated by intravenous fluconazole, oral itraconazole with plasma level monitoring and surgical resection of the focus for 3 months after clinical diagnosis of fungal infection was made. Once she had recovered from surgery we attempted to induce remission with anti-fungal treatment. She developed fever and neutropenia and appeared unlikely to remit with conventional chemotherapy. Unrelated one-antigen-mismatched cord blood (CB) transplantation was performed 2 months after the induction therapy. Her pulmonary aspergillosis was reactivated during subsequent conditioning. Anti-fungal drugs were switched to amphotericin B and granulocyte colony-stimulating factor-mobilized granulocyte concentrates were transfused. She obtained engraftment and has maintained complete hematological and molecular remission without signs of aspergillus infection for 13 months so far after transplantation. Even very high-risk transplantation in pediatric patients could be successfully supported by carefully designed intensive comprehensive medical care.

Key words: pulmonary aspergillosis – cord blood transplantation – Philadelphia chromosome – acute lymphoblastic leukemia

INTRODUCTION

The Philadelphia chromosome (Ph)/bcr-abl positive acute lymphoblastic leukemia (ALL) subgroup of ALL carries the worst prognosis in both children and adults. However, a recent report suggests that the prognosis for this type of ALL depends on the age and leukocyte count at the time of diagnosis (1). Treatment strategies to cure acute leukemia are now risk-directed and Ph positive ALL requires more intensive chemotherapy. Complete remission is achieved in nearly 80% of patients but half of those who remit subsequently relapse, mainly in the bone marrow (1). Regimen-related toxicity such as severe myelosuppression causing infection might also occur and invasive aspergillosis is an important cause of infectious morbidity and mortality during neutropenic periods. Allogeneic bone marrow transplantation (BMT) from either related or unrelated donors is the only current treatment that results in a cure in a substantial proportion of patients (1–3).

We report successful unrelated one-antigen-mismatched cord blood (CB) transplantation in a child with Ph positive ALL during non-remission and pulmonary aspergillosis, treated with conventional anti-fungal agents, granulocyte colony-stimulating factor (G-CSF)-mobilized granulocyte transfusion and surgical resection.
A 3-year-old girl was admitted to our hospital in May 1999 with ALL (Figure 1). Her initial white blood cell (WBC) count was 159 × 10^9/l. She was first treated by the ER induction protocol, which consists of prednisolone (PDL), vincristine (VCR), cyclophosphamide (CY), daunomycin (DM), L-asparaginase (L-Asp) and intrathecal methotrexate (MTX) and cytosine arabinoside (Ara-C) in a Japan Association Childhood Leukemia study (JACLS). Bone marrow examination on day 14 revealed 7.5% blasts. Ph was identified in her peripheral blood 4 weeks later and fluorescence in situ hybridization (FISH) and reverse transcriptase–polymerase chain reaction (RT–PCR) confirmed the bcr-abl rearrangement. Then the treatment was switched to the F protocol, which is acute myeloid leukemia-oriented therapy and assigned for induction failure or Ph positive ALL in JACLS. She developed severe neutropenia after four-drug re-induction phase with PDL, Ara-C, mitoxantrone (MIT) and etoposide (VP-16) and was placed in a clean laminar airflow and given anti-fungal prophylaxis with amphotericin B orally, but had a high fever. Blood cultures were negative. Empirical treatment with broad-spectrum antibiotics produced an initial improvement but 3 days later fever recurred. Despite a combination of synergistic antibiotics and intravenous fluconazole, her fever persisted while her neutropenia failed to respond to G-CSF. Repeated fungal cultures remained negative throughout. Two weeks later her β-D-glucan level was increased to 38.2 (from <20.0) ng/l. Itraconazole 8 mg/kg/day by mouth was started. Thereafter she complained of cough and sputum in addition to continued fever. A chest X-ray revealed right lung infiltrates and a computed tomography (CT) scan demonstrated nodular opacities in the right lower lobe. Aspergillus antigen was not detected by latex examination. However, a clinical diagnosis of pulmonary aspergillosis was made in July 21, 1999. The dose of itraconazole was increased to 12 mg/kg/day to achieve effective plasma concentrations (>0.25 mg/l). Three weeks later her fever resolved with recovery of neutropenia. Despite sustained clinical improvement, a CT scan of the chest 4 weeks later still showed a nodular shadow in the right lower lobe (Figure 2). Gallium scintigraphy revealed a round ‘hot’ area in the right chest corresponding to the shadow seen on CT (Figure 3). The right lower lobe was resected surgically and histology confirmed a fungus ball formation surrounded by granuloma and aspergillus hyphae (Figure 4). She remained clinically stable but a bone marrow aspirate revealed that 60% of the nucleated cells were blasts.

Once she had recovered from surgery we attempted to induce remission with anti-fungal treatment with fluconazole and itraconazole. She developed fever and severe neutropenia during re-induction and appeared unlikely to remit with conventional chemotherapy. However, there were no compatible donors among the family members and, given the urgent need
for stem cell transplantation, the Hokkaido Cord Blood Bank was consulted. An unrelated one-antigen-mismatched CB unit was identified consisting of 96.4 ml of CB containing $9.14 \times 10^7$/kg nucleated cells, $2.55 \times 10^5$/kg CD34+ cells, $7.51 \times 10^4$/kg colony-forming unit-granulocyte macrophage and $5.98 \times 10^4$/kg burst-forming unit-erythroid. Her human leukocyte antigen (HLA) type was A11.1, 24; B52, 55.1; DR04, 12 while that of the CB was A11.1, 24; B52, 55.1; DR04, 08. The unit was serologically negative for hepatitis B and C, human immunodeficiency virus, human T cell leukemia virus type 1 and syphilis.

After signed informed consent had been obtained we transplanted the CB during non-remission urgently on December 19, 1999. The conditioning regimen consisted of busulfan (BU) 140 mg/m²/day orally for 2 days (days –8 and –7), Ara-C 2 g/m² twice daily intravenously for 3 days (from day –6 to –4), idarubicin (IDA) 12 mg/m²/day as a continuous intravenous infusion for 3 days (from day –6 to –4), VP16 15 mg/kg/day intravenously for 3 days (from day –6 to –4) and CY 50 mg/kg/day intravenously for 2 days (days –3 and –2). She received standard cyclosporin A and methylprednisolone graft-versus-host disease (GVHD) prophylaxis, intravenous acyclovir and cytomegalovirus (CMV) high-titer immunoglobulin as CMV prophylaxis. She also received non-absorbable oral antibiotics and amphotericin B. Before conditioning, the WBC count was $2.5 \times 10^9$ with 1% neutrophil, 36% lymphocyte and 63% blasts. On day –7 she complained of cough and rapidly deteriorated. A chest X-ray revealed diffuse infiltrate in the right lower lobe and her $\beta$-D-glucan level increased to 312 ng/l, indicating reactivation of her pulmonary aspergillosis. Anti-fungal agents were replaced by intravenous amphotericin B starting with 0.25 mg/kg/day and increasing to 1.0 mg/kg/day. Granulocytes were transfused on days –5 and –4. Aphereses were performed from her mother, who shared the same blood group. The mother received 75 µg of G-CSF once a day for 2 days after giving informed consent. Granulocytes were collected using an AS104 Blood Cell Separator (Fresenius, Germany) on days 2 and 9 yielding $(7.7–8.4) \times 10^9$ granulocytes. The patient’s fever resolved quickly after the granulocyte transfusions and the $\beta$-D-glucan level fell to normal on day 1. On day 0, cryopreserved CB was thawed and transfused without further manipulation. G-CSF 5 µg/kg was given intravenously from day 1 to 37. She became febrile again from day 2 to 13 due to either reactivation of her aspergillosis and/or other infection.
By day 19 her WBC count was 1.0 × 10⁹/l with an absolute neutrophil count of 0.5 × 10⁹/l and by day 32 her reticulocyte count rose above 1% of the total red cell count. Her platelet count exceeded 20 × 10⁹/l on day 45 and reached over 50 × 10⁹/l on day 52. Red cells were last transfused on day 30 and platelets on day 41. A maculopapular rash and diarrhea developed on day 26 and 46, respectively. A skin biopsy confirmed the existence of acute GVHD, overall grade II, which was treated with high-dose methylprednisolone (20 mg/kg/day) from day 46 to 48 and methotrexate (7 mg/m² weekly, orally) from day 66 to 137. Amphotericin B was continued until day 40 in a reduced dosage due to hypokalemia. On day 40 she was switched toitraconazole until day 65, when it was discontinued. A bone marrow test on day 30 showed that 99.7% of sex chromosome was male type of donor origin by FISH. The bcr-ABL rearrangement was undetectable by either FISH or RT-PCR. Thirteen months after transplantation she remains in good condition with no evidence of recurrence of aspergillosis and maintained molecular remission.

**DISCUSSION**

Aspergillosis in immunocompromised patients is usually treated with amphotericin B, although its efficacy is limited with a response rate ranging from 33 to 54% in patients with leukemia or in BMT recipients (4). Caillot et al. (5) reported that early diagnosis using CT along with earlier initiation of anti-fungal treatment improves the outcome and that the overall response rate toitraconazole was 75%. Our patient was first treated with fluconazole anditraconazole, because she had infiltration-related side effects of amphotericin B such as fever and nausea at initial test dose. Itraconazole has the disadvantage of only being available in oral formulation and having variable absorption depending on the relationship of administration to meals. Blood levels ofitraconazole should be monitored to ensure therapeutic concentrations. This was important in our patient who suffered from oral mucositis, which frequently prevented her from taking oral medications and who also had diarrhea and malabsorption secondary to chemotherapy effects on the bowel.

Surgical resection of pulmonary aspergillosis is effective in properly selected patients. Salerno et al. (6) recently reported successful treatment in 69% of their patients by combining resection of the focus with appropriate anti-fungal drugs. Preemptive resection in patients liable to become neutropenic secondary to subsequent immunosuppression may be beneficial. Granulocyte transfusion was also effective in our patient. Acute pulmonary reactions were known to occur most typically during or shortly after an infusion of amphotericin B that followed granulocyte transfusion (7). We separated the infusion of this drug as far as possible from the time of granulocyte transfusion.

Invasive aspergillosis is not a universal contraindication to BMT. Ozsahin et al. (8) successfully treated invasive aspergillosis in chronic granulomatous disease by BMT, G-CSF-mobilized granulocytes and anti-fungal drugs. They showed that pre-existing invasive multifocal aspergillosis could be controlled during aplasia after BMT and cellular immune reconstitution. The advantages of CB transplants for leukemic patients, especially for those between unrelated individuals, include prompt availability of hematopoietic stem cells and less stringency respecting absolute HLA identity between donor and recipient (9). The efficacy of an HLA-mismatched unrelated CB transplant, especially in a patient who has failed to enter remission, is unproven and may carry a high risk in a patient with aspergillosis. Nevertheless, we decided to perform a CB transplant in our patient. The outcome was successful with respect to both the leukemia and the fungal infection. Use of IDA in a conditioning regimen in children with high-risk acute leukemia was reported to reduce the relapse rate (10). Our regimen contains idarubicin but modified from theirs and we are currently evaluating the efficacy. We are encouraged by this case in which both intravenous and oral anti-fungal agents, combined with surgical resection and G-CSF-mobilized granulocytes, controlled and possibly eradicated the fungal infection while CB transplantation restored immunity.

**References**