Singapore Med J 1997; Vol 38(8):344-346

Autologous Bone Marrow Transplantation in a Child with Acute Promyelocytic Leukemia in Second Remission

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ABSTRACT

Acute myeloid leukemia (AML) comprises 15%-20% of childhood acute leukemia cases. The long-term disease free survival (DFS) in childhood AML is poor with standard chemotherapy alone. Early intensive chemotherapy is generally regarded to be necessary for achieving high complete remission (CR) rates. Recent experience has shown that incorporation of early intensification with high-dose melphalan conditioning and autologous bone marrow transplantation (BMT) during the first remission significantly improves long-term DFS in children with AML. In this article, we report the use of autologous BMT for treatment of a three-and-half year old child with acute promyelocytic leukemia (APL or M3) in second remission. The patient was conditioned with high-dose melphalan of 180 mg/kg prior to bone marrow reinfusion. A total of 4.0 x 10^7/kg mononuclear cells and 1.07 x 10^5/kg granulomonocytic colony forming units (CFU-GM) were infused. Haematopoietic stem cells were enriched by almost 20-fold after the separation and cryopreservation procedures. Haematological recovery was achieved four-and-a-half weeks post-BMT. She has remained in complete remission 18 months after transplantation. Our experience in this patient indicates that this procedure can be used in second remission and it may provide a better alternative for the management of childhood AML in Singapore.

Keywords: acute myeloid leukemia (AML), autologous bone marrow transplantation (BMT)

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INTRODUCTION

Acute myeloid leukemia (AML) comprises 6% of childhood malignancy. It is a heterogeneous group of diseases which comprises about 15% - 20% of childhood acute leukemia cases[1,2]. The prognosis is still poor with standard chemotherapy alone. Only 40% - 50% long-term event-free survival has been achieved from a few studies involving intensive chemotherapy with cyclic myelosuppressive treatment for one to two years[3-7]. Early intensive therapy is generally regarded to be necessary to achieve better CR rates[3-9]. Allogeneic bone marrow transplantation (BMT) during first complete remission (CR) of AML has produced disease-free survival rates of 5% - 65% with low relapse rates of 8% - 20%[10,12]. Although allogeneic BMT after first CR is considered by some as the treatment of choice in paediatric patients, only 30% of patients with AML have HLA-compatible sibling bone marrow donors. Different regimens, including "megadose" therapy and autologous bone marrow transplantation, have been attempted in those with AML who do not have HLA-compatible bone marrow donors. Autologous BMT in complete remission has produced post-transplantation relapse-free rates ranging from 22% - 75% with variable follow-up periods[13-16]. The only series of an exclusive population of patients with AML who have undergone uniformly intensive chemotherapy and autologous BMT have been reported recently[17]. A 68%, 5-year event free survival (EFS) from diagnosis and 87%, 5-year EFS post-autologous BMT have been reported in this study. However, our local experience in long-term EFS is still poor with chemotherapy alone[18]. We have adapted the intensive chemotherapeutic protocol, melphalan conditioning and autologous BMT regimen in a paediatric patient with acute promyelocytic leukemia (APL, M3) during second remission in August, 1993. The patient is currently free of leukemia, 18 months post-bone-marrow transplantation.

CASE REPORT

CJK, a 16-month-old Chinese girl, presented to the National University Hospital, Singapore, in March, 1992 with one week's history of cough, intermittent fever, spontaneous bruising, gum bleeding, fatigue, pallor and malaise. Physical examination revealed an afebrile and pale child. Multiple bruises were seen on her limbs and abdomen. There was mild cervical lymphodenopathy and the liver was enlarged to 2 cm below the costal margin. There was mild gingival swelling and a few small haematomas in the tongue.

Laboratory investigations

Haemoglobin was 10.5 g/dL; platelets 30 x 10^9/L, total white cell count 3.5 x10^9/L with 60% circulating blasts on peripheral blood films. Bone marrow smears were stained with May-Grünwald-Giemsa staining which showed hypercellular marrow with almost complete replacement of normal haematopoiesis by

Table I - Certain important parameters

Parameters	Total	Units/kg
Bone marrow harvested	180 mL	15 mL/kg
Nucleated cell obtained	8.06×10^9	6.2×10^8/kg
Cell recovery after Percoll separation	1.17×10^9 (recovery = 14.5%)	9×10^7/kg
CD34 + cells:		
Fresh harvested BM	1.43%	
Thawed BM	24.34%	
Mononuclear cells reinfused	5.2×10^8	4×10^7/kg
CFU-GM reinfused	1.39×10^6	1.07×10^5/kg

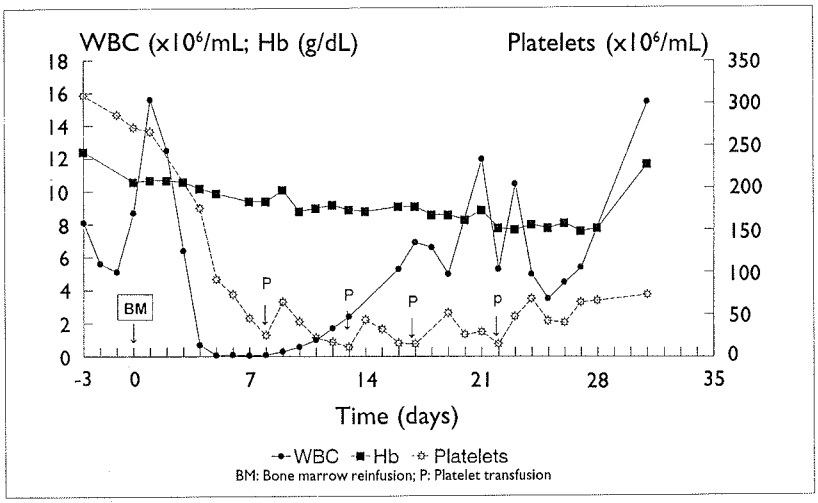

Fig 1 - Post-BMT progress of patient

leukemic blasts. The blasts were uniformly small in size with heavy cytoplasmic granulation, abundant Auer rods and "faggots". The blasts were strongly positive for myeloperoxidase (MPO) and Sudan black stains, but negative for periodic acid Schiff's (PAS), acid phosphatase (AcPh) and α-naphtol butyrate esterase (NSE). The leukemic blasts were positive for CD13 and CD33, but negative for surface markers of T-, B-lymphocytes and monocytes by immunophenotyping. Prothrombin time (PT) and partial thrombin time (PTT) were normal. Liver and renal functions were both normal. Cytogenetic study showed a karyotype of 46, XY, t(15;17). The diagnosis of acute promyelocytic leukemia (APL or M3) was made.

Pre-BMT treatment and clinical progress

The patient was started on chemotherapy including all-trans-retinoic acid as described elsewhere[17]. She developed heart failure from anthracycline-related cardiomyopathy after the fourth course of chemotherapy with a cumulative daunorubucin dose of 250 mg/m^2 and the treatment had to be discontinued. She was treated with digoxin with resolution of heart failure. Further treatment for AML was stopped as complete haematological remission was documented by morphological examination. In March 1993, 12 months from the initial diagnosis, the patient became pancytopenic and bone marrow relapse was confirmed. She was re-induced with the same chemotherapy protocol, as the initial treatment with the addition of oral all-trans retinoic acid. Second complete remission was achieved 3 months later. Bone marrow was harvested after two courses of consolidation therapy. Two further courses of maintenance therapy were given according to the protocol described previously[17]. The patient was conditioned with high-dose melphalan of 180 mg/kg 72 hours prior to bone marrow reinfusion.

Bone marrow processing for autologous BMT

Bone marrow was harvested under general anaesthesia after the confirmation of second CR by haematologic and cytogenetic examinations. A total of 15 mL/kg bone marrow was harvested which yielded 6.2 x 10^8/kg nucleated cells. The bone marrow was processed by discontinuous density gradient using 50% and 60% Percoll (Pharmacia) according to the method described previously[17]. A total of 9.0 x 10^7/kg mononuclear cells (MNCs) were recovered from the interface between 50% and 60% Percoll solution. The BM mononuclear cells were washed twice with Hank's balanced salt solution (HPSS) and resuspended in freezing medium containing 90% heparinised autologous plasma and 10% dimethyl sulfoxide (DMSO). The bone marrow cells were then frozen by a controlled freezer and stored in liquid nitrogen (LN2) at -196°C. The stored bone marrow MNCs were quickly thawed at 42°C and reinfused into the patient intravenously. Bone marrow haematopoietic stem cells were analysed by monoclonal antibody against CD34 and flow cytometry (FACScan). Colony-forming-units were assessed by semi-solid cultures as described previously. Certain important parameters are summarised in Table I.

Post-BMT management and progress

The patient received G-CSF 5 μg/kg/day (Neupogen, Amgen) for the first three days after bone marrow reinfusion. She recovered satisfactorily with minimal supportive therapy and was discharged four-and-a-half weeks after bone marrow reinfusion. Post-BMT progress is summarised in Fig 1. She remained in complete remission 18 months after the procedure without any further treatment and is leading a normal life.

DISCUSSION

The previous experience in survival of paediatric patients with AML in Singapore was poor[18]. Early intensive myelosuppressive chemotherapy has been demonstrated to be necessary to achieve higher rates of CR[19,20]. The recent experience in incorporation of early intensive chemotherapy with autologous bone marrow transplantation and high-dose melphalan conditioning regimen has opened a new frontier in management of AML in children. It is believed that melphalan is an effective conditioning agent for both allogeneic and autologous bone marrow transplantation[21,22]. Melphalan may be more effective

than standard cyclophosphamide and busulphan for pre-BMT conditioning in eliminating residual leukemia in children[17]. The major concern in autologous BMT is the reinfusion of leukemic cells in the harvested bone marrow. This has led to different modalities for *in vitro* purging of leukemia cells[18-25]. However, the role of *in vitro* purging so far remains unclear despite many single institutional studies. There was no *in vitro* purging involved in this report, neither was there, in the original study[17]. The low post-BMT relapse rate of 13.0% at 5 years was comparable to that seen in allogeneic BMT for AML in first remission[26]. The intensification of chemotherapy was crucial to achieve CR in AML. Melphalan conditioning and Percoll separation may contribute to leukemic cytoreduction as a whole. It has been speculated that cryopreservation itself may have a purging effect[27]. Percoll is a more effective density gradient separation reagent compared to the commonly used Ficoll-hypaque[28,29]. An almost 20-fold enrichment in CD34+ cells was seen in the thawed bone marrow compared to the fresh bone marrow in our laboratory. The post-transplantation recovery was satisfactory with the administration of G-SCF in this patient. Only four units of platelets were required for supportive therapy. No red cell transfusion was required. There was no life-threatening complications experienced in this patient. The experience in this patient as well as in the original study, suggests that this procedure can also be used as an alternative for BMT in children with AML in second remission.

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