Autologous bone marrow/stem cell transplantation: Initial experience at a north Indian referral centre

S. GUPTA, L. KUMAR, G. M. K. RAJU, V. KOCHUPILLAI, D. K. SHUKLA

ABSTRACT

Background. High-dose chemotherapy and/or radiation therapy rescued by autologous bone marrow or peripheral blood stem cells is being increasingly used for the treatment of haematological and solid malignancies. While few centres in India use this modality of therapy, the worldwide experience is encouraging. We, therefore, analysed the results of our initial experience with this therapeutic modality.

Methods. Forty-two patients [multiple myeloma (17), Hodgkin’s disease (4), non-Hodgkin’s lymphoma (3), chronic myeloid leukaemia (2), acute myeloid leukaemia (2), acute lymphoblastic leukaemia (2), epithelial ovarian cancer (6), breast cancer (4), primitive neuroectodermal tumour and testicular germ cell tumour (1 each)] underwent high-dose chemotherapy followed by either autologous bone marrow transplant (n = 9), peripheral blood stem cell transplant (n = 30) or both (n = 3). The indications for transplant included either advanced stage at diagnosis, other adverse prognostic indicators during the course of their disease, or relapse. The data were analysed retrospectively in December 1998 using hospital records. Follow up data of all the patients were available.

Results. Thirty-four of the 42 patients (81%) showed stable engraftment. Eight patients (19%) died in the early post-transplant period (day 5 to day 52 post-transplant). Seven patients died due to neutropenic infections and one due to acute renal failure. Of the 34 surviving patients, 20 were alive at the time of analysis. Eighteen patients experienced grade 2-4 acute toxicity. Disease remission was achieved in 27 patients, while 20 patients were alive. Of the 20, 17 were alive without evidence of disease and three were in remission. The median overall survival for all patients was 17 months and for the 34 engrafted.
patients it was 27 months. An analysis of factors affecting survival revealed that patients with chemosensitive disease had a longer overall survival (20.9 v. 6.1 months, p=0.04) compared to those with chemoresistant disease.

**Conclusion.** Autologous bone marrow or peripheral stem cell transplantation is a feasible procedure in India with an acceptable morbidity and mortality. It should be offered more frequently to properly selected patients.

**INTRODUCTION**

Progress in cancer treatment has led to an improved outcome in many haematological and solid malignancies. Nevertheless, even with combined modality treatments patients with advanced cancers have poor results. To improve these results, higher doses of chemotherapy have been administered, with or without haematopoietic stem cell rescue. For many chemotherapeutic agents, the dose-response curves are available in vitro and in animal models. The steepness of these curves is related to the sensitivity of a tumour to a given drug, with a distinct plateau above which a higher dose of the drug does not kill more tumour cells. In humans, it is often not known whether this plateau has been reached when the standard maximum tolerated doses are administered. Myelosuppression is one of the dose-limiting side-effects of escalating doses of chemotherapy. This limitation has been overcome with the availability of recombinant haematopoietic cytokines, granulocyte colony stimulating factor (G-CSF) and granulocyte–macrophage colony stimulating factor (GM-CSF), and the development of techniques to support haematopoiesis with autologous peripheral blood stem cells (PBSC) and/or bone marrow (BM).

**PATIENTS AND METHODS**

Forty-two patients underwent autologous bone marrow transplantation between April 1990 and October 1998 at the Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi. The data were collected retrospectively from hospital records and follow up information was available on all patients.

**Patients**

We had a heterogeneous group of patients with both haematological and solid cancers. The largest group of 17 patients had multiple myeloma (MM). The others included 6 with epithelial ovarian cancer, 4 each with Hodgkin's disease (HD) and breast cancer, 3 with non-Hodgkin's lymphoma (NHL), 2 each with chronic myeloid leukaemia (CML), acute myeloid leukaemia (AML), and acute lymphoblastic leukaemia (ALL), and 1 each with primitive neuroectodermal and testicular germ cell tumour. The indications for autologous transplant in these patients included:

1. Relapse of disease after initial complete remission (n=4),
2. Less than optimal response to frontline therapy (n=15),
3. Advanced stage at diagnosis, including patients with metastatic disease [n=19; MM (16) and breast cancer (3)], and
4. Lack of allogeneic donors for patients with AML and CML (n=3).

**Procedure**

Informed consent was obtained from all patients after a detailed explanation of the risks and benefits involved. All patients were admitted to the hospital for treatment. The source of haematopoietic progenitor cells was:

1. Autologous bone marrow in 9 patients (BMT),
2. Mobilized autologous peripheral blood stem cells in 30 patients (PBSC), and
3. Both in 3 patients.

Autologous bone marrow harvest was carried out under general anaesthesia using the standard technique of multiple iliac crest punctures. The volume of marrow aspirated varied, depending on the nucleated cell count of the harvest.

For PBSC, stem cells were mobilized into the peripheral blood using recombinant haematopoietic growth factors G-CSF (22 patients) and GM-CSF (11 patients) given in a dose of 600 μg/day in two divided doses subcutaneously. These growth factors were given for a median of 6 days (range 4–9 days). At the end of this period, stem cells were harvested from the peripheral blood using an apheresis machine (Hemotronics MCS-3P). Stem cells were collected in disposable bags. The harvest (bone marrow or peripheral blood) was either stored unprocessed in a refrigerator at 4 °C for up to 24–48 hours or cryopreserved at −70 °C following the addition of cryoprotective solution containing 7.5% DMSO and 4% albumin. Cryopreserved grafts were thawed at the bedside in a waterbath at 37 °C at the time of re-infusion. The use of harvest cryopreserved at −70 °C and those stored at −4 °C was dictated by the half-lives of drugs used and the time taken to administer the high-dose chemotherapy regimen.

**Conditioning protocols**

All patients received high-dose chemotherapy for conditioning and no patient was given total body irradiation. The following regimens were used:

1. High-dose melphalan alone (n=28) at a dose of 120–187 mg/m² of body surface area. The mean dose was 158 mg/m² [MM (17), epithelial ovarian cancer (5), NHL (2), HD (1), AML (2) and ALL (1)].
2. Cyclophosphamide (dose 5–6 g/m², mean 5.6 g/m²) and high-dose carboplatin (dose 1.1–1.8 g/m², mean 1.3 g/m²) were used in 6 patients [breast cancer (4), epithelial ovarian cancer (1), primitive neuroectodermal tumour (1)].
3. Cyclophosphamide (120 mg/kg body weight) and busulfan (16 mg/kg) were used in 3 patients (CML 2, ALL 1).
4. Cyclophosphamide (6 g/m²), BCNU (300 mg/m²) and etoposide (600 mg/m²) were used in 2 patients (NHL 1, HD 1).
5. Melphalan (140 mg/m²) and etoposide (800 mg/m²) were used in 1 HD patient.
6. Cyclophosphamide (4 g/m²), etoposide (400 mg/m²) and cytarabine (800 mg/m²) were used in 1 HD patient.
7. Cyclophosphamide (6 g/m²), etoposide (800 mg/m²), carboplatin (1.2 g/m²) were used in 1 testicular germ cell tumour patient.

Aggressive hydration, antiemesis and an alkaline urine were maintained during conditioning and patients were frequently monitored for complications.

**Supportive care**

A single lumen indwelling central venous catheter was placed in all patients and they were cared for in isolation rooms with barrier nursing. Meticulous hand washing was practised routinely by all staff. Antibiotic prophylaxis included oral ciprofloxacin and oral fluconazole or itraconazole. Blood components were given as and when indicated (packed red blood cells to maintain a haemoglobin level above 6 g/dl and platelets to maintain counts above 20 000 per cmm). At the time of initiation of this study, blood compo-
nents were not routinely irradiated but this has been practised since early 1998. Complete blood counts were done daily and serum chemistry at least thrice weekly, post-transplant.

**Engraftment**

Engraftment was defined as the achievement of an absolute neutrophil count (ANC) >500/cmm for 3 consecutive days and unsupported platelet count >20,000/cmm for at least 7 days.

**Statistical analysis**

Standard definitions of complete remission (CR) and partial remission (PR) were used according to the diseases in question. All survival times were recorded to the nearest month in December 1998 and data censored at that point. The probability of overall (OS), disease-free survival (DFS) and event-free survival (EFS) was estimated by the Kaplan–Meier method. The log rank test was used to compare survival curves of the 2 groups of patients. All survival times were calculated from the date of transplant. Analysis was carried out using the BMDP statistical software.

Events were defined as (i) death due to any cause for OS, (ii) relapse in patients with CR for DFS, and (iii) any progression from CR or PR, or death, whichever was earliest, for EFS. Due to the small numbers in other disease categories, EFS was estimated in MM patients only (n=17).

**RESULTS**

The median age of the patients was 42 years (range: 12–62 years). There were 22 men and 20 women. All patients had been previously treated with chemotherapy; 17 (42%) had also received radiotherapy. At the time of transplantation, 34 patients (81%) were either in CR (n=6) or PR (n=28) and 7 patients (17%) had stable/progressive disease. Thirty-one patients (74%) had chemosensitive and 10 (24%) chemoresistant disease. In 1 patient with MM the pre-transplant chemosensitivity and remission status were not known. The median age of the 17 patients with MM was 50 years (range: 33–62 years); 10 were men and 7 women. Fifteen patients had advanced disease [Salmon–Durie stage III A (10) and IIIB (5)] at the time of diagnosis. One patient each was in stage IA and IIA. Pre-transplant, 1 patient was in CR, 12 in PR, 3 had stable/progressive disease while 13 were chemosensitive and 3 chemoresistant.

**Cell dose**

The median nucleated cell count per kg recipient body weight (n=41) was 4.6x10⁸/kg (mean: 4.69x10⁸/kg, range: 1.6–11x10⁸/kg). The median mononuclear cell count (n=18) was 2.84x10⁸/kg (mean: 3.04x10⁸/kg, range: 0.8–6.5x10⁸/kg).

**Engraftment**

Stable engraftment occurred in 34 (81%) patients while 8 (19%) died before engraftment. Table I shows the haematopoietic engraftment, blood component requirement and growth factors used post-transplant. All patients except 1 needed some blood component support (RBC and/or platelet transfusions). Thirty-seven (88%) patients received growth factors post-transplant [G-CSF (11), GM-CSF (20) and both (6)] for a median of 17 days. Factors affecting the rate of haematopoietic recovery were analysed (Table II). G-CSF use was associated with a significantly faster neutrophil and platelet recovery compared to GM-CSF (pooled ‘t’ test). As discussed later, this finding needs to be interpreted with caution. Patients who underwent PBSCT or PBSCI and BMT had faster neutrophil and platelet recovery compared to patients who underwent BMT only, but was not statistically significant. Nucleated cell dose (<3x10⁸/kg v. >3x10⁸/kg) also had no significant impact on haematopoietic recovery.

**Transplant-related toxicity**

Eight patients (19%) died due to transplant-related complications. Six died due to sepsicaemia in the post-transplant neutropenic phase, 1 due to mucormycosis of the lung after secondary graft failure with pancytopenia, and 1 following irreversible acute renal failure due to the conditioning regimen. The median duration to death was 13 days (5–52 days). Thirty-nine (93%) patients had one or more febrile episodes unrelated to antibiotics or blood component transfusions.

The early post-transplant febrile episodes and antibiotic use are shown in Table III. Twelve patients developed sepsicaemia in the post-transplant period out of whom 6 died. The commonest site of infection was the lung (20 patients), followed by blood (15), urinary tract (4), subcutaneous (3) and perianal (2). Both Gram-positive and Gram-negative organisms were isolated from these sites but in only 20 patients. In the rest, no organism could be cultured. Two patients had documented fungal infection while Amphotericin B was used empirically in 29 patients.

### Table I. Haematopoietic engraftment and supportive treatment

<table>
<thead>
<tr>
<th>Item</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to absolute neutrophil count &gt;500/cmm</td>
<td>15</td>
<td>8–36</td>
</tr>
<tr>
<td>Days to absolute neutrophil count &gt;1000/cmm</td>
<td>19.5</td>
<td>10–70</td>
</tr>
<tr>
<td>Days to platelet count &gt;20,000/cmm (unsupported)</td>
<td>19</td>
<td>0–47</td>
</tr>
<tr>
<td>Red blood cell transfusions per patient</td>
<td>2</td>
<td>0–18</td>
</tr>
<tr>
<td>Platelet transfusions per patient (occasions)</td>
<td>3</td>
<td>1–13</td>
</tr>
<tr>
<td>Use of growth factors post-transplant (days)</td>
<td>17</td>
<td>3–44</td>
</tr>
<tr>
<td>Post-transplant hospital stay (days)</td>
<td>23</td>
<td>5–52</td>
</tr>
</tbody>
</table>

* in 3 patients platelet counts were never <20,000/cmm

### Table II. Factors affecting engraftment

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mean days to Absolute neutrophil count &gt;500/cmm</th>
<th>Platelet count &gt;20,000/cmm</th>
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<tbody>
<tr>
<td>Stem cell source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow (n=9)</td>
<td>21.1</td>
<td>22.1</td>
</tr>
<tr>
<td>PBSC, PBSCI and BM (n=33)</td>
<td>16.3</td>
<td>20.0</td>
</tr>
<tr>
<td>p value</td>
<td>0.09</td>
<td>0.70</td>
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<tr>
<td>Post-transplant growth factors</td>
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<td></td>
</tr>
<tr>
<td>GM-CSF (n=20)</td>
<td>17.9</td>
<td>22.8</td>
</tr>
<tr>
<td>G-CSF (n=11)</td>
<td>12.3</td>
<td>12.4</td>
</tr>
<tr>
<td>p value</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Nucleated cell dose/kg body weight</td>
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<td></td>
</tr>
<tr>
<td>&lt;3x10⁸/kg (n=13)</td>
<td>18.4</td>
<td>19.9</td>
</tr>
<tr>
<td>&gt;3x10⁸/kg (n=29)</td>
<td>16.8</td>
<td>20.7</td>
</tr>
<tr>
<td>p value</td>
<td>0.54</td>
<td>0.87</td>
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### Table III. Post-transplant febrile days and antibiotic use

<table>
<thead>
<tr>
<th>Item</th>
<th>Median</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>No. of parenteral antibiotics/patient</td>
<td>5</td>
<td>2–10</td>
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<tr>
<td>Days of parenteral antibiotics/patient</td>
<td>17</td>
<td>1–44</td>
</tr>
<tr>
<td>Febrile days/patient</td>
<td>11</td>
<td>2–30</td>
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TABLE IV. Pre- and post-transplant remission status

<table>
<thead>
<tr>
<th>Pre-transplant status</th>
<th>CR</th>
<th>PR</th>
<th>PD</th>
<th>Death*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete remission</td>
<td>3</td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Partial remission</td>
<td>12</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Stable/progression</td>
<td>1</td>
<td>4</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17 (40)</td>
<td>12 (29)</td>
<td>5 (12)</td>
<td>8 (19)</td>
</tr>
</tbody>
</table>

CR complete remission  PR partial remission  PD progressive disease  Death*  
* transplant-related

Oral mucositis was the most common non-infectious complication and occurred in 38 (90%) patients. Grade IV mucositis occurred in 16 (57%) of 28 patients who received only melphalan and in none of the 14 patients who received other regimens (p=0.001). Although bleeding occurred in 16 patients (38%), it was minor in all but one. Diarrhoea, probably due to intestinal mucositis, was also common (74%). Organ system toxicity due to the conditioning regimen (acute renal failure following high-dose cyclophosphamide and carboplatin) was the primary cause of death in only 1 patient of breast cancer. Post-transplant cardiac and renal toxicity occurred in 5 patients each, 3 patients had hepatic toxicity, while 2 patients each had pulmonary toxicity, seizures and paralytic ileus.

Response
The remission status was assessed 6 to 12 weeks post-transplant (Table IV). Post-transplant, 8 patients with MM were in CR compared to only 1 patient pre-transplant, 5 patients were in PR and 1 patient had stable/progressive disease. Three patients with MM died post-transplant.

Survival
In December 1998, after a median follow up of 15 months in the surviving patients, 20 (48%) patients were alive and 22 (52%) had died. Eight died due to procedure-related complications, 13 due to a progressive primary malignancy and 1 from an unrelated cause.

For all patients (n=42), the median OS was 17 months (Fig. 1). After excluding patients who had died due to a transplant-related cause (n=8), the median OS was 27.3 months. The median OS for patients in CR or PR after transplant (n=29) was 41.6 months. The median OS and EFS had not been attained in the MM patients at the time of analysis (Fig. 1)

Among the factors affecting survival, patients with chemosensitive disease had significantly longer OS compared to chemoresistant patients (median OS 20.9 v. 6.1 months, p=0.04; Fig. 2). Patients in PR or CR before transplant (n=34) had a longer survival compared to patients with stable/progressive disease (n=7). However, this difference was not statistically significant (median OS 18.0 v. 5.4 months, p=0.27).

DISCUSSION
The validity of these comparisons may be compromised by the diversity of our patient population, with many other variables potentially affecting the outcome. This is a preliminary observation regarding the feasibility of autologous transplants in the Indian context. No inference can be drawn from it regarding the advisability of undertaking this procedure in any patient subgroup. Only large, well designed randomized studies can answer that question.

Our patients were heterogeneous with respect to diagnosis, stage, grade and other prognostic variables, making comparison with other studies difficult. All non-MM patients had some poor prognostic feature(s) which predicted poor outcome with standard therapy, and except 1 MM patient all had advanced disease at diagnosis. It is in these advanced and poor prognostic malignancies that autologous transplants may have their greatest application. To date, few completed randomized trials of autologous transplants in both haematological and solid malignancies are available. Barring a few exceptions, the utility of autologous transplants has not been proven in these studies. Most workers have studied autologous transplants in two settings: (i) as consolidation therapy integrated into the frontline treatment, for patients with poor prognostic features at initial presentation; and (ii) as salvage therapy for relapsed malignancies.

Improvement in OS is considered to be the most valid endpoint for trials evaluating new cancer therapies. Improved OS with autologous transplants has been shown in some randomized trials in patients with poor prognostic NHL, relapsed NHL, metastatic breast cancer, and multiple myeloma. Other randomized studies in poor prognostic NHL, poor prognostic...
breast cancer with extensive axillary lymphadenopathy,21 metastatic breast cancer,22 testicular cancer,23 and small cell lung cancer24 have failed to demonstrate any benefit. One randomized study in recurrent Hodgkin’s disease25 showed a significantly better 3-year EFS but closed before an improved OS could be demonstrated. The results in acute leukemias have also been inconclusive or negative.26,27 The use of autologous transplants outside randomized controlled studies has increased substantially in the last decade28 despite lack of confirmation of its efficacy in many situations. This is unfortunate as it has been argued that the apparently improved outcome with autologous transplants in uncontrolled studies could reflect a selection bias.29-31

As has been the trend worldwide,32 the majority of our patients underwent blood stem cell transplants. Their neutrophil and platelet counts recovered earlier than in BMT patients. However, statistical significance was not attained probably because of the small numbers. Apart from avoiding general anaesthesia, PBSCCT has certain advantages over BMT: faster haematopoietic reconstitution33 and immune recovery,34 lower requirement of antibiotics,35 fewer infectious complications,36 fewer days of post-transplant hospital stay and ability to harvest stem cells even in patients with previous pelvic irradiation. However, the long term outcome is no different.37

We mobilized and harvested blood stem cells after administration of growth factors (G-CSF or GM-CSF) as has been done by others.38,39 Stem cells can also be harvested during the recovery phase after myelosuppressive chemotherapy when stem cell concentration is known to increase in the peripheral blood.40 We also used growth factors post-progenitor cell infusion in the majority of our patients to hasten haematopoietic recovery.41-43 In our experience, patients who were given G-CSF post-transplant, had significantly faster neutrophil and platelet recovery compared to the GM-CSF group.

We found a significantly longer OS in those with chemosen- sitive as compared to chemoresistant disease (21 v. 6 months, p=0.04). It was also longer in patients in PR or CR at the time of transplant, compared to those with stable/progressive disease (median OS 18 months v. 5.4 months, p=ns). These results are similar to the findings reported by others.44-47

Eight procedure-related deaths (19%) occurred in our patients which is slightly higher than the <15% mortality reported in most recent series.37,48 This could be partly explained by the patient selection: 3 patients had poor performance status at the time of transplant (ECOG PS IV) while 3 others had co-morbid diseases. Only 2 deaths (one each due to acute renal failure and pulmonary mucormycosis) occurred in otherwise healthy patients in good general condition with no co-morbid illness. Oral mucositis was the most common non-haematological toxicity and was significantly associated with melphalan-based regimens.

In conclusion, high-dose chemotherapy along with autologous transplantation is a feasible procedure in patients with both haematological and solid malignancies. PBSCCT offers some advantages over BMT. However, we need to define the indications in our socio-economic milieu through well designed randomized trials. Good patient selection and supportive care are vital for obtaining optimal results.

ACKNOWLEDGEMENTS

We sincerely thank all the residents, nursing and technical staff for their excellent care of transplant patients.

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Mycoplasma pneumoniae and community-acquired pneumonia

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ABSTRACT

Background. Community-acquired pneumonia is an important cause of mortality and hospitalization in all age groups. In temperate climates, Mycoplasma pneumoniae is a common respiratory pathogen causing pneumonia. Information on human Mycoplasma infection in India is scarce.

Methods. We aimed to determine the frequency of Mycoplasma pneumoniae infection among patients with community-acquired pneumonia in a prospective cross-sectional study. The assessment included clinical and radiological evaluation followed by microbiological evaluation for the specific pathogen. Microbiological investigations included aerobic and anaerobic blood culture, anti-Mycoplasma IgM antibody detection by gelatin particle agglutination test and ELISA, culture of respiratory tract secretions for Mycoplasma pneumoniae and other organisms, and detection of specific Mycoplasma pneumoniae antigen by indirect immunofluorescence.

Results. Sixty-two patients (42 men and 20 women; mean age 41.7 years) with community-acquired pneumonia were investigated prospectively. They included 42 immunocompetent and 20 immunocompromised patients. Six patients had definitive

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