original research report

A simplified method for autologous stem cell transplantation in multiple myeloma

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BACKGROUND AND OBJECTIVES: We evaluated the efficacy and safety of non-cryopreserved storage of autologous hematopoietic stem cells with no post-transplant granulocyte colony-stimulating factor (G-CSF) support in adult patients undergoing autologous stem cell transplantation (ASCT) for multiple myeloma (MM).
DESIGN AND SETTING: Retrospective review of patients undergoing ASCT from May 2009 to July 2011.
PATIENTS AND METHODS: Autologous stem cell were mobilized using G-CSF. Leukapheresis to harvest stem cells was performed on day -2 and -1. The grafts were kept in a conventional blood bank refrigerator at 4°C until reinfusion on day 0. The conditioning regimen consisted of melphalan 200 mg/m^2 in all patients. The post-chemotherapy myeloablative phase was managed without growth factors.
RESULTS: Between May 2009 to July 2011, 54 adults with MM were treated in our center in Oran. The median age at ASCT was 55 years (range, 35-65). There were 37 males and 17 females. The median harvested CD34+ cell count was 3.60×10^6/kg (range, 1.90 to 10.52). All patients had neutrophil engraftment on the median of day 10 (range, 6-17) and platelet transfusion independence on the median of day 13 (range 9-24). In the 47 evaluable patients the median post-transplant overall survival had not been reached; the estimated overall survival at 30 months was 93.8% (0.05%) , and the estimated disease-free survival at 27 months was 93.6% (0.05%).
CONCLUSION: High-dose chemotherapy and ASCT using non-cryopreserved stem cells and no G-CSF support is safe and feasible in the treatment of MM under our work conditions in developing countries.

In multiple myeloma (MM), the strategy of combining therapeutic intensification with autologous stem cell transplantation (ASCT) may improve outcomes in terms of response rate and survival.[1] Currently, the majority of autologous transplants are performed using peripheral stem cells.[2] After the collection of autologous stem cells the conventional practice is to freeze each collection product through cryopreservation. This procedure requires expensive equipment and trained personal. To avoid the high costs of establishing and maintaining a cryopreservation facility, efforts have been made to perform autografts without the use of cryopreservation devices.. Although non-cryopreserved storage of hematopoietic stem cells is not a standard practice, several reports have shown that autografts can be performed safely without cryopreservation devices, keeping the stem cells in a conventional blood bank refrigerator, at 4°C, for up to 4 days.[3-10]

In Europe and in the United States, the standard of care is administration of growth factors to accelerate neutrophil recovery after ASCT. The need for growth factors after transplant has been investigated recently.[11] Most of these studies are from the advanced countries, and data from developing countries such North Africa are scanty. We report our experience in patients with MM treated with a simplified autografting procedure in a single institution (Department of Hematology and cell therapy, Etablissement Hospitalier Universitaire [EHU], 1st November, Oran, Algeria) using non-cryopreserved stem cells.

PATIENTS AND METHODS

From May 2009 to July 2011, 78 ASCT were carried out in our center, of which 54 were for patients younger than the age 65 years, presenting with MM. Diagnosis of MM was based on the following findings:[12-14] (a)

increased numbers of abnormal, atypical or immature plasma cells in the bone marrow or histological proof of plasmacytoma; (b) presence of an M-protein in the serum or urine or (c) bone lesions consistent with those of MM. ASCT was indicated in all patients younger than the age of 65 years suffering from stage II or III symptomatic MM, with no major comorbidities. Normal renal and liver function tests were required for inclusion. Written informed consent was obtained before any procedure. All patients had been treated before the autograft as follows: 17 patients with vincristine, doxorubicin and dexamethasone (VAD), 14 with bortezomib-dexamethasone (VD), 11 with VAD/VD, and 12 with bortezomib, thalidomide, dexamethasone (VTD). Patients were admitted to a single room and reverse barrier nursing was practiced.

The source of stem cells in all patients consisted of mobilized autologous PBSCs. For mobilization of PBSCs, patients received granulocyte colony-stimulating growth factor (G-CSF) at a dose of 15 mcg/kg subcutaneously once daily for 5 days. Leukapheresis was performed on days -2 and -1. A volume of 8-10 L of blood was processed in each sitting, using the aphaeresis machine OPTIA (Cobe, Spectra). A median of one leukapheresis was performed (range, 1-2). Leukapheresis was done from the femoral vein in all patients using a dialysis catheter. A sample of the stem cell harvest was obtained and total cell counts were determined using an automated cell counter; the differential cell count was done manually. For CD34 counts, cells were labeled with fluorescein-conjugated anti-CD34 and analyzed using a FACS scan flowcytometer to yield an absolute CD34+ count. The viability of cells was determined by a trypan blue dye exclusion test.

The products of the aphaeresis and 1-mL aliquots were kept in ACD-A (Baxter Healthcare, Deerfield, IL) and stored in a conventional blood bank refrigerator at 4°C, in 300-mL transfer packs (Baxter Healthcare) composed of gas impermeable, polyvinyl chloride plastic film. Stem cells were re-infused intravenously to the patient on day 0, 24 hours after melphalan chemotherapy. To do this, stem cells were removed from the conventional blood bank refrigerator and thawed at room temperature.

The myeloablative regimen consisted of melphalan 200 mg/m^2 (Glaxo-SmithKline, Uxbridge, Middlesex, UK) in a single intravenous dose, on day -1 in all patients, followed by ondansetron (8 mg intravenously every 12 h) and forced alkaline diuresis. Autologous PBSCs were re-infused on day 0 through a central venous catheter preceded by the administration of pheniramine maleate 50 mg intravenously. Post-transplant no patients received growth factor (G-CSF). All patients received prophylactic ciprofloxacin (250 mg twice daily), acyclovir (500 mg/day) and fluconazole (200 mg twice daily). Antibiotics were used until granulocytes were greater than 0.5×10^9/L. All patients had daily laboratory workup and clinical evaluation. Blood products transfused during the post-transplant period were irradiated with 25 Gy.

Cases of non-hematological dysfunction were considered regimen-related toxicities unless they could be clearly explained by another cause. A grading scale[15] was used for toxic complications of transplant: grade 0 represented no toxicity; grade 1 toxicity was fully reversible without specific intervention; grade 2 toxicity was not life threatening, but required specific measures to be reversed; grade 3 was life threatening but reversible; and grade 4 toxicity was fatal.

Engraftment was calculated from day 0 of the SCT. Neutrophil engraftment required a sustained absolute neutrophil count of 500/μL for 3 consecutive days. Engraftment of platelets required a platelet count of 20,000/μL (un-transfused), with no platelet transfusions within the preceding 48 hours. Evaluation of response to transplantation was assessed on day-100 post-transplant. The criteria for response were the following: Complete response (CR) was defined as the absence of paraprotein on serum electrophoresis and 5% or fewer plasma cells in the marrow. Very good partial response (VGPR) was defined as a decrease of 90% in the serum paraprotein level, partial response (PR) as a decrease of 50% in the serum paraprotein level and minimal response as a decrease of 25% in the serum paraprotein level.[16]

Overall survival (OS) was defined as the time from date of transplant until death or date of censor. Disease-free survival (DFS) was evaluated in all patients who have achieved a complete response (CR) or VGPR after transplantation. It was calculated from the day of first documentation of CR or VGPR to the day of relapse or death. The analyses were performed using SPSS software version 15.0 (IBM Corp, Armonk, NY USA). The Kaplan-Meier method was used to assess survival. The median follow-up was 10 months (range, 3-30 months). The data were censored on 31 July 2011.

RESULTS

From May 2009 to July 2011, 78 ASCT were performed in our center in Oran including 54 patients with MM, 37 men and 17 women, with a median age of 55 years (range, 35-65). **Table 1** shows some of the clinical

characteristics of these patients. The type of paraprotein was IgG in 29 cases, IgA in 16 cases and light chain disease in 9 cases. According to the International Staging System[17] 43 patients had stage III MM. The status of patients pre-transplant after induction treatment was as follows: 21 patients in CR (39%); 18 VGPR (33%) and 15 PR (28%).

A median of one aphaeresis sessions was needed to collect a minimum of 1×10^6 CD34+ viable cells per kg of the recipient; the range was 1 to 2 sessions to obtain enough CD34+ cells. The median CD34+ cells harvested was 3.60×10^6/kg body weight; (range 1.90 to 10.52×10^6/kg). As mentioned above, grafts were preserved in conventional blood bank refrigerator at +4°C for one day. In all cases, the viability of the CD34+ cells was above 85% before being re-infused to the patients. There were neither febrile episodes nor other side effects of the PBSC re-infusion in any case.

The median time to neutrophil engraftment) was 10 days (range, 6-17 days) and median time for platelet transfusion independence was 13 days (range, 9-24 days). After transplant, patients received a median of 2 units of red blood cells (range, 0-9) and 1 unit of single donor platelet transfusion (range, 0-3) (**Table 2**). Post-transplant no patients received growth factors (G-CSF).

Of the 54 autografted patients, 47 were assessable as being beyond the 100th post-transplant day. Of the latter, 42 achieved a CR (89%), 1 VGPR (2%) and 4 a PR (9%). The number of CR+ VGPR increased from 72% before to 91% after ASCT. The 100-day mortality was 0%. In these 47 assessable patients, the median post-transplant overall survival had not been reached; the 30 month-overall survival was 93.8% ±0.05 (**Figure 1**) and the 27 months DFS was 93.6% ±0.05 (**Figure 2**).

Grade II mucositis (n=26), grade II-III nausea/vomiting (n=54) and grade II alopecia (n=54) were the common non-hematologic toxicities. No patient had evidence of engraftment syndrome[18] as evidenced by weight gain, fever, dyspnea, pleural effusion, skin rash and impaired liver and renal functions.

DISCUSSION

In Algeria, the prevalence of MM was 1.1/100 000 and the frequency of patients under the age of 40 years was 25%,[19] due to the youth of population.[20] Among 13 hematology centers in Algeria, only two centers can offer ASCT to MM patients.[21] The EHU 1st November from Oran is the second center. Our ASCT program started in May 2009 and has implemented the practice of keeping the stem cells in a conventional blood bank refrigerator.

Firstly, evaluation of our results obtained in terms

Table 1. Patient characteristics.

	Number	Range
All patients	54	-
Patient's sex		
Male	37	-
Female	17	-
Age (years)	55	35-65
ISS myeloma stage		
II	11	-
III	43	-
Monoclonal paraprotein type		
IgG	29	
IgA	16	
Light chain	9	
Kappa/lambda	29/25	
Aphaeresis sessions performed	1 (median)	1-2
Autologous CD34+ve cells harvested ($\times10^6$/kg)	3.65 (median)	1.9-10.52

Table 2. Results.

	Number	Range
All patients	54	-
Days of hospitalization after transplant	14 (median)	10-25
Granulocyte recovery (days)	10 (median)	06-17
Platelet recovery (days)	13 (median)	09-24
Red blood cells units transfused	2 (median)	00-9
Number of single donor platelets transfused	1 (median)	00-3
Mucositis grade		
Grade 0	7	
Grade 1	22	
Grade 2	19	
Grade 3	6	
Transplant related mortality	0*	

*Applicable to 47 accessible patients

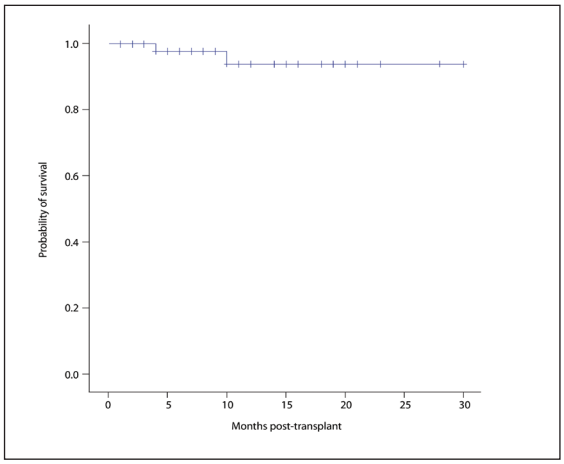

Figure 1. Overall survival.

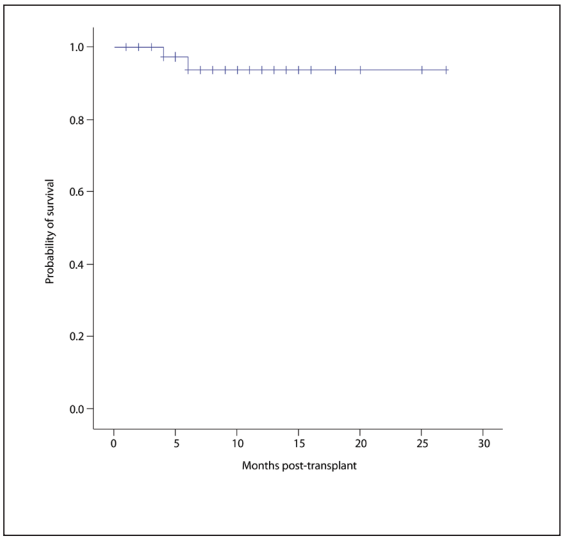

Figure 1. Disease free survival.

of engraftment (duration of hospitalization, neutrophil and platelets recovery), showed the feasibility and safety of ASCT without prior cryopreservation of hematopoietic stem cells (HSC), and thus validates this technique in our center. The advantages of this technique lie in the fact that it "avoids" the use of cryopreservation, an expensive process that requires special equipment as well as liquid nitrogen for freezing to −180°, the consumables, albumin and dimethylsulfoxide (DMSO) (fluid retention CSH) . Moreover, the simple preservation of HSC in a blood bank refrigerator at +4°C until re-infusion into the patient is devoid of any toxic side effects that are described with DMSO.[22] This technique also has the advantage of reducing the time between the last induction chemotherapy and ASCT. The only constraint to this method is the need for a rigorous organization of scheduling patients on this protocol.

Secondly, the standard of care after myeloablative chemotherapy and reinfusion of autologous PBSCs is the initiation of growth factors (typically G-CSF) on day 5, continuing this agent daily until a neutrophil count of 500/μL has been achieved for three consecutive days.[23] However, growth factors may potentially increase the number and severity of some of the fluid-related complications after transplant, such as acute respiratory distress syndrome, allergic reactions, alveolar hemorrhage and, rarely, splenic rupture.[24] Furthermore, growth factors come with substantial costs; as of 26 January 2010 the retail price for a single dose of pegfilgrastim was $3526.88 and that of 10 syringes of 480 mcg filgrastim was $4621.83 (http://drugstore.com, accessed January 2010).

The need for growth factors after ASCT in MM has been investigated recently. Gertz et al showed that a neutrophil count of 500/μL was achieved in a median of 12.5 days in patients receiving growth factor, compared with 13.5 days in those not receiving growth factor ($P<.001$). Platelet engraftment was identical (median, 14.5 days; $P=.12$) in both groups.[11] Of note, the median hospital stay was 3.5 days shorter in the group not receiving growth factor in this study and the authors concluded that it is feasible and reasonable to perform autologous SCT for multiple myeloma without administering growth factors. Our study show similar results of satisfactory engraftment with a median time to neutrophil recovery of 10 days (range 6-17) and median time to platelet transfusion independence of 13 days (range, 9-24) without the use of growth factors.

The results of our study in MM patients are acceptable and clearly superior to those obtained with conventional chemotherapy in the same institution.[25,26] We have been able to reproduce the results obtained by autografting MM patients in more advanced countries and, as a result, to offer this therapeutic option to patients living in Algeria who could not afford the cost of a conventional autografting procedure.

Using this approach, the cost of ASCT in our country is expected to be substantially lower than that reported in Europe.[27,28] This cost saving procedure is critical in developing countries.[29] These data are to be taken into account when considering treatment options in MM patients, mainly in developing countries.[30,31]

This study demonstrates the feasibility of intensified therapy followed by autologous non-cryopreserved HSCs infusion in MM patients. Furthermore, it is feasible not to administer growth factors post ASCT in this setting without adversely affecting outcome.

This method of ASCT in MM is cheaper, and may potentially enable the widespread use of autologous SCT activities in other hematology centers in Algeria and in developing countries.

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