

# Autotransplant in Multiple Myeloma with Oral Melphalan, without Cryopreservation

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#### Abstract

Multiple Myeloma (MM) is the second most frequent hematologic cancer in adults with a marked increase in incidence; therefore, the costs related with the treatment are considerable in all the health systems worldwide. Pharmacological developments have made of Myeloma a chronic, yet incurable, disease. Hematopoietic transplantation is still essential for controlling this disease. The strategies for reducing costs without decreasing the therapeutic efficacy are very important for health systems as they increase the efficiency of the procedure.

**Purpose:** To retrospectively describe evolution of patients with MM treated with hematopoietic transplantation at Hospital de Especialidades of the Mexican Social Security Institute in Puebla, Mexico.

**Material and Methods:** Out of 388 transplants performed without cryopreservation, 22 patients with MM were analyzed, all of them mobilized without chemotherapy, only with filgrastim. Cell harvests were performed at 4°C, within 4-6 days. Conditioning prior to hematopoietic autotransplant (HAT) was oral melphalan at 225 mg/m<sup>2</sup> in 77% of the cases. Three patients received allogenical transplant after the autologous transplant.

**Results:** Twenty-two HAT were performed in that many patients. Three of them later received an allotransplant due to a compatible related HLA donor. Sixty-eight percent (15) were men and 31% (7) women, between 29-57 years old, with a mean age of 44.4 years. An average of 1.5x10<sup>6</sup> CD34+ cells/kg with a range of 0.5-4.0×10<sup>6</sup>/kg and an average viability after refrigeration of 90%. The average number of days in hospital was 29.2. The average number of days for obtaining neutrophilic graft (>500/mm<sup>3</sup>) was 12.3, 18.9 for effective erythropoiesis, and 20.3 days for effective plateletpoiesis. Mortality for autotransplant was 0%. With an 87-month follow-up, 8 patients (36.3%) remain in complete response (CR), 7 (31.8%) in very good partial response (VGPR), and 4% (18.1) in progressive disease (PD). The autologous post-transplant progression rate was 36.3%. Time to progression was 64.3 months in average. Overall survival of the series was 86.4%.

Conclusions: HAT can be performed safely even without cryopreservation of autologous hematopoietic cells.

**Keywords:** Multiple myeloma; Allogenical transplant; Cryopreservation

## Introduction

Multiple Myeloma (MM) is a plasma cell neoplasia derived from a series of genetic alterations and hematopoietic environment summed up throughout the life of the subject. There is no blank therapy capable of reverting clonal reproduction of this type of cancer. There are multiple-drug schemes with different mechanisms of action to achieve a better control of the disease. Hematopoietic autotransplant (HAT) has been established as standard treatment for maintaining the response obtained with initial cytoreductive therapy. However, it can be assumed that the leading role of the procedure will diminish next to new drugs with different mechanisms of action than cytotoxic chemotherapy [1]. New drugs are hard to obtain in underdeveloped countries such as ours due to their high cost; thus, hematopoietic transplantation is still very important to maintain the quality of life of these patients [2].

Cryopreservation is the common method for long-term conservation of hematopoietic stem cells harvested in an autologous manner used for transplant. The most used cryopreservative is dimethyl sulfoxide (DMSO), which prevents cell damage due to freezing, and has variable toxicity in humans. After thawing, 20-30% of the stored cells are expected to lose viability due to early irreversible apoptosis [3].

Few centers around the world perform autotransplant in spite of not having cryopreservation [4]. Different studies have proven the safety of storing stem cells at  $4^{\circ}$ C up to 5 days because the viability of collected cells begin to lower after this day; this period allows the patient to receive high chemotherapy doses and preferably to receive the transplant between the 4th and 5th days [5,6].

Since 1957 there have been interesting pre-clinical studies in mice that had achieved effective hematopoiesis after being subject to lethal doses of total body irradiation, being later instilled with autologous hematopoietic cells stored at 25°C for 11 days [7]. Also, reports from "in vitro" studies have proven that bone marrow in liquid state can be stored 2-9 days under refrigeration without significantly losing stem cells; so, it is feasible to achieve hematopoietic reconstitution after myeloablative doses of chemo-/radiotherapy [8].

The use of non-cryopreserved stem cells has the following advantages: it avoids toxicity produced by DMSO, including nausea, vomit, abdominal pain, diverse degrees of cardiovascular, renal, respiratory, hepatic and hemolytic adverse effects; it reduces procedure-related costs; it decreases the period since last antineoplastic therapy and the transplant by simplifying the procedure; it avoids excessive loss of stem cells due to thawing. It has, on the other hand, some disadvantages: there must be a tight coordination in the work team for the mobilization, collection by apheresis, storage, and infusion of collected units in a short time; besides, there is no possibility of a second transplant once the time limit has elapsed [9,10].

High doses of Melphalan have constituted standard chemotherapy in autologous pretransplant conditioning in patients with MM. The dose range proposed is 140-220 mg/m<sup>2</sup> IV; by this means, plasma and urine levels are undetectable within the hour and after 6 hours, respectively; therefore, hematopoietic stem cells are instilled after 8-24 hours, thus obtaining effective grafts.

The hematopoietic transplantation program in our center began in 1995, without having cryopreservation. Up to date we have performed 388 hematopoietic transplantations with these storing conditions. The main cause of bone marrow transplantation was acute leukemia, followed by lymphomas. In our hospital, MM is the fourth cause of transplant. We included this neoplasia for transplantation until year 2005, mainly due to lack and cost of intravenous melphalan in Mexico. Due to an increase of incidence in patients with indication of transplant due to MM, we've decided to implement strategies to allow its performance.

We are reporting the experience obtained from a single hospital in the province belonging to the Mexican Social Security mainly using high doses of oral Melphalan followed by autologous infusion of noncryopreserved hematopoietic stem cells in patients with multiple myeloma.

## **Material and Methods**

The data and evolution of 22 patients with MM receiving HAT were analyzed between January 2005 and April 2014 in the Hematology Service at Hospital de Specialties of the Mexican Social Security Institute (IMSS) in Puebla, Mexico.

Patients were mobilized with subcutaneous filgrastim at doses of 15-20 mcg/kg/day during 6 days without previous chemotherapy or any other mobilizer. Cell harvests were routinely obtained at the 5th and 6th day by processing 3 blood volumes daily using apheresis machines. These were stored under refrigeration at 4°C for 4-6 days before their infusion. Quantification of CD34+ cells was performed in a third party laboratory for the 5 first patients transplanted before 2008; cellularity quantification was only obtained on the day of the transplant and it is the one reported. For the rest of the patients, quantification was performed in the laboratory at our hospital.

The pretransplantation conditioning scheme was oral melphalan at doses of 225 mg/m<sup>2</sup>/2 days in 17 patients; BEAM (carmustine, etoposide, cytarabine y melphalan) in 3 patients, also with oral melphalan, and BUCY-2 (busulfan, cyclofosfamide) in 2 patients (in these cases busulfan was oral). All the conditionings had a duration of 48-72 hours. Antiemetic therapy used was ondansetron in 19 cases and

Most of the patients were referred to our center due to MM type IgG [11-14], 5 patients type IgA (significantly the youngest, between 29-42 years old), 2 patients with non-secretory MM, and 4 patients with Kappa light chain disease.

Only 8 patients had complete remission, 12 patients "very good partial remission", and 2 patients had partial remission at the moment of the autotransplant.

An average of  $1.5 \times 10^6$  CD34+ cells/kg with a range of  $0.4-4.0 \times 10^6$ /kg were infused. Six out of 22 patients keep autologous units of cryopreserved peripheral stem cells in close-by reference hospitals for future use.

Patients were subject to infection prophylaxis since the day after the transplant with aciclovir, non-liposomal amphotericin B, non-modified immunoglobulin (6 g weekly in 4 doses) and trimethoprim with sulfamethoxazole for preventing pneumocystis carinii infection.

If neutropenic fever appeared, the empiric wide spectrum antibiotic therapy protocol, current in the hospital, was followed, including in an escalated manner cephepime/amikacin, Tazobactam, imipenem or Meropenem, associated to Teicoplanin or Linezolid. Bacterial cultures were taken routinely once a week and whenever fever appeared. All the patients received filgrastim since the day after the transplant to accelerate the graft. Blood products used as support were infused always irradiated and/or with filter for white cells. Patients were kept in rooms with bathroom using reverse isolation techniques. In no case environmental air filtration or laminar flow were available.

## Results

Twenty-two HAT were performed in patients. Three of them later received an allotransplant due to a compatible related HLA donor. Sixty-eight percent [15] were men and 31% (7) women, between 29-57 years old, with a mean of 44.4 years (Figures 1 and 2).



Thirty-one percent (7) of patients were transplanted after two years of the diagnostic due to the time of referral to the transplant unit. The average number of previous antineoplastic schemes was three, in most of them prednisone-associated with melphalan at conventional dose and later on in combination with other drugs, including other alkylating agents for long time. Likewise, 4 cases received radiotherapy to vertebral bodies. In the rest of the 15 cases, the range of time for HAT after diagnostic was 8-12 months. In them, thalidomide and dexamethasone were used for initial cytoreduction of the disease; Bortezomib was also used, only in four cases.



**Figure 2:** Status of disease at the time of the autologous transplant. CR: complete remission; PR: partial response; VGRPR: very good partial response

The average number of days hospitalization was 29.2 (range 20-45). The days passed before achieving effective granulopoiesis were 12.3 (range 9-17), effective erythropoiesis 18.9 (range 15-25), and effective plateletpoiesis 20.3 (range 15-28). Patients living in the city returned to their houses once the neutrophilic graft was achieved, and went to the blood bank for platelet infusion every 5 days until reaching transfusion independence.

Complications during the procedure were as follows: Secondary pneumotorax after placing a Mahurkar catheter in one case; catheter infections in 3 patients; hospital-acquired pneumonia in 2; transient neutropenic colitis in 2; subclavian vein thrombosis in one; perianal abscess in one; acute myocardial infarction with transient hemodynamic instability in one patient that resolved without after effects. Ninety-five percent of the patients suffered neutropenic fever of variable duration. No case had bacterial development in routine cultures. All the patients suffered grade I-II mucositis. There were no deaths associated to HAT procedure. All the patients received nonmodified human immunoglobulin in the period of aplasia provided a suboptimal IgG level to be documented of outpatient follow-up.



All patients received maintenance therapy with thalidomide from day +100 after transplant; also, 12 patients received pegylated interferon 100 mg sc every 2 weeks for two years; two suspended it due to intolerance and one due to progression of the disease.

# Current status of patients

With an average 87-month of follow-up, 8 patients (36.3%) maintain complete response (CR), one of them after allotransplant; 7 (31.8%) in very good partial response (VGPR), and 4% (18.1) in progressive disease (PD) (Figures 3 and 4).

A total of three patients (13.6%) died; two deaths were caused by progressive disease and one by chronic extensive graft vs. host disease six years after the allotransplant. The autologous post-transplant progression rate is 36.3%. With a median time to progression of 64.3 months (46.6-82). Overall survival of the series is 86.4%. With a median follow-up of 41.7 months (1-101).



## Discussion

Multiple Myeloma is still an incurable disease, with an increase in prevalence, data from SEER (Surveillance Epidemiology and End Results) suggest that there are approximately 54,000 patients with MM in the US. Due to its high prevalence, it is the first cause of hematopoietic transplantation worldwide. Recently, in 2007, 20,000 new patients were diagnosed in the United States; therefore 4000 transplants are performed yearly, and 11,000 deaths occur annually due to this disease [16-19].

Melphalan was first used in 1962 as the first effective therapy in MM control achieving a survival time lower than 3 years [20,21]. Combination of chemotherapy was widely studied without improvement in the survival time [22]. High doses of melphalan have proved to be effective against Multiple Myeloma since the decade of 1980. Multiple Myeloma transplant has been consolidated as an effective therapy since then, eliminating excessive myelotoxicity with expedites implant of autologous hematopoietic stem cells [23,24].

During the last decade we observed huge advances in the understanding of cytogenetics, genomics and proteomics of multiple myeloma, which have allowed new classification and prognosis stratification systems as well as the design of new drugs with mechanisms of action addressed to pathogenic mechanisms of the disease, improving the promptness and depth of neoplastic responses.

The transplant keeps benefiting patients by consolidating and extending the response obtained, with the consequential quality of life supposing an extended remission of the disease. The increase of progression-free periods is highly valuable, mainly among young patients [1,10,11]. It is still controversial to achieve a substantial increase of global survival for causes attributable to autotransplant because the relapse of the disease is expected in most of the patients, and the periods free of relapse are explained by molecular and cytogenetic factors of the disease [24,25].

Multiple myeloma is, due to its incidence, the second hematologic neoplasia in the world, [12] the third cause of death by hematological neoplasia in Mexico; it's the fourteenth cause of death due to cancer in the country and the twentieth in incidence of malign neoplasia [13].

The age of presentation of this neoplasia, published in developed countries, is over 69 years with only 15% of patients debuting before 50 years [14]. In contrast, the mean age of presentation reported by Latin American authors is lower in over 10 years [10,15].

Mexico occupies the 3rd place of incidence of multiple myeloma among Latin American countries. The age of presentation reported by the national epidemiology organism in a single year was: under 14 years old: 2 patients, 15-39 years old: 33 patients, 40-44 years old: 53 patients, 45-49 years old: 80 patients, over 50 years old: 888 patients [16]. These data force the national health system to offer antineoplastic therapeutics longer than other countries.

Oncological centers that have hematopoietic transplantation in the country are scarce and concentrate in the capital. The hospitals in the Mexican province lack resources to pay the technological needs required by hematologic ailments.

In the Italian study Comim, analyzed 236 patients with MM treated "during normal clinical practice", the costs generated per strata were compared retrospectively: symptomatic patients not receiving transplant: &21, 707.8  $\pm$  21, 785.3. Receiving transplant without complete remission: &59, 243.7  $\pm$  24, 214.0. Transplant in complete remission &8.130, 7  $\pm$  15, 092.5. In this study, the main factors for total cost of the disease were drugs and hospital admissions (46.1% and 29.4%, respectively) [26]. It is clear that performing the autotransplant in initial stages to obtain complete remission is better than transplanting without complete remission or not transplanting the patients. Therefore, crucial for improving costs is a prompt referral of patients to transplant centers with a short waiting time for the procedure.

The procedure for transplant without cryopreservation may increase the number of low-infrastructure centers performing it and, consequently, the number of patients benefited by the low cost procedure [9]. In another scenario, a first transplant can be used using refrigeration and, the subsequent, cryopreserved cells [17].

In Mexico, there are hospitals using autologous transplant technique without cryopreservation with reproducible results in patients with Multiple Myeloma [18]. Our group has accumulated experience in autotransplants using the cell refrigeration method in several diseases. Lack of cryopreservation and the reduced number of transplanted patients at the same time under the responsibility of a very small medical team allow properly coordinating short periods of refrigeration, storing and identification of the units to be transplanted, condition that may be risky in big hospitals due to the short expiration of the viability of cell units, which is the obstacle for performing this method massively [27-30].

This document includes a small series of cases of patients with diagnostic performed in different areas of the Mexican Republic, pretransplant therapeutics and heterogeneous risk factors, as well as different stages of disease, with time for a wide range transplant. It is notable in our results that time of relapse is notoriously higher than in the series with more patients and longer follow-up; the authors consider two probable causes: the first one refers to the retrospective nature of screening and the second is based in the well described "Postransplant Humoral Reconstitution". Production of antibodies is posterior to the generation of new B lymphocytes derived from transplanted cells. Humoral immunity after myeloablative transplant recovers very slowly after 6 months of the transplant and can commonly extend even more for IgA. Melphalan is an alkylanting agent considered myeloablative that in doses over 200 mg/m<sup>2</sup>; due to erratic absorption we decided to increase the doses of melphalan in 25 mg/m<sup>2</sup>. Likewise, it can be seen that transplanted cell cultures are quite poor regarding CD 34+/kg because the parameter we used was, for safety, quantification of total nucleated cells at the moment of deciding the conditioning, which is translated into the possibility of the graft in our patients is mostly constituted by stem cells with lineage conditioning and that immune reconstitution and generation of new plasma cells took longer than expected in patients mobilized and cryopreserved with the usual methods. It is important to remark that two out of the three patients who recived allogenic transplant had a very long relapse-free survival, which would modify the behavior of survival curve and the time of progression. No doubt, about allogenic transplantation, drastically modified the outcome of any hematologic disease, whether dramatically extending or shortening overall survival. Because the purpose of our report is based in proving feasibility of a procedure, we decided to describe the behavior of all the patients.

We pretend to prove experience of one hospital without suggesting changes in the standards of proved treatment in hospitals with more budget and equipment.

## Conclusions

Autologous transplant of alloperipheral cells in multiple myeloma is benefit most of the patients. By dispensing cryopreservation, the procedure can be performed with lower costs in more hospitals with scarce technological equipment, safely and effectively. In an age in which the cost analyses gain importance in the decision making process of health programs involving cancer, it is vital to acknowledge that survival of patients increased and need to return them to their work environment as soon as possible is a priority, for quality of life, and to avoid work days absences that should be summed up to the total cost of antineoplastic treatments.

In a country as Mexico, where the access to modern and effective drugs against MM is scarce and restricted (bortezomib, lenalidomide, carfilzomib, pomalidomide, etc.,) it is of high importance to develop strategies to widen hematopoietic transplantation programs through its simplification.

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