Early chemosensitivity to VAD regimen predicts a favorable outcome after autologous stem cell transplantation in multiple myeloma

We report that early chemosensitivity, defined by a greater than 50% reduction of M-component and plasma-cell marrow infiltration, after 2 cycles of VAD was correlated with a favorable outcome following autologous stem cell transplantation in 46 patients with newly diagnosed multiple myeloma submitted to high-dose therapy.

haematologica 2002; 87:779-781
(*http://www.haematologica.ws/2002_07/779.htm*)

High-dose treatment with autologous stem cell transplantation (ASCT) has been widely applied in the management of multiple myeloma (MM) in the last decade and has shown a clear advantage in terms of complete remissions and outcome in comparison with results from conventional-dose treatment.[1,2] Analysis of prognostic factors that were predictive of a favorable outcome underlined the role of some parameters associated with the course of the disease before the transplantation, such as the length of the interval from diagnosis, the number of conventional-dose regimens pre-transplant and the sensitivity to conventional chemotherapy,[3-5] but most of the studies focused on the effect of myeloablative treatment on a cohort of patients who had previously received different regimens of induction therapy for different periods of time. In a homogeneous group of 46 newly diagnosed MM patients transplanted in our Center after 4 cycles of VAD and collection of peripheral blood stem cells (PBSC) primed with cyclophosphamide and granulocyte colony-stimulating factor (G-CSF), we retrospectively investigated whether the response to induction chemotherapy could predict subsequent outcome to high-dose therapy.

Between January 1996 and December 1999 a total of 46 MM patients ≤ 65 years with stage II and III disease or stage I with marrow plasmacytosis > 50% started on high-dose therapy. All the patients had a measurable M-component in serum or urine at diagnosis. All the patients received 4 monthly cycles of VAD induction chemotherapy, then underwent a collection of PBSC after administration of cyclophosphamide 7 g/m^2 plus recombinant G-CSF at the dose of 5 μg/kg /day s.c. The patients from whom it was possible to collect ≥ 2×10^6/kg CD34$^+$ cells proceeded to the myeloablative treatment consisting of busulfan 12 mg/kg plus melphalan 120 mg/m^2. We defined early chemosensitivity (ES) as a reduction of greater than 50% of the measurable paraprotein and the bone marrow plasma cell infiltration at the evaluation performed before the 3rd VAD cycle. All the other cases, showing a reduction lower than 50% or an increase of M-component and/or marrow plasmacytosis, were scored as having early chemoresistance (ER). Responses after autotranspantation were evaluated with EBMT, IBMT and ABMTR criteria.[6]

Out of the 46 patients, 23 (50%) showed ES and the other 23 had ER after 2 cycles of VAD. All the patients were treated with 2 other cycles of VAD and with cyclophosphamide. Six out 46 patients (13%) failed to mobilize an adequate number of CD34$^+$ cells and did not proceed to high-dose therapy. All these 6 patients belonged to the ER group. Forty patients (23 ES and 17 ER) were analyzed after ASCT. Twelve of 23 (52%) ES patients obtained a complete response after ASCT, in comparison with 1/17 (6%) of the ER patients (p<0.001). Analyzing the kinetics of the reduction of the M-component and plasma cell infiltration at the different steps of high-dose treatment, we observed that a significant difference between the 2 groups appeared after the 2nd cycle of VAD and persisted after cyclophosphamide administration and transplantation (Figure 1). Figure 2 shows a prolongation of the median event-free survival in ER group in comparison with that in the ES patients, while no difference was detected in overall survival (*data not shown*).

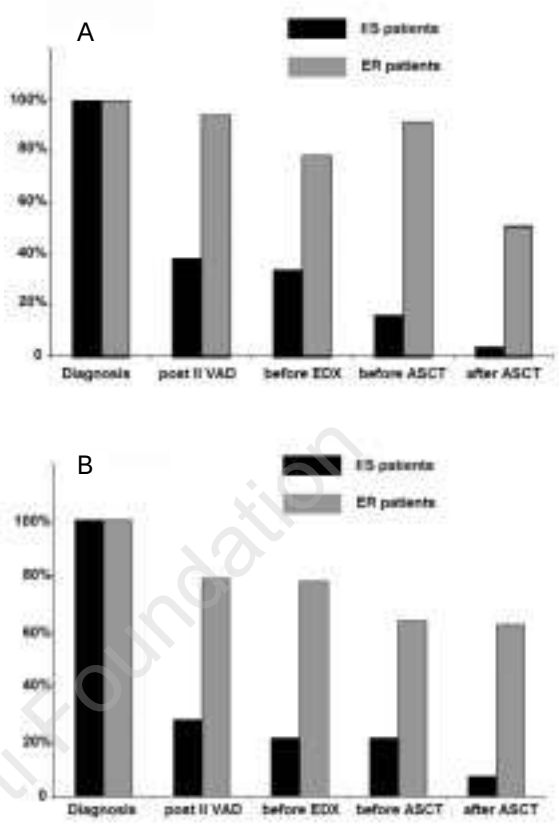

Figure 1. Reduction of M-component (A) and plasma cell infiltration (B) at the different steps of high-dose treatment in ES patients (early sensitivity after 2 cycles of VAD) and ER patients (early resistance after 2 cycles of VAD) are showed. At diagnosis the amount of M-component and plasma cell infiltration was taken to be 100% for all patients. At every step of the treatment we calculated the percentage of residual M-component and plasma cell infiltration and we represented the mean value ± SD. The difference calculated by the Student's t-test between the mean value of M-component and plasma cell infiltration of ES and ER patients was significant (p<0.01) at every step of the treatment.

There are specific problems with the mobilization of patients with MM, in whom there is a decrease of progenitors in the bone marrow due to a variety of reasons, such as a defect of the monocyte/macrophage activation pathway, the previous repeated courses of chemo-radiotherapy or, finally, the persistence of massive bone marrow plasmocytosis.[7] The rate of failure of mobilization ranges from 5%[2] to 10%[1] in the literature and was slightly higher in our study (13% of the patients). VAD is widely preferred to melphalan as conventional-dose therapy in MM patients who are candidates for transplantation, since VAD produces a faster cytoreduction[8] and does not alter the release of marrow progenitors into the peripheral blood.[7] We confirm that

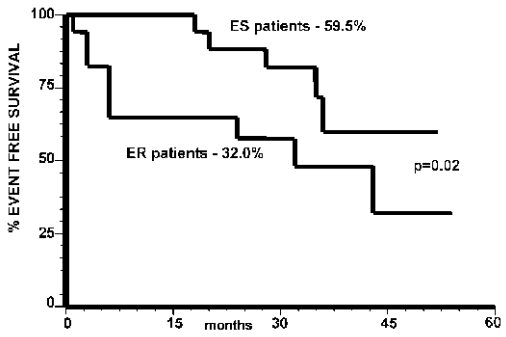

Figure 2. Event-free survival of ES patients (early sensitivity after 2 cycles of VAD) and ER patients (early resistance after 2 cycles of VAD). Event-free survival was calculated with the method of Kaplan-Meier from the date of the transplant until the date of last follow-up or event (death or relapse for patients in complete remission or progression of disease defined by > 50% increase of M-component and / or plasma cell infiltration or new bone lesions for patients who were not in complete remission).

the response to VAD, based on the reduction of M-component together with plasma cell marrow infiltration, appears very early and, indeed, could be recognized after just 2 cycles in all the sensitive cases. Moreover, we observed that the patients who had early chemosensitivity were more likely to have a lower percentage of residual marrow plasma cell infiltration after induction therapy and to yield an adequate PBSC collection.

Early chemosensitivity appeared to be a crucial factor not only for mobilization but also for the outcome of the transplant. Sensitive disease has had a prognostic relevance in all the major high-dose therapy trials, whether the ASCT was applied as first-line treatment[2-5] or as salvage therapy in MM patients.[9] The kinetics of the response has rarely been investigated in MM; however, a recent paper[10] underlined the importance of a rapid response in candidates for ASCT, since those who had a M-component which halved in < 0.5 months or reached a serum myeloma protein < 1 g/dL by the end of the induction therapy, were more likely to achieve a complete response after ASCT.

In our study a new concept of *sensitivity* based on the response to the first 2 cycles of induction therapy, was associated with a significantly higher complete response rate after transplantation and a prolongation of event-free survival. In the literature the achievement of a complete response after high-dose therapy is considered one of the most important prognostic factor for long-term disease control and the overall survival.[1-5] In our study the lack of a significant relationship between early chemosensitivity and survival is probably due to the follow-up being too short and to the fact that the group of patients was small.

We think that our report could have a practical significance and could help to modify the design of clinical trials, which commonly indicate that both sensitive and resistant cases after induction therapy proceed to mobilization and high-dose treatment. Since it is possible to discriminate after 2 VAD cycles patients who are likely to have a benefit from autotransplantation and patients who are not, it is reasonable to consider a therapeutic strategy in which sensitive cases will go straight to ASCT and resistant cases will receive an alternative treatment. The addition of thalidomide to induction chemotherapy could be worthy of study in patients with early resistance in an attempt to improve response prior to intensive treatment.

Francesca Patriarca, Alessandra Sperotto, Carla Filì, Francesco Zaja, Simonetta Prosdocimo, Renato Fanin

Clinica Ematologica e Dipartimento per il Trapianto di Midollo Osseo, Policlinico Universitario, Udine, Italy

Key words: multiple myeloma, autologous stem cell transplantation, induction therapy, VAD.

Correspondence: Francesca Patriarca, MD, Clinica Ematologica, Policlinico Universitario, p.le S. Maria della Misericordia, 33100 Udine, Italy. Phone: international +39.0432.559662. Fax: international +39.0432.559661. E-mail: ematologia@drmm.uniud.it

Manuscript processing
This manuscript was peer-reviewed by two external referees and by Professor Jesús San Miguel, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Prof. San Miguel and the Editors. Manuscript received March 21, 2002; accepted May 6, 2002.

References

1. Attal M, Harousseau JL, Stoppa AM, Sotto JJ, Fuzibet JG, Rossi JF, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Français du Myelome. N Engl J Med 1996; 335:91-7.
2. Barlogie B, Jagannath S, Vesole DH, Naucke S, Cheson B, Mattox S, et al. Superiority of tandem autologous transplantation over standard therapy for previously untreated multiple myeloma. Blood 1997; 89:789-93.
3. Bjorkstrand B, Goldstone AH, Ljungman P, Brandt L, Brunet S, Carlson K, et al. Prognostic factors in autologous stem cell transplantation in multiple myeloma: an EBMT Registry Study. European Group for Bone Marrow Transplantation. Leuk Lymphoma 1994; 15:265-72.
4. Vesole DH, Tricot G, Jagannath S, Desikan KR, Siegel D, Bracy D, et al. Autotransplants in multiple myeloma: what have we learned? Blood 1996; 88:838-47.
5. Majolino I, Vignetti M, Meloni G, Vegna ML, Scimé R, Tringali S, et al. Autologous transplantation in multiple myeloma: a GITMO retrospective analysis on 290 patients. Gruppo Italiano Trapianti di Midollo Osseo. Haematologica 1999; 84:844-52.
6. Blade J, Samson D, Reece D, Apperley J, Bjorkstrand B, Gahrton G, et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated with high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. Br J Haematol 1998; 102:1115-23.
7. Cappio FC, Cavo M, DeVincentiis A, Lanuta L, Lemoli RM, Majolino I, et al. Peripheral blood stem cell transplantation for the treatment of multiple myeloma: biological and clinical implications. Haematologica 1996; 81:356-75.
8. Alexanian R, Barlogie B, Tucker S. VAD-based regimens as primary treatment for multiple myeloma. Am J Hematol 1990; 33:86-9.
9. Fermand JP, Ravaud P, Chevret S, Divine M, Leblond V, Belanger C, et al. High-dose therapy and autologous peripheral blood stem cell transplantation in multiple

myeloma: up-front or rescue treatment? Result of a multicenter sequential randomized clinical trial. Blood 1998; 92: 3131-6.
10. Alexanian R, Weber D, Giralt S, Dimopoulos M, Delasalle K, Smith T, et al. Impact of complete remission with intensive therapy in patients with responsive multiple myeloma. Bone Marrow Transplant 2001; 27:1037-43.

Mixed chimerism after bone marrow transplantation for thalassemia major

Thirty-four thalassemia patients were studied for chimerism by fluorescent *in situ* hybridization or variable number tandem repeats after bone marrow transplantation. Mixed chimerism was detected in 9 patients with host cells ranging from 4 to 56%. One had graft rejection and the others were transfusion independent. Mixed chimerism was common but mostly without deleterious effect.

haematologica 2002; 87:781-782
(*http://www.haematologica.ws/2002_07/781.htm*)

After allogeneic bone marrow transplantation (BMT), some patients still have residual recipient hematopoiesis. The co-existence of recipient's and donor's hematopoietic systems is called mixed chimerism. Some patients may remain in stable mixed chimerism while others may convert to complete donor chimerism or graft rejection. The prediction of final outcome of mixed chimerism may help to decide interventions and prevent graft rejection.

Forty-four thalassemia major patients underwent transplantation in the Prince of Wales Hospital. Two patients received umbilical cord blood, four received peripheral blood stem cells and the others received a bone marrow transplantation. Conditioning was busulphan 16 mg/kg, cyclophosphamide 150-200 mg/kg, anti-thymocyte globulin 90 mg/kg.[1] One had primary graft rejection after a cord blood transplant. Chimerism state was monitored using peripheral blood leukocytes at 1, 3, and 6 months and then annually. For sex-mismatched donor-recipient pairs , fluorescent *in situ* hybridization (FISH) for X and Y probes was adopted. For sex-matched pairs, the variable number tandem repeat (VNTR) was utilized to test for DNA polymorphism. Five patients died early. Four patients did not have informative DNA markers for chimerism study and one had graft rejection at 71 days.

Thirty-four patients were serially followed up for chimerism and 21 developed complete donor chimerism. Thirteen patients (35.1%) were detected to have residual host cells during follow-up. Four had a low level of recipient cells (<10%) and all converted to complete donor chimerism. Nine patients with > 10% recipient cells were considered as having definite mixed chimerism. One patient (PT40) rejected the donor's graft at 9 months and PT17 achieved complete donor chimerism at 2 years. Seven patients (20.5%) had persistent mixed chimerism with 4 to 56% recipient cells at the follow-ups between 3 to 8.5 years (Table 1). All patients were transfusion independent. PT42 had an increasing percentage of recipient cells (60%) and severe anemia (Hb 5.9 g/dL). Donor leukocyte infusion with 3×10^8/kg CD3 cells was given at 8 months. The latest study at 34 months showed 47% recipient cells and Hb 7.9 g/dL. Age at BMT, sex matching, splenectomy, donor's thalassemia trait status, serum ferritin, liver enzymes, number of nucleated cells and CD34 cells infused did not have an impact on development of mixed chimersm. Four patients had a major mismatch for ABO blood group with their donors but none had mixed chimerism. Busulphan levels were measured in 18 patients. The areas under the curve of busulphan levels in patients with mixed chimerism were lower than those of patients with complete donor chimersim, on day 1, 2, 3 or 4 of the measurements. The mean area under the curve of the busulphan levels of the 4 days was 774 and 908 μmol × min/L for patients with mixed and complete chimerism, respectively (p=0.067) (Table 2). Hb F level was increased in 23 patients (67.6%) and was more common in patients with donors who were thalassemia trait carriers, 91% versus 27.3% (p<0.001).

Table 1. Mixed chimerism: recipient cell percentage after BMT.

	1 m.	*2 m.*	*3 m.*	*6 m.*	*1 y.*	*2 y.*	*3 y.*	*4 y.*	*5 y.*	*6 y.*	*7 y.*	*8 y.*
PT1	6	–	–	50	–	31.1	30	20	49.7	35	31	36
PT17	4.3	–	11	14	6.7	0.5	0	0	0	0	–	–
PT21	1.3	5.2	25	20.5	12.3	17.5	–	21.5	18.2	–	–	–
PT27	16	24	32	47	51	50	58	–	–	–	–	–
PT29	12	10	6	14	14	11	18	16	–	–	–	–
PT32	10	7.5	5	22.7	61.2	77	77	–	56	–	–	–
PT39	0	0	–	–	4	11.2	4.8	4	–	–	–	–
PT40	2.8	–	1.3	5	38.4	–	–	–	–	–	–	–
PT42	6.5	–	18.8	60.3	75.8	48.7	46.5	–	–	–	–	–

Abbreviations: m. = months; y. = years.

Table 2. Median busulphan area under curve level (μmol x min/L).

	Mixed chimerism (n = 6)	*Complete chimerism (n = 12)*
Day 1	596	697 (p = 0.177)
Day 2	844	918 (p = 0.083)
Day 3	848	876 (p = 0.864)
Day 4	539	947 (p = 0.060)
Average day 1-4	774	908 (p = 0.067)

In malignant diseases, persistent mixed chimerism is always associated with relapse of leukemia. In non-malignant diseases, residual recipient cells may stay in harmony with the donor's system and rejection does not occur.[2,3] The mechanism of maintaining stable mixed chimerism is unknown. In this study, 26% of patients were found to have mixed chimerism and this is similar to the 36% previously reported.[4] Thalassemia patients have a higher incidence of mixed chimerism. The hypercellular bone marrow may be more resistant to ablative conditioning. Repeated blood transfusion before BMT may stimulate patients' immune system, thus rendering them more resistant to immunoablation.[2]

Intensity of conditioning regimen was reported to have an impact on mixed chimerism.[5] Manna reported that thalassemia patients who received lower doses of cyclophosphamide had a higher incidence of mixed chimerism.[6] One study showed that busulphan levels did not affect transplant outcome.[7] In this study, however, there was a trend towards patients with mixed chimerism having had lower levels of busulphan and this deserves further study. The incidence of graft rejection in thalassemia patients with mixed chimerism was reported to be high, 20 out of 55 patients.[8] More than >25% residual host cells early after transplant is highly predictive of graft rejection.[3] In this study, only one patient with mixed chimerism developed graft rejection. We used a more intensive conditioning and this may account for the low rejection rate. We observed a high percentage of elevated Hb F production and this is in concordance with a previous report that