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Early changes in bone marrow morphology induced by thalidomide in patients with refractory myeloma

Bone marrow morphology and the number of CD34+ cells were evaluated in 17 patients with refractory multiple myeloma at the start of therapy with low-dose thalidomide and after 3 months. All responding patients showed an evident increase of cellularity, reappearance of erythroblasts and myeloid precursors in various phases of differentiation, and an increase of megakaryocytes. Nine of the ten responders also had increase of bone marrow CD34+ cells.

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Although high-dose therapy produces high response rates and overall survival in multiple myeloma (MM), recurrence of disease usually develops and options of salvage therapy are limited.[1,2] Thalidomide has proven to be very effective in MM[3,4] through several biological pathways.[5,6] We have previously reported that clinical and hematologic recovery can be observed even in patients who obtain less response.[7] Moreover, we described the early morphologic changes in two refractory MM patients treated with thalidomide.[8]

Based on these observations, we performed this study on a cohort of patients with refractory MM treated with thalidomide in order to evaluate the morphologic bone marrow changes and to estimate the variation of bone marrow CD34+ cells.

The 17 patients included in the study were refractory to at least 2 previous lines of therapy, which in 6 patients had been one or two autologous bone marrow transplantations. Thalidomide was given at a dose of 100 mg/day and escalated to 200 mg/day in case of resistance. Four patients, because of severe disease-related symptoms, also received dexamethasone 20 mg/day for two days every two weeks for the first two months. Response to therapy was assessed according to the criteria of the European Bone Marrow Transplantation Group.[9] A minimum of three months of uninterrupted therapy was necessary to evaluate the response. The clinical and laboratory characteristics of the 17 evaluable patients recorded at the start of thalidomide were: median age 55 (37-67) years; 13 IgG, 2 IgA, 2 Bence Jones; 1 stage I, 4 stage II, 12 stage III. The evaluation of the bone marrow morphology was performed on May-Grünwald Giemsa smears before and after three months of thalidomide. Bone marrow smears were evaluated by three independent investigators, and by an external morphologist. Percentages of erythroblasts, myeloid precursors, and plasma cells were calculated on a minimum of 500 cells. Cytometric evaluation of bone marrow CD34+ cells was performed as previously reported.[10]

Thalidomide was well tolerated and side effects were always mild. After three months of thalidomide, 10 out of 17 patients (59%) had achieved a response (6 partial, 1 minor, 3 stable disease), and 7 (41%) had progressed. Table 1 describes the morphologic changes in detail. As shown, an increase of bone marrow cellularity was evident in 8 of the 10 responders. A striking increase in megakaryocyte number accompanied by reappearance of myeloid precursors, very rare in pre-therapy smears, at various stages of differentiation was observed in all responding patients. The percentage of erythroblasts was significantly higher after therapy, increasing from a mean value of 3.21% to 10.14% ($p<0.0001$). An increase of the bone marrow eosinophils was also noted. The bone marrow CD34+ cells, evaluated as absolute numbers and percentages, showed a parallel increase in 9 of 10 responding patients (Figure 1), from a mean of 134.4±194/mcrL (0.6%) before starting therapy to 462± 335/mcrL (1.8%) after thalidomide ($p=0.02$ and $p=0.007$, respectively). By contrast, in non-responding patients bone marrow cellularity very often appeared reduced. Similarly, in these

Table 1. Bone marrow morphology and CD34+ count before and after three months of thalidomide in 10 responding and 7 non-responding (*) MM patients.

Patient	*Months*	*Cellularity*	*Erythroblasts*	*Megakaryocyte*	*BMPC*	*Lymphocyte*	*CD34/μL*	*CD34%*
MC	0	low	6.0	rare	66.5	4.5	16	0.4
	3	normal	23.5	normal	17.5	9.5	540	1.0
LF	0	normal	11.5	rare	51.0	4.5	189	1.0
	3	low	55.0	increased	3.5	8.5	61	0.6
RF	0	scarce	15.0	rare	10.0	14.0	75	0.5
	3	normal	46.5	increased	10.0	6.0	83	3.3
AR	0	low	30.0	normal	28.5	14.0	12	2.0
	3	normal	49.0	increased	5.0	16.5	450	2.5
BG	0	scarce	1.0	rare	72.0	14.0	45	0.6
	3	high	24.5	normal	9.5	4.0	201	1.0
GA	0	normal	12.0	rare	66.5	4.5	480	0.8
	3	high	55.0	increased	11.5	7.5	598	2.3
LA	0	low	22.0	normal	23.5	9.0	496	0.6
	3	normal	57.0	normal	12.5	5.5	735	1.0
PP	0	high	7.0	rare	58.5	15.5	18	0.3
	3	normal	51.0	decreased	7.0	12.5	450	3.0
TD	0	scarce	3.0	rare	22.0	38.0	10	0.1
	3	normal	38.5	increased	3.0	1.0	1185	1.5
TM	0	low	6.0	decreased	29.0	18.0	3	0.1
	3	normal	46.5	normal	7.5	13.5	320	2.0
*VP	0	high	63.0	decreased	19.0	4.0	137	0.7
	3	normal	41.0	rare	36.0	17.0	108	1.2
*NE	0	scarce	24.0	rare	45.5	5.5	78	0.1
	3	high	6.0	rare	87.0	1.0	96	0.4
*CM	0	normal	31.5	normal	39.0	6.5	290	1.2
	3	scarce	7.0	rare	37.0	27.0	74	0.8
*LC	0	normal	32.5	normal	15.5	1.5	357	0.8
	3	low	40.5	rare	12.0	8.0	325	5.5
*FG	0	low	22.0	rare	27.5	10.0	162	4.0
	3	scarce	23.0	rare	21.0	30.0	5	0.2
*MF	0	high	9.0	normal	74.5	5.5	72	0.6
	3	normal	2.0	rare	62.0	12.0	13	0.2
*GR	0	low	2.0	rare	63.0	15.0	33	0.3
	3	high	2.0	rare	92.0	3.0	21	0.3

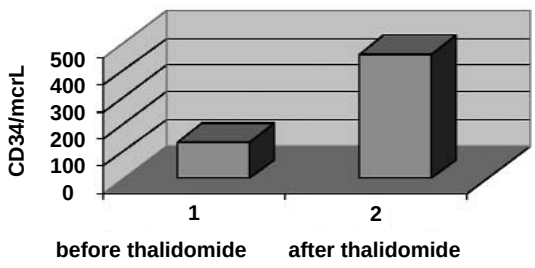

Figure 1. Mean values of bone marrow CD34+ cells before and after thalidomide treatment in 10 responding patients.

patients the number of bone marrow CD34+ cells appeared to decrease, although not significantly, from a mean value of 161±120/μL (0,75%) before thalidomide treatment to 97±123/μL (0,40%) after (*p*=NS).

We report the first study on the bone marrow morphologic changes in patients with refractory MM treated with thalidomide. Patients with refractory myeloma are difficult to treat because of the resistance to therapy acquired by plasma cells and the fragility of the patients. The massive bone marrow plasmacytosis, often present in this phase, affects the normal hematopoietic compartment causing peripheral blood cytopenias. Conventional chemotherapy can reduce the percentage of plasma cells but also damages the normal hematopoietic compartment.

In this study, before starting thalidomide all patients showed different grades of hypocellularity with a prevalence of plasma cells and plasmablasts, and a marked reduction or absence of

megakaryocytes. After three months of thalidomide therapy, an increase in bone marrow cellularity was found in all responding patients, with reappearance of three-lineage precursors in different phases of maturation. A significant increase of bone marrow CD34+ cells was recorded. These changes produced an amelioration of the peripheral cytopenias and in four patients eliminated the need for transfusions. In contrast, non-responding patients did not show any morphologic changes.

In conclusion, in myeloma patients, response to thalidomide is characterized by an early increase of bone marrow cellularity with an expansion of hematopoietic stem cells. This could be related to an inhibition of the neoplastic clone and, with respect to chemotherapy, to less damage to normal hematopoietic stem cells.

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