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Original article

Effect of thalidomide on bone marrow angiogenesis in multiple myeloma patients

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ABSTRACT

Background: Bone marrow angiogenesis is increased in multiple myeloma (MM) patients, prompting the rationale for using antiangiogenic drugs in the treatment of these patients.
Objective: To assess angiogenesis in patients with MM at diagnosis and following treatment with an antiangiogenic drug.
Patients and Methods: Twenty-three patients with newly diagnosed MM were treated with thalidomide-based regimens. Bone marrow evaluation was made before and following treatment and included angiogenesis assessment, which was quantified through microvessel density (MVD) determination, by means of anti-CD34 immunohistochemical labeling, and classified either as high MVD or low MVD, according to the mean CD34 count: above or below the median of 12.6.
Results: The pre-therapy median MVD was 12 (7.5–18.3) versus 8.7 (5.35–18.5) post-therapy, $p = 0.2114$.
Conclusions: Our study found no reduction in MVD before and following treatment and, accordingly, we could establish no relationship between MVD and response to therapy in the sample we studied.

Introduction

Multiple myeloma accounts for 1% of all neoplasms and 10% of all onco-hematological diseases.[1] The overall survival of MM patients has significantly increased over the last 15 years with

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the incorporation of the new agents into their treatment, such as immunomodulatory drugs,[2,3] proteasome inhibitors,[4] and monoclonal antibodies.[5] Thalidomide was the first of these drugs to have its clinical activity shown, and has been proven effective against the disease throughout its stages.[6] Despite its proven antiangiogenic effect, its detailed mechanism of action remains unclear.[7] The interactions between MM cells and the bone marrow microenvironment are fundamental to MM pathogenesis[8]; MM was the first hematological neoplasm in which angiogenesis was shown to play a relevant role.[9,10] The formation of new blood vessels is essential for the survival and proliferation of plasma cells and is possibly mediated by growth factors and cytokines, such as VEGF and IL-6.[11] Previous studies have shown that angiogenesis is not significantly altered by therapies that are not based on immunomodulatory drugs.[12,13] In Brazil, thalidomide is a widely used agent for treating MM, being readily accessible and used in various combinations. Our study aimed to evaluate the effect of thalidomide upon bone marrow angiogenesis in patients newly diagnosed with MM.

Methods

Twenty-three patients with newly diagnosed MM received treatment, between August 2008 and May 2012, with combinations containing thalidomide. Bone marrow biopsy samples were collected before initiating the treatment and then again 3–4 months into treatment, according to the protocol of our institution. Five marrow biopsy samples from patients without hematological disease were used as the control group. All patients gave their voluntary and informed consent in writing prior to participating in any in the study procedures, which were approved by the Local Ethic Committee.

Marking microvessels

The samples were histoprocessed in a conventional fashion and assayed immunohistochemically in order to determine MVD counts upon reaction to CD34 on the BenchMarck automated system (Ventana medical systems, Inc. Roche USA). Tissue sections were dewaxed, hydrated and then had their endogenous peroxidase activity blocked through successive baths and treatment with hydrogen peroxide. They were incubated with the primary antibody (anti-CD34; Dako, clone QBEnd10, title 1/400) for 20 min. The reaction was revealed with Diaminobenzidine (DAB) and counterstained with the Harris' hematoxylin.

Determining microvessel density (MVD)

Bone marrow angiogenesis was estimated by assessing the MVD with the aid of optical microscopy.[14] All slides were analyzed by two independent pathologists. Anti-CD34 labeling was assessed under 100× magnification in order to identify the three most hypervascularized fields, i.e. those with the greatest number of microvessels. Those three fields were then evaluated under 400× magnification, using a 40× objective and a 10× eyepiece. Large vessels and vessels located in the periosteum were excluded. MVD counts were then determined as the mean of the number of vessels contained in each one of the three fields.

Determining the degree of angiogenesis

Patients were divided into two groups[9]: the low MVD group, or the patients having a mean CD34 below the median of 12.6 and the high MVD group, or the patients having a mean CD34 above the median of 12.6.

Therapy against Multiple Myeloma

All patients underwent a therapy regimen based on thalidomide. Thalidomide was initially given at a dose of 100 mg/day for 2 weeks and then increased to 200 mg/day orally. Twelve patients received a triplet regimen with cyclophosphamide, thalidomide and dexamethasone; one patient, melphalan, thalidomide and dexamethasone, and; 10 patients, a double regimen with thalidomide and dexamethasone.

Statistical analysis

Continuous variables were summarized, such as variation (minimum and maximum values), mean, standard deviation (SD), median, and interquartile range. Categorical variables were described as absolute and relative frequencies. The Kolmogorov–Smirnov test was used, when evaluating the distribution pattern of the numerical variables in the sample. T-test pairs were used in MVD count comparisons before and following therapy. Survival analyses were performed by using the Kaplan–Meier technique. All analyses were conducted with the aid of the MedCalc software (Mariakerke, Belgium, v.11.3.3.0). As a rule of thumb, 5% two-tailed significance levels were considered indicative of a statistical difference between the groups.

Results

Of the 23 patients included in the study, 16 patients underwent an adequate biopsy for pre- and post-treatment CD34 analyses.

Patient characteristics are listed in Table 1.

Thalidomide-based regimen therapy resulted in 9.1% of complete response (CR), 31.8% of very good partial response (VGPR), 40.9% of partial response (PR), 4.5% of minimal response (MR) and 13.6% of disease progression (DP). The overall treatment response rate was 81%.

The median MVD of five cases having no hematological disease, as analyzed with CD34, was 3.67.

The median pre-treatment MVD was 12 (7.5–18.3), as compared to 8.7 (5.35–18.5) following treatment ($p = 0.2114$). (Figure 1)

In patients who achieved VGPR, the median pre-treatment MVD was 13.3 (9.98–15.6), as opposed to the post-treatment 10.3 (7.6–20.5), $p > 0.99$, whereas in patients with PR, the pre-treatment median was 8.3 (5.7–20.3), as opposed to the post-treatment 5.7 (5–8.7), $p = 0.218$. (Figures 2 and 3) The median MVD was 12.6; 56.2% were classified as low MVD and 43.75%,

Table 1 – Baseline patient characteristics.

Characteristic	Value or N (%)
Age at diagnosis, years	N = 23
Median (IIQ)	57.7 (52.0–66.0)
Gender	N = 23
Male	15 (65.2%)
Female	8 (34.8%)
Durie Salmon	N = 23
II	1 (4.3%)
III	22 (95.7%)
ISS	N = 23
1	9 (39.1%)
2	6 (26.1%)
3	8 (34.8%)
Monoclonal component	N = 23
IgG	15 (65.2%)
IgG Kappa	12
IgG lambda	3
IgA Kappa	3 (13%)
Light chain	5 (21.8%)

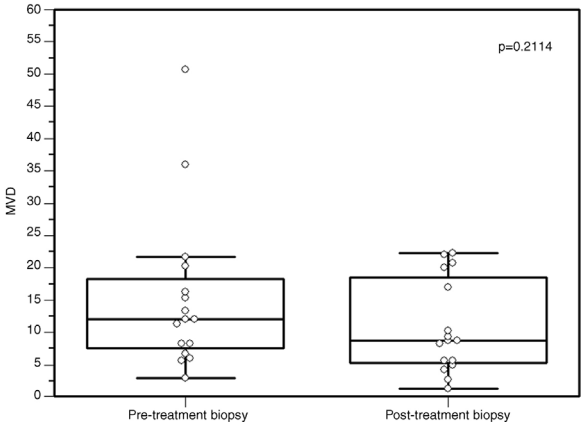

Figure 1 – Comparison of median MVD at diagnosis and after therapy in the 16 patients.

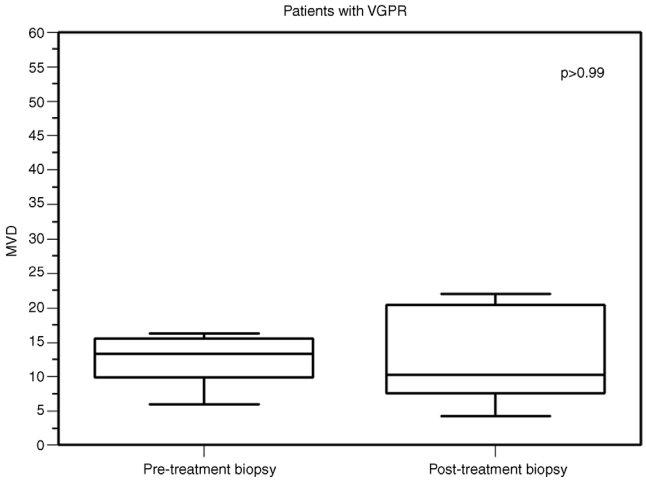

Figure 2 – Comparison of median MVD values pre- and post-treatment in patients who achieved VGPR.

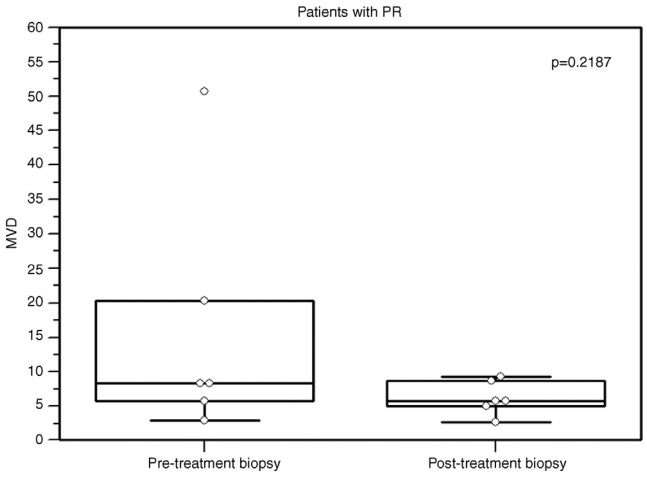

Figure 3 – Comparison of median MVD values pre- and post-treatment in patients who achieved PR.

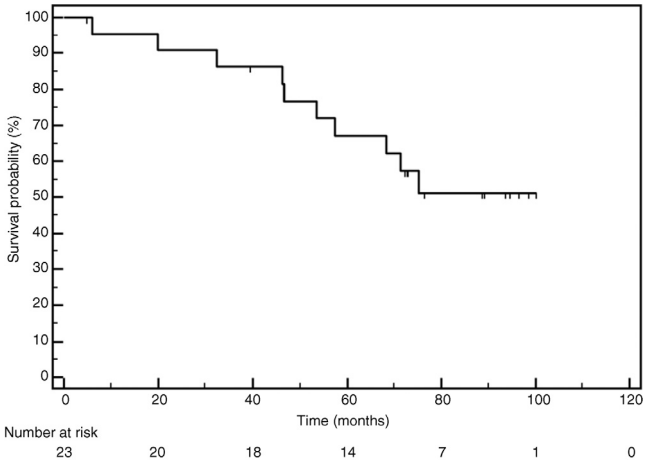

Figure 4 – Overall survival from diagnosis.

as high MVD, at the first biopsy. At the second one, 68.7% were classified as low MVD and 31.25%, as high MVD.

The median overall survival (OS) for 23 patients was not reached (Figure 4).

The high MVD group had an OS of 75.1 m at diagnosis, whereas in the low MVD group, the median OS was not reached, $p = 0.3410$ (Figure 5).

The progression-free survival (PFS) for the entire group was 30.1 months. In the high MVD group, it was 28.9 m, versus 40.95 months in the low MVD group, $p = 0.222$ (Figure 6).

Discussion

The important role played by angiogenesis in tumor growth was initially described in solid tumors. Only in the 1990s was this role shown in hematological malignancies, with MM as its first model.[9]

Successive genetic mutations and changes in the bone marrow microenvironment cause normal plasma cells to transform into malignant ones. This process, in turn, results in cytokines being released into that microenvironment, cell

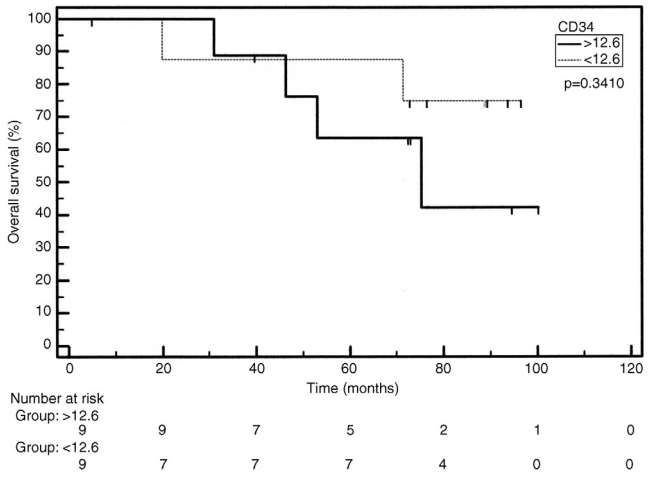

Figure 5 – Overall survival from diagnosis in the two groups categorized as low and high MVD.

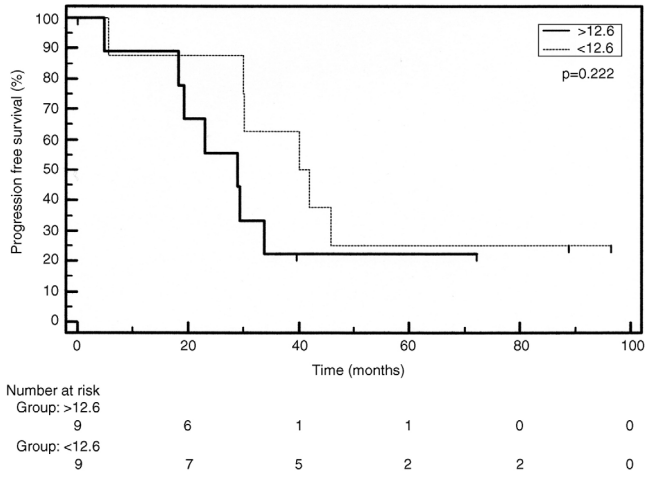

Figure 6 – Progression-free survival from induction in the two groups categorized as low and high MVD.

proliferation and increased angiogenesis, which are the fundamental stages in disease pathogenesis.[15]

Increased bone marrow angiogenesis in MM patients, as compared to patients with non-active monoclonal gammopathy of undetermined significance (MGUS) and MM, had been reported in 1994 in a publication by Vacca et al.[16]

The increased angiogenesis in MM and the well-known antiangiogenic action of thalidomide are reasons for this drug having been included in the therapy for MM patients. Previous studies had shown that MVD did not significantly change with thalidomide-free regimens.[12,13] However, in 2001, Sezer et al.[7] found a decrease in bone marrow angiogenesis in patients who had achieved remission following conventional chemotherapy, and suggested conducting a study on angiogenesis with an antiangiogenic drug.

This study aimed mainly to assess the MVD following treatment with thalidomide.

Our study confirmed that microvessel counts in MM patients are greater than in the control cases, as found by Vacca and collaborators, who showed that the MVD were greater among MM patients, when compared to healthy or MGUS patients,[16] thereby confirming increased bone marrow angiogenesis in MM patients.

In the sample we studied, we observed a non-statistically significant reduction in the median MVD among patients treated with thalidomide, while Kumar et al.,[11] who conducted a similar study, reported a significant difference in the MVD reduction. Factors that may account for the differences observed in these studies could be the sample size and the time interval between bone marrow biopsies. In our study, post-treatment biopsies were performed 3–4 months after the initial biopsy. In the study conducted by Kumar et al.,[11] the biopsy collection was 4–6 months after the first biopsy.

Angiogenesis as a prognostic factor is consistent across several studies.[9,10] In ours, patients whose MVD was low at diagnosis did not reach the median OS, whereas in patients with a high MVD at diagnosis, it was 75.1 months. Although not statistically significant, patients whose MVD was high had a lower overall survival rate, as reported by Rajkumar et al.,[9] thus suggesting a worse prognosis.

The PFS was 41.05 months in low MVD patients and 28.9 months in the high MVD group. Although not statistically significant, there was a 12-month difference between the two groups, which was found to be suggestive of an unfavorable prognosis. In 2015, Lee et al. showed that high MVD at diagnosis was considered an independent factor of poor prognosis.[17]

In summary, our study did not find any reduction in MVD following thalidomide therapy. Probably, with a larger number of patients and biopsies over a longer period of time, it should be possible to show the effect of thalidomide upon angiogenesis and treatment response.

Conflicts of interest

The authors declare no conflicts of interest.

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