

The association between COX-2 expression and survival in myeloma patients

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Abstract. – **BACKGROUND:** Increased cyclooxygenase-2 (COX-2) expression has been associated with poor prognosis in multiple myeloma (MM).

AIM: This study examined the relationship between COX-2 expression in bone marrow and prognosis in MM patients.

PATIENTS AND METHODS: Bone marrow biopsy samples of 67 newly diagnosed MM patients were examined immunohistochemically for COX-2 expression. Mean age of the patients was 52.69 years (52.69 ± 9.17) and median follow-up time was 99.5 months (range: 6-170 months).

RESULTS: Of all patients, 30 (44.8%) were COX-2 positive and 37 (55.2%) were COX-2 negative. Median overall survival (OS) was 78 months (range: 54.07-101.92 months) among all patients, 75 months (range: 45.61-104.38 months) in COX-2-positive patients, and 98 months (range: 50.36-145.63 months) in COX-2-negative patients. Median progression-free survival (PFS) was 30 months (range: 3-134 months) in all, 29.5 months (range: 3-68 months) in COX-2-positive and 35 months (range: 3-134 months) in COX-2-negative patients. Statistically significant differences in OS and PFS between COX-2-positive and COX-2-negative patients were not observed ($p = 0.84$ and $p = 0.22$, respectively). Differences between the COX-2-positive and COX-2-negative patients in gender, hemoglobin, β_2 -microglobulin (β_2M), creatinine, albumin, and disease stage were not statistically significant.

CONCLUSIONS: COX-2 expression neither had a role in prognosis nor significantly affected OS and PFS. We conclude that stem cell transplantation might eliminate the detrimental effects of COX-2 positivity. Larger series of patients are needed to investigate this observation.

Key Words:

Cyclooxygenase-2, Multiple myeloma, Transplantation

Introduction

Cyclooxygenases (COX) are a group of enzymes that catalyze prostaglandin synthesis from

arachidonic acid. They are involved in many physiological and pathological processes. Unlike COX-1, which is expressed in many cells, COX-2 is not expressed in resting cells, and its secretion is induced by inflammatory cytokines and growth factors produced during pathological processes such as inflammation^{1,2}. It has been argued that COX-2 plays a role in angiogenesis by controlling vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) levels in many cancers^{3,4}. In various solid tumors and hematological cancers increased COX-2 expression has been associated with poor prognosis⁵⁻¹⁹.

Earlier research on hematological cancers showed that increased COX-2 expression was associated with poor prognosis in chronic leukemias, such as chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML)⁹⁻¹¹. Increased COX-2 expression was reported both in Hodgkin's and non-Hodgkin's lymphoma (NHL), and was observed to be associated with poor treatment response in NHL¹²⁻¹⁶. Similarly, increased COX-2 expression has been associated with poor prognosis in multiple myeloma (MM)¹⁷⁻¹⁹. In those studies patients received different treatment regimens and 25-66% of the cases underwent autologous stem cell transplantation.

In this study, bone marrow samples that were obtained from the patients at the time of diagnosis were embedded in paraffin blocks and examined for COX-2 expression in order to determine its relationship to prognosis.

Patients and Methods

The study included 67 MM patients who were diagnosed at Faculty of Medicine, Ege University between 1998 and 2007. The study was performed with the principles of the Declaration of Helsinki and approved by the institutional local Ethic Committee. Bone marrow samples obtained at the time of diagnosis and embedded in paraffin

blocks were removed from the archive of the Pathology Department and examined for COX-2 expression with immunohistochemical staining. Patients' clinical and laboratory findings were obtained from the hospital files. After 3-6 courses of the VAD protocol (vincristine, doxorubicin, and dexamethasone), responses of the patients were assessed according to the European Group for Bone and Marrow Transplant (EBMT) criteria before transplantation²⁰. Five patients who had no response to induction therapy were treated with thalidomide $\pm$ dexamethasone and 3 patients received bortezomib $\pm$ dexamethasone before transplantation. Of all patients, 8 achieved complete response (CR), 53 partial response (PR), 4 minimal response (MR) and 2 had progressive disease (PD) before transplantation. All patients underwent autologous stem cell transplantation. Stem cells were mobilized with cyclophosphamide (2-4 g/m²) and granulocyte colony-stimulating factor. Sixty one patients received 200 mg/m² of melphalan and 6 patients received 140 mg/m² of melphalan as a pre-transplant conditioning regimen. Response to therapy was re-assessed according to the EBMT criteria after transplantation²⁰.

Immunohistochemistry

From formalin-fixed bone marrow paraffin blocks, 5- μ m tissue cross-sections were taken and placed on polylysine-coated slides (X-traTM, Surgipath Medical Industries, Richmond, IL, USA). The cross-sections were dehydrated at 60°C for at least 2 h. All procedures, including deparaffinization and antigen-obtaining processes, were performed using a BenchMark XT[®] automatic staining machine (Ventana Medical Sys-

tems, Oro Valley, AZ, USA). After the sections were contrast-stained with hematoxylin using an automatic machine, dehydration, incubation with xylene, and assembly procedures were performed manually. Monoclonal mouse anti-human COX-2 antibody (code: M3617; dilution: 1/75; clone: CX-294, Dako SA, Glostrup, Denmark) was used as the primary antibody. Five colorectal adenocarcinoma tissue sections were used as the positive control. Negative controls were obtained with the same immunohistochemical method after extracting the primary antibodies. Cells were enumerated by examining 10 areas that contained the least immunopositive cells at 40x magnification under a light microscope. The percentage of COX-2 was calculated as the ratio of immunopositive cells to total cells. COX-2 expression was defined as positive when at least 10% of neoplastic cells were stained positive.

Statistical analysis

Survival was analyzed using the Kaplan-Meier method. PFS (Progression-Free Survival) was measured as the time between diagnosis and progression or death and Overall Survival (OS) was defined as the time from diagnosis to death. Progression was defined according to the EBMT criteria. The difference between the 2 groups in terms of COX-2 expression was analyzed using the chi-square and Fisher's exact tests. *p* values < 0.05 were regarded as statistically significant. All statistical analyses were performed using SPSS (Statistical Package for Social Sciences Inc., Chicago, IL, USA) for Windows.

Table I. Demographic and laboratory findings of patients with respect to COX-2 expression status.

	Number (%)	COX-2 (+)	COX-2 (-)	p
Total	67 (100)	30 (44.8%)	37 (55.2%)	
Mean Age (SD)	52.69 ($\pm$ 9.17)			
Gender (F/M)	29/38 (43.3/56.7)	14/16	15/22	0.61
M component				0.93
IgG	35 (52.3)	15	20	
IgA	9 (13.4)	4	5	
Light chain	23 (34.3)	11	12	
K/L	30/37 (44.8/55.2)	15/15	15/22	0.43
β_2 M > 3.5 μ g/l	29 (43.2)	11	18	0.23
Hb < 8 g/l	11 (16.4)	6	5	0.47
Creatinine > 2 mg/l	16 (23.9)	6	10	0.5
Stage III	64 (95.5)	27	37	0.08
Albumin < 3.5 g/dl	43 (64.1)	22	21	0.17

Hb: hemoglobin β_2 M: beta-2-microglobulin Ig: immunoglobulin K: kappa L: lambda

Table II. Pre-transplantation and post-transplantation response with respect to COX- 2 expression.

	Pre-transplantation response		p	Post-transplantation response		p
	COX-2 (+) (%)	COX-2 (-) (%)		COX-2 (+) (%)	COX-2 (-) (%)	
CR	4 (13.3)	4 (10.8)	0.62	10 (33.3)	18 (50)	0.59
PR	24 (80)	29 (78.4)		19 (63.4)	17 (47.2)	
Minimal response	2 (6.7)	2 (5.4)		0	0	
Progressive disease	0	2 (5.4)		1 (3.3)	1 (2.8)	

CR: complete response PR: partial response

Results

Laboratory findings are summarized in Table I. Mean age of the patients was 52.69 years (52.69 ± 9.17) and median follow-up time was 99.5 months (range: 6- 170 months). According to the Durie-Salmon staging system, 3 patients (4.5%) were stage II and 64 (95.5%) were stage III at the time of diagnosis. Renal failure was observed in 16 patients (23.9%). In all, 5 patients received thalidomide $\pm$ dexamethasone and 3 patients received bortezomib $\pm$ dexamethasone treatment before mobilization. After autologous transplantation, 28 patients were in CR (complete response), 36 were in PR (partial response), while 2 patients were in PD (progressive disease) and one patient died after transplantation due to pneumonia.

Among the patients, 30 (44.8%) were COX-2 positive and 37 (55.2%) were COX-2 negative. Pre-transplantation and post-transplantation responses with respect to COX-2 expression status are shown in Table II. Pre-transplantation treatment response was similar between COX-2- positive and negative groups ($p = 0.62$). Median OS was 78 months (range: 54.07-101.92 months) in all patients, 75 months (range: 45.61-104.38 months) in COX-2-positive patients, and 98 months (range: 50.36-145.63 months) in COX-2-negative cases.

Median PFS was 30 months (range: 3- 134 months), 29.5 months (range: 3- 68 months) and 35 months (range: 3-134 months) in all, COX-2 positive and COX-2 negative patients, respectively. The differences in OS and PFS between COX-2 positive and COX-2 negative patients were not statistically significant ($p = 0.84$ and $p = 0.22$, respectively). Kaplan Meier plots of OS according to COX-2 expression status are shown in Figure 1.

No statistically significant differences with respect to COX-2 expression were observed in the factors of known prognostic significance in MM, such as gender, hemoglobin, Beta-2-microglobulin ($\beta 2M$), creatinine, albumin levels, and disease stage (Table I).

Discussion

We retrospectively examined the COX-2 expression in bone marrow biopsy specimens of newly diagnosed myeloma patients and looked into its relation with survival. COX-2 positivity at the time of diagnosis was 44.8% and this is in agreement with the literature (31-70%)¹⁷⁻¹⁹. However, even though both PFS and OS were longer in COX-2 negative patients compared to COX-2 positive patients, the differences were not statistically significant. Review of the literature showed that both PFS and OS were higher in COX-2 negative patients in 3 studies and the differences between groups were statistically significant¹⁷⁻¹⁹.

In our investigation, we assessed COX-2 in 67 newly diagnosed patients while there were 51, 57 and 94 patients in other studies, respectively¹⁷⁻¹⁹. With 99.5 months, median follow-up time in our study was considerably longer than other reports (30-38 months)^{18,19}.

Median OS was 98 months in the COX- 2 negative group and 75 months in the positive group in our study while it was in the range of 48-71 and 30- 46 months, respectively in the others^{17,19}. OS was markedly longer in both COX-2 positive and negative groups than the other studies. In our patients, median PFS was 35 months and 29.5 months in COX-2 negative and COX-2 positive groups, respectively while those in Ladetto et al work¹⁷ were 36 and 18 months, respectively. PFS in our COX-2 negative group was comparable with the literature while in the COX-2 positive group was longer. The difference in both OS and PFS may be attributed to the patient selection and their treatments. At the first line of treatments all our patients had autologous stem cell transplantation following VAD chemotherapy. All patients of Cetin et al study¹⁹ also had VAD chemotherapy while 25-66% of the patients had autologous stem cell transplantation, 29.8-65% had conventional treatment and 4.2-10% had

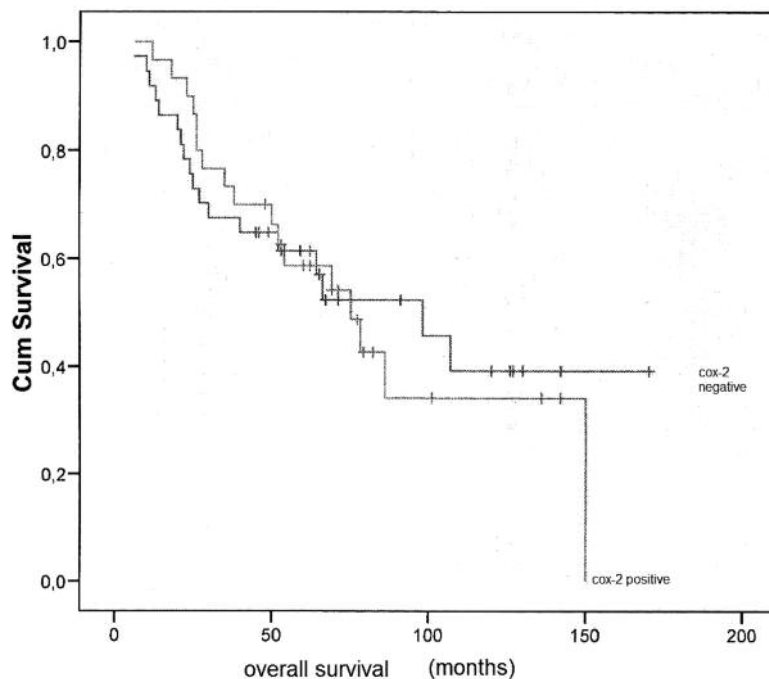


Figure 1: Kaplan Meier plots of overall survival according to COX-2 expression status.

thalidomide and conventional treatment¹⁷⁻¹⁹. A relation between maximal responses achieved during or following stem cell transplantation and long term outcome has been shown by Van De Velde et al²¹. The unfavorable effect of COX-2 on prognosis could be alleviated by transplantation of autologous stem cell in all patients. However, to prove this hypothesis, we need studies that assess COX-2 expression and prognosis in patients with or without stem cell transplantation.

COX-2 positivity in bone marrow has generally been tested immunohistochemically using polyclonal antibodies¹⁷⁻¹⁹. In this study, we determined COX-2 positivity in 44.8% of the cases using monoclonal antibodies which are known to have better sensitivity²². In Çetin et al study¹⁹, COX-2 positivity, as determined immunohistochemically, was 70% while that in Trojan et al report was 47%¹⁸. Immunohistochemically, Ladetto et al¹⁷ found that 51% of their patients' bone marrows were positive for COX-2 but Western Blotting showed COX-2 positivity in 31% of the patients¹⁷. It is possible that testing COX-2 using monoclonal antibodies decreased false positivity.

Retrospective design doesn't permit to determine whether patients who were COX-2 positive before the transplantation became COX-2 negative afterwards. Moreover, we are lacking of cytogenetic analysis, an important prognostic deter-

minant. Higher OS and PFS with respect to the other investigations could due in our patients to a better prognostic cytogenetics.

Conclusions

We showed that about half of the patients with multiple myeloma were COX-2 positive. However, conversely to the literature, we did not find an unfavorable effect of COX-2 positivity on OS or PFS. It is possible that detrimental effect of COX-2 on prognosis is reduced by autologous stem cell transplantation, which has an important place in myeloma treatment. Therefore, we need prospective studies with larger sample size.

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Conflict of interest

The Authors declare that they have no conflict of interests.

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