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Clinical significance of cyclooxygenase-2 (COX-2) in multiple myeloma

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Summary

Several biological and clinical considerations suggest the involvement of cyclooxygenase-2 (COX-2), the key enzyme of prostaglandin (PG) synthesis, in the pathogenesis and progression of haematological malignancies. Despite the wealth of data concerning COX-2 expression, only limited information is available on multiple myeloma (MM). Using standard immunohistochemistry we therefore evaluated COX-2 protein expression in samples from 57 patients with a primary diagnosis of MM. Time to progression and a variety of clinicopathological features were evaluated by the Kaplan-Meier method and the Cox regression model. In addition, COX-2 expression was evaluated by staining bone marrow from healthy donors and 11 patients with MGUS. Overall, 31 MM samples (54%) expressed COX-2. Positivity for COX-2 was unrelated to stage or clinical or molecular features of the disease. However, patients with COX-2 positive tumours experienced a significantly shorter time to progression (17 vs 30 months, p = 0.037). In summary, COX-2 is frequently expressed in MM and correlates with shorter progression-free survival.

Key words: multiple myeloma; cyclooxygenase-2 (COX-2); immunohistochemistry; prognosis

Introduction

Multiple myeloma (MM) is a B-cell malignancy characterised by clonal expansion of plasma cells. It accounts for some 12% of all malignant haematological neoplasms, and despite the availability of novel treatment modalities it is still an incurable disease with variable median survival of a few years. Many attempts have been made in the past to identify routinely applicable prognostic and predictive factors in this disease. Recently COX-2, which is involved in a variety of inflammatory processes and syntheses of prostaglandins, has become a focus of attention in the pathogenesis and prognosis of MM. The COX-2 gene, an immediate-early response gene, is rapidly induced by mitogenic or inflammatory stimuli including tumour promoters, cytokines, growth factors and others. Among these, cytokines such as IL-6 are produced by cells of the bone marrow (BM) microenvironment. These cytokines have been implicated in plasma cell survival and growth, drug resistance and migration of MM cells, as well as myeloma-related phenomena such as the development of bone destruction and anaemia [1]. COX-2 expression as a key mediator of prostaglandins has become a prognostic factor associated with a poor clinical outcome in many solid cancers. To date its role in haematologic malignancies has not been well established. We were recently the first to demonstrate expression of COX-2 in a heterogeneous collection of MM samples from patients at different stages also treated for refractory and recurrent disease. In this study, labour intensive PCR methodology for RNA transcription and Western blotting (WB) showed COX-2 expression to be an independent prognostic factor for both progression-free and overall survival [2]. The aim of the current study was therefore twofold: 1) to evaluate whether COX-2 expression assessed solely by routinely applicable immunohistochemistry (IHC) offers an alternative to the molecular

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methods described for clinical prognosis in MM, and 2) investigate COX-2 expression in an independent and largely homogeneous group of previously untreated patients from three different institutions with monoclonal gammopathy of undetermined significance (MGUS) and MM.

Methods

Standard IHC was performed on a total of 70 specimens (59 MM, 11 MGUS) and the corresponding COX-2 expression was analysed for clinical significance. 12 of the 70 specimens had already been evaluated in our previous study [2]. Samples were obtained either by bone marrow puncture of the posterior iliac crest or surgical resection/biopsy of an extra-medullary lesion for diagnostic procedures. Varying fixation and decalcification procedures were used in the individual institutions before paraffin embedding of the bone-containing samples. Clinical records and follow-up information were available for all patients, as well as demographic data, M gradient and assigned stage according to Durie and Salmon 1975, information being obtained after informed consent and coded for anonymous analysis. β2-microglobulin at diagnosis was available in 38 patients and chromosome 13 deletion assessed by fluorescence in situ hybridisation (FISH) in 14 patients. None of the patients had received chemo-, radio- or immunotherapy before diagnosis. Time to progression was calculated from the time of diagnosis to evidence of progressive disease as defined by the criteria proposed by Blade et al. Patients' clinical characteristics at diagnosis are shown in table 1. To determine COX-2 expression in MM patients at diagnosis, biopsies were stained using the COX-2 murine polyclonal affinity-purified antibody (Ab) (CaymanChemical, Ann Arbor, USA). Immunohistochemistry (IHC) was performed according to the manufacturers' recommendations. Briefly, after deparaffinisation antigen retrieval was performed by pretreatment with cell conditioning solution CC1-Buffer pH 6.0 (Ventana Medical Systems, Tucson, Arizona). Tissue sections were then incubated with the primary COX-2 Ab for 32 minutes (dilution 1:60). Immunoreactivity was visualised using NIEW DAB detection kit (Ventana Medical Systems) developing solutions, containing biotinilated streptavidin horse radish peroxidase and 3`3-diamonobenzidine tetrahydrochloride and Hämalaun for counterstaining. A negative control was performed without primary antibody. HE sections of all biopsies were reviewed independently by the local haematopathologist of the participating institutions. As positive control for the stainability of the different biopsies, which were subjected to various decalcification procedures, an established cytoplasmic plasma cell marker (VS38c, monoclonal, DAKO, Glostrup, Denmark) was applied. COX-2 staining was finally assessed by two different investigators who had no prior knowledge of the clinical parameters (MT and BJ). The proportion of immunostained plasma cells was calculated by evaluating the entire bone marrow trephine or biopsy. When more than 10% of the malignant plasma cells stained moderately to strongly positive for COX-2, the case was scored as positive. Staining intensity was evaluated using a range from 0 (none), 1 (faint), 2 (moderate) to 3 (strong). For comparison, we included four tissue samples containing reactive plasma cells due to inflammation or autoimmune disease, three normal as well as 11 bone marrow trephines from patients diagnosed with MGUS. COX-2 expression was correlated in patients at diagnosis with widely employed prognostic parameters such as age, sex, BM plasmocytosis, Salmon and Durie clinical stage (stage I–III), creatinine (<2 or >2 mg/dL) Hb level (<8 g/dl or >8 g/dl), presence of bone lesions, β_2-microglobulin (>2.4 or <2.4 µg/L), and chromosome 13 deletion assessed by FISH. The Mann-Whitney test and Fisher's exact test were used to compare COX-2 positive and negative cases. A difference between the two groups was considered to be significant for $p < 0.05$. Progression-free survival (PFS) was calculated from the date of diagnosis to the date of progression and was analysed using the Kaplan-Meier method. The log-rank test and Cox regression were applied to analyse the role of clinicopathological parameters and COX-2 staining as predictors for time to progression (TTP). A multivariate Cox regression (backward stepwise likelihood ratio) was used to determine independent predictors. All statistical analyses were performed using SPSS 11.0 (SPSS Inc. Chicago IL).

Results

COX-2 in reactive tonsils was found to be expressed strongly and exclusively in plasma cells, but not in other B-cell or T-cell areas. Plasma cells infiltrating inflamed tissue also stained strongly positive for COX-2 (figure 1 A, B). In contrast, only scattered COX-2-positive cells were detected in normal bone marrow trephines. In patients with MGUS we observed no (5 cases) to faint (6 cases) COX-2 expression in plasma cells, but never such strong expression as that observed in reactive tissues or in MM. Due to reduced quality of trephines only 57 of the initial 59 MM samples were finally evaluable for COX-2 expression. Overall, immunoreactivity for COX-2 protein in MM was scored positive in 31 out of 57 tumours (54%), of which four (7%) were from extramedullary tumour manifestations (one skin, one nasopharyngeal and two soft tissue, all of which exhibited strong homogeneous immunostaining). In the decalcified bone marrow trephines staining intensity varied from faint to strong. The VS38c was strongly positive in all bone-containing biopsies, except in two which were excluded from the study. In contrast, MM from extramedullary localisations presenting large tumour areas all exhibited moderate to strong VS38c expression in most of the tumour cells. The staining pattern of COX-2 in MM is shown in figure 1C–F. Correlations were sought between COX-2 expression at diagnosis and clinicopathological parameters. The results of the statistical analysis for a correlation between COX-2 expression and clinical parameters are shown in

Table 1

Clinical characteristics of patients at diagnosis.

	No	%
Total	57	100
Age median (range)	60 (35–86)	
Sex (M/F)	37/20	65/35
M component		
IgG	46	80
IgA	7	12
Light chain	2	4
Non-secretory	2	4
K/L	45/11	
Salmon and Durie stage		
I	14	25
II–III	43	75
Extramedullary disease	4	7
β_2-MG >2.4 mcg/L	21	55*
Creatinine >2 mg/L	4	7
Hb level <8 g/dL	3	5
Bone lesions >1	34	60
Del13q**	7	50 ***
First line treatment		
Conventional	37	65
Autologous stem cell transplantation	14	25
Thalidomide + conventional	6	10

* β_2-MG (microglobulin) available in 38 pts.
** Assessed by FISH.
*** Del13q available in 14 pts.

Table 2

COX-2 expression and clinical parameters.

	COX-2 POS	COX-2 NEG	p
Age median (range)	64 (36-86)	63 (35-78)	0.97
Sex M/F	19/12	18/8	0.58
Salmon and Durie Stage			
Stage I	9/31 (29%)	5/26 (19%)	0.38
Stage II	9/31 (29%)	5/26 (19%)	0.38
Stage III	12/31 (42%)	17/26 (62%)	0.12
β_2-MG >2.4 mcg/L	9/16 (56%)	12/22 (54%)	0.95
Creatinine >2 mg/L	2/31 (6%)	2/26 (8%)	0.92
Hb level <8 g/dL	1/31 (3%)	2/26 (8%)	0.52
Bone lesions >1	18/31 (58%)	16/26 (62%)	0.95
Del13q*	4/7 (57%)	3/7 (43%)	1.00

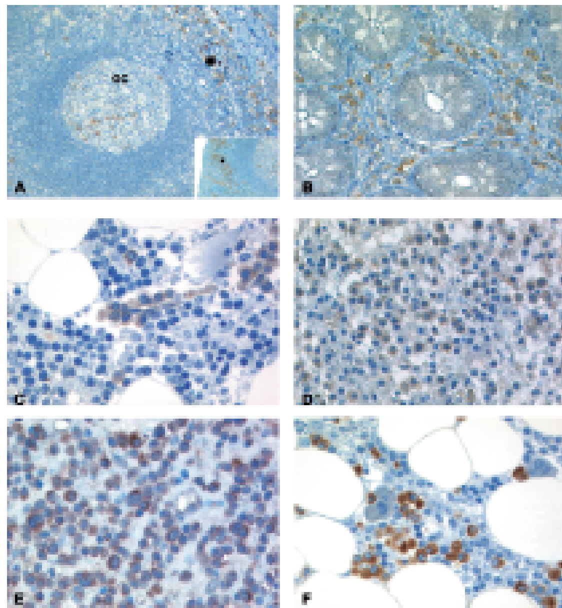
Table 3

Prognostic factors for time to progression by univariate Cox regression.

Prognostic factors	Hazard ratio	95% CIa	p
Age	0.99	0.96–1.03	0.78
Sex	1.53	0.78–3.00	0.22
Stage	0.96	0.64–1.43	0.84
β_2-MG	1.33	0.62–2.86	0.47
COX-2 pos	2.01	1.01–4.01	0.046

[a] 95% CI, lower and upper limits of the confidence interval

Figure 1



COX-2 expression in reactive tissues, MGUS and multiple myeloma (MM).
A Tonsil with strong positivity in plasma cells in the sub-endothelial area (asterisk) and in germinal centre (GC).
B Scattered plasma cells expressing COX-2 in an inflamed appendix.
C Faint but distinct positivity in MGUS.
D Faint expression of COX-2 in MM.
E and **F**: As positive scored MMs with **E** moderate and **F** strong staining intensity.

Figure 2

Kaplan Meier estimate of probability of TTF in previously untreated multiple myeloma patients according to COX-2 expression status at diagnosis. Dotted line indicates COX-2 positive and solid line COX-2 negative patients by immunohistochemical expression analysis (p = 0.037).

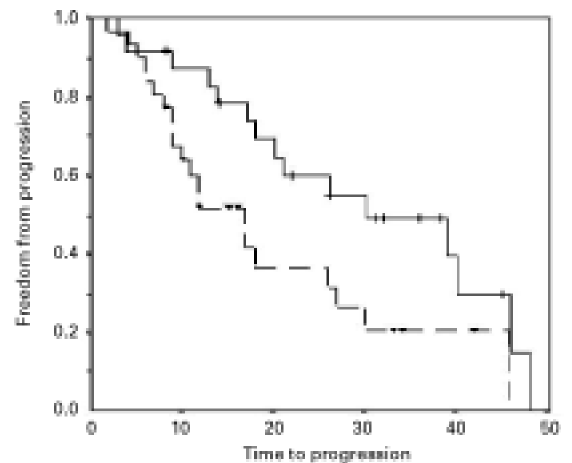


table 2. COX-2 positivity was not distributed differently in relation to age, gender, stage, creatinine, Hb level and presence of bone lesions. Neither could we find a correlation between COX-2 expression on tumour and elevated (>2.4 mg/l) β_2-microglobulin values, and no difference occurred with respect to deletion of chromosome 13q (assessed by FISH), although our analysis may be limited due to the number of genetic analyses available with and without deletion of chromosome 13q respectively. An outcome analysis based on PFS was performed in patients assessed at diagnosis. The median follow up period was 30 months (range 2–60 months). During the follow-up period disease progression was observed in 39 patients (67%). The results obtained from the analysis of clinicopathological parameters predicting progression-free survival are shown in table 3. According to univariate analysis neither age, sex, stage nor β_2-microglobulin showed statistical significance.

However, there was a significant difference for progression-free survival according to COX-2 expression on tumours. Patients with COX-2 positive tumors exhibited a significantly shorter time-to-progression interval: at diagnosis the median time to progression was 17 months in COX-2-positive and 30 months in COX-2-negative patients (p = 0.037, log-rank test). Figure 2 shows the TTP curves in our population according to COX-2 status. Of note is the finding that when backward stepwise Cox regression was used for multivariate analysis of prognostic factors, COX-2 expression (HR 2.01, 95% CI 1.01–4.01; p = 0.046) remains the only significant prognostic factor as well.

Discussion

Among blood-related neoplastic disorders, MM is an interesting disorder to evaluate for a disturbance of the COX-2 and PG pathways, since malignant plasma cells are strictly dependent on microenvironmental factors for survival [3]. Employing standard IHC, evaluation of sections from previously untreated MM patients showed that progression-free survival is significantly impaired in patients who scored positively for COX-2 immunoreactivity as compared to COX-2 negative tumours (17 months vs 30 months; p = 0.037). These data are in line with TTP in our previous molecular study (18 months vs 36 months; P <0.001) performed in 122 patients, as well as with the results from solid cancers and from one study investigating a variety of non-Hodgkin lymphomas presented so far [2–4]. However, COX-2 was not associated with any of the commonly used clinicopathological and prognostic indicators evaluated, an observation which also remains unclear in tumours other than MM. Although a correlation between COX-2 and survival analysis in MM could be established in patients at different phases of the disease, the present study appeared to be limited due to the number of events. As could be expected from its biological role, COX-2 was found to be expressed in reactive plasmocytosis and to a slight extent in MGUS, and so does not necessarily represent a suitable marker to discriminate reactive from pre-malignant or malignant plasma cell disorders. Future analysis may focus on identification of MM patients with primary resistance to chemotherapy, possibly induced by the expression of a multidrug resistance gene in the malignant clone, and correlation to parameters with established prognostic relevance in MM [5]. In addition, the functional status and impact of COX-2 and its modification by different factors such as NF-kappaB, HIF-1 and cyclin D1, as well as PG-receptor expression in the tumour microenvironment of MM, is currently being explored [6–8].

In summary, we were able to reproduce earlier mRNA findings on COX-2 expression merely by IHC on a smaller number of initially untreated MM patients. Since paraffin immunohistochemistry is routinely available, robust and inexpensive, we suggest that COX-2 IHC in multiple myeloma should be evaluated in a prospective study for routine staining using a stringent protocol for fixation and decalcification steps. Patients with COX-2-positive multiple myeloma may then benefit from new targeted inhibitory therapy [9].

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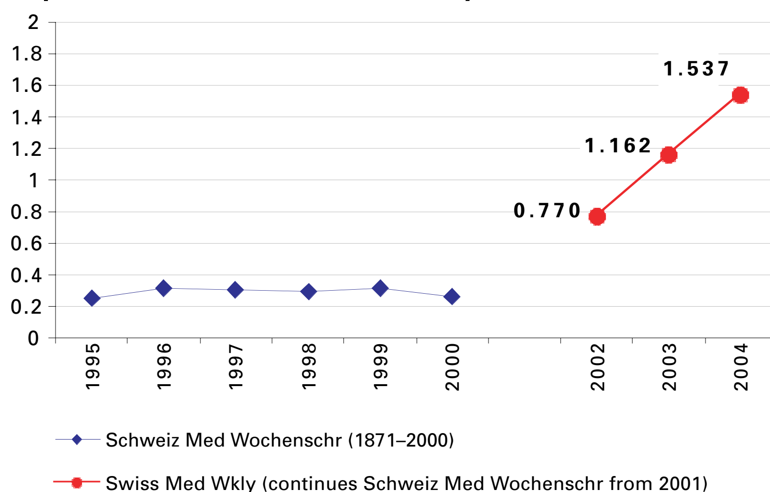

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