ORIGINAL PAPER

Immunohistochemical expression of the cyclooxygenase-2 (COX-2) in gastric cancer. The correlations with the tumor angiogenesis and patients' survival

DANIELA LAZĂR[1)], SORINA TĂBAN[2)], CARMEN ARDELEANU[3)], CRISTIANA SIMIONESCU[4)], I. SPOREA[1)], MARIOARA CORNIANU[2)], CORINA VERNIC[5)]

[1)]*Department of Gastroenterology*

[2)]*Department of Pathology*

"Victor Babeş" University of Medicine and Pharmacy, Timisoara

[3)]*Department of Pathology,*
"Victor Babeş" National Institute for Research and Development in Pathology and Biomedical Sciences, Bucharest

[4)]*Department of Pathology,*
University of Medicine and Pharmacy of Craiova

[5)]*Department of Biostatistics,*
"Victor Babeş" University of Medicine and Pharmacy, Timisoara

Abstract

Introduction: The mechanisms by which COX-2 contributes to the carcinogenesis are not known until present. It seems that the COX-2 enzyme stimulates the cell proliferation, inhibits the apoptosis, increases the malignant cells' invasiveness and induces the angiogenesis by elaborating some angiogenic factors. *Material and methods*: In the present study, we intend to evaluate the immunohistochemical expression of COX-2 in gastric carcinomas, keeping track of the correlations between the clinicopathologic factors, the tumor angiogenesis (evaluated by microvascular density – MVD – determination and by VEGF expression) and the patients' survival. In addition, we have tracked the immunoreactions' positivation in the peritumoral mucosa with various lesions, with the purpose to establish the contribution of COX-2 to the gastric carcinogenesis during the pre-invasive stages. A prospective study was realized, regarding the evolution and aggressiveness of the gastric cancer, with a duration of five years, 61 patients operated of gastric cancer being included. *Results*: The COX-2 immunoreactions have been significantly more frequent noticed in the gastric carcinomas included in the study (57.4%) and in the epithelial dysplasia areas adjacent to the carcinomas of intestinal type (35.5% of the cases), than in the normal peritumoral mucosa (4.9%) ($p<0.001$ ES). The COX-2 immunoreactions have turned positive more frequently in gastric carcinomas of intestinal type (68.4%), in comparison to the carcinomas of diffuse type (29.4%) ($p<0.001$ ES). The COX-2 expression is significantly correlated with the invasion level, the presence of the metastases in the regional lymph nodes and the pTNM stage, but without influencing the prognosis of the gastric cancer patients. The negative VEGF carcinomas have turned positive for COX-2 only for 19% of the cases. Different from those, the positive VEGF carcinomas have associated COX-2 immunoreactivity in 77.5% of the cases. *Conclusions*: The results obtained are suggestive for the predominant expression of COX-2 in the carcinomas of intestinal type and its precursory lesions. Our results show a tight correlation between the immunohistochemical expressions of COX-2 and VEGF in gastric carcinomas ($r = 0.562$, $p<0.001$ ES) and also a MVD average value significantly higher in the positive COX-2 carcinomas, suggesting an intense angiogenesis activity in that group of tumors ($p<0.001$ ES).

***Keywords*:** COX-2, gastric cancer, tumor angiogenesis, survival.

Introduction

The cyclooxygenase (COX) represents the key enzyme with bifunctional activity (peroxydasic and cyclooxygenasic), implicated in the formation of the prostaglandins from the arachidonic acid. Two COX forms have been described: the COX-1 isoenzyme, a component of the normal cells, and the COX-2 isoenzyme, frequently undetectable in most normal tissues, but quickly induced by cytokines, growth factors and carcinogenous agents. The mechanisms by which COX-2 contributes to the carcinogenesis are not known until present. It seems that the COX-2 enzyme stimulates the cell proliferation, inhibits the apoptosis, increases the malignant cells' invasiveness and induces the angiogenesis by elaborating some angiogenic factors.

Material and methods

In the present study, we intend to evaluate the immunohistochemical expression of COX-2 in gastric

carcinomas, keeping track of the correlations between the clinicopathologic factors, the tumor angiogenesis (evaluated by microvascular density – MVD – determination and by VEGF expression) and the patients' survival. In addition, we have tracked the immunoreactions' positivation in the peritumoral mucosa with various lesions, with the purpose to establish the contribution of COX-2 to the gastric carcinogenesis during the pre-invasive stages.

A prospective study was realized regarding the evolution and aggressiveness of the gastric cancer, with a duration of five years, 61 patients operated of gastric cancer in the Surgical Sections of the No. 1 County Hospital of Timisoara being included. The surgical interventions, of curative or palliative intention, were not preceded by chemo- or radiotherapy treatment. The patients' survival was tracked for a variable period, between one month and 68 months. For each case, clinical and morphological data were collected.

The gastric operational pieces have been morphologically analyzed, by microscopic and macroscopic examination with usual histological, histochemical and immunohistochemical staining. The gastric carcinomas were classified and interpreted according to the evaluation protocol recommended by *The American Joint Committee on Cancer* (AJCC) and *The International Union Against Cancer* (IUAC) from January 2005. The survival period was calculated starting with the month when the surgical intervention took place and up to the month of the demise or of the confirmation of survival, and the survival rate was represented by the survivals percentage at the end of the tracked interval (in years and months).

The immunohistochemical reactions were performed using the LSAB+ (DAKO, Denmark) technique, the overnight incubation in moist room, at room temperature, with the COX-2 primary antibody, in 1 : 50 dilution, and then the applying of the secondary antibody for 30 minutes, 45 minutes incubation with the ABC complex, DAB visualization and Meyer's Hematoxylin counterstaining.

The COX-2 immunohistochemical expression was evaluated using the semi-quantitative analysis, establishing for each case a corresponding score for the sum between:

- the positive cells' percentage: 0 = 0% immuno-positive cells; 1 = <25% positive cells; 2 = 26–50% positive cells; 3 = >50% positive cells;
- the intensity of staining: 0 = negative immuno-reaction; 1 = weak intensity; 2 = moderated intensity; 3 = strong intensity.

The sum of the two parameters for three representative microscopic fields varied between 0 and 6. In our study, we considered:

- a negative immunoreaction (-), for a score between 0 and 2;
- a weakly positive immunoreaction (+), for a score between 3 and 4 (Figure 1);
- a strongly positive immunoreaction (++), for a score between 5 and 6 (Figure 2).

The cases with scores higher than 3, have been quantified as positive immunoreactions.

Results

The positive COX-2 immunoreactions were predominantly localized in the cytoplasm of the tumor cells, in some cases having perinuclear pattern. We have noticed different types of immunostainings: diffuse or focal, of various intensities (Figure 3).

Generally, the most COX-2 positive malignant cells were identified in the invasion front. In some carcinomas, the vascular endothelium, the fibroblasts and the inflammatory cells were stained in various shades of brown.

The quasi-normal gastric mucosa in the carcinomas' vicinity expressed COX-2 in a limited number of cases, especially in the profound glands. In exchange, we have noticed some cases with immunostainings at the level of some epithelial cells' groups or modified glands in the peritumoral area.

The COX-2 immunoreactions have been observed significantly more frequent in the gastric carcinomas (35 cases – 57.4%) than in the peritumoral normal mucosa (three cases – 4.9%) (Table 1).

Table 1 – ***The COX-2 expression in the gastric cancer and the normal peritumoral tissues***

Lesion	Total no. of cases	COX-2 expression		P
		-	+~++ *(%)*	
Gastric carcinomas	61	26	35 (57.4%)	<0.001 ES
Normal peritumoral mucosa	61	58	3 (4.9%)	
Intestinal metaplasia	34	31	3 (8.8%)	0.09014 FS
Epithelial dysplasia	31	20	11 (35.5%)	

In three cases (8.8%), we have encountered weakly positive and focal immunoreactions at the level of the metaplastic glands associated to the gastric carcinomas of intestinal type.

The epithelial dysplasia from the vicinity of the carcinomas of intestinal type expressed COX-2 in 35.5% of the cases (Figure 4), significantly more frequent in comparison with the normal peritumoral mucosa (p = 0.000384 ES), as well as with the intestinal metaplasia areas.

Our results do not show a correlation between the sex and age of the patients and the COX-2 expression (Table 2).

Table 2 – ***Correlations between the COX-2 expression and the clinicopathologic factors***

Clinicopathologic factors		COX-2 expression		P
		- *(n = 26)*	+~++ *(%) (n = 35)*	
Sex	Men	17	26 (60.5%)	0.202778 NS
	Women	9	9 (50%)	
Age	≤60 years	12	17 (58.6%)	0.714253 NS
	≥61 years	14	18 (56.3%)	
Location	Antrum	14	17 (54.8%)	>0.05 NS
	Body	7	8 (53.3%)	
	Pangastric	4	6 (60%)	
	Cardia	0	2 (100%)	
	Gastric remnant	1	2 (66.7%)	

Depending on the tumors location, we have obtained positive immunoreactions in 54.8% of the antral

carcinomas, 53.3% of the corporeal carcinomas, 60% of the pangastric carcinomas and 66.7% from the carcinomas developed on the gastric remnant. We would like to mention that both carcinomas developed in the proximal area of the stomach have become positive for COX-2.

The COX-2 immunoreactions have become significantly positive more frequently in gastric carcinomas of intestinal type (68.4%), in comparison to the carcinomas of diffuse type (29.4%, Figure 5 and Table 3).

Table 3 – ***The relation between the COX-2 expression and the gastric cancer***

Clinicopathologic factors		COX-2 expression - (n = 26)	COX-2 expression +~++ (%) (n = 35)	P
Lauren classification	Intestinal type	12	26 (68.4%)	0.00014 ES
	Diffuse type	12	5 (29.4%)	
	Mixed type	2	4 (66.7%)	
Histologic type	Tubular adenocarcinoma	7	21 (75%)	0.004108 FS
	Papillary adenocarcinoma	2	3 (60%)	
	Mucinous adenocarcinoma	3	5 (62.5%)	
	Signet ring cell carcinoma	12	5 (29.4%)	
	Undifferentiated carcinoma	2	1 (33.3%)	
Histologic grade	G1	0	2 (100%)	<0.001 ES
	G2	6	14 (70%)	
	G3	20	19 (48.7%)	
Lympho-vascular invasion	Present	15	23 (60.5%)	0.361114 NS
	Absent	11	12 (52.2%)	

The data obtained are suggestive for the predominant COX-2 expression in the carcinomas of intestinal type and the lesions that forerun them (epithelial dysplasia and in a smaller amount, the intestinal metaplasia). The COX-2 immunopositivity appears in our study as a precocious event in the sequence involved in the gastric carcinoma of intestinal type development.

Among the histological forms, we have obtained positive reactions in 75% of the tubular adenocarcinomas, 60% of the papillary adenocarcinomas and 62.5% of mucinous adenocarcinomas. The COX-2 expression was significantly rarer in the signet-ring cell carcinoma (29.4%) and the anaplastic carcinoma (33.3%).

The immunoreactivity for COX-2 has been significantly higher in strongly differentiated carcinomas (100%, Figure 6) and moderately differentiated carcinomas (70%), in comparison with the weakly differentiated carcinomas (48.7%).

The obtained results do not show a relation between the COX-2 expression and the lymphovascular invasion.

The COX-2 expression is significantly correlated with the level of invasion. We have noted positive immunoreactions in 25% of the pT1 carcinomas, 55.6% of the pT2 carcinomas, 58.8% of the pT3 carcinomas and 63.3% of the pT4 carcinomas (Table 4).

Table 4 – ***The relation between the COX-2 expression and the gastric cancer's clinicopathologic factors***

Clinicopathologic factors		COX-2 expression - (n = 26)	COX-2 expression +~++ (%) (n = 35)	P
pT	Tis	1	0 (0%)	0.04269 S
	T1	3	1 (25%)	
	T2	4	5 (55.6%)	
	T3	7	10 (58.8%)	
	T4	11	19 (63.3%)	
pN	N0	11	7 (38.9%)	0.031167 S
	N1	7	9 (56.3%)	
	N2	7	16 (69.6%)	
	N3	1	3 (75%)	
pM	M0	20	27 (57.4%)	0.983895 NS
	M1	6	8 (57.1%)	
pTNM	0	1	0 (0%)	0.035729 S
	IA	2	1 (33.3%)	
	IB	3	2 (40%)	
	II	4	3 (42.9%)	
	IIIA	4	7 (63.6%)	
	IIIB	3	5 (62.5%)	
	IV	9	17 (65.4%)	

In addition, the COX-2 expression is significantly correlated with the presence of the metastases in the regional lymph nodes (38.9% of the pN0 carcinomas, 56.3% of the pN1 carcinomas, 69.6% of the pN2 carcinomas and 75% of the pN3 carcinomas). The presence of the distant metastases does not influence the COX-2 expression (positive immunoreaction in 57.4% of the pM0 carcinomas and 57.1% of the pM1 carcinomas).

The positive COX-2 immunoreactions have been encountered more frequently in the advanced pTNM stages: IIIA (63.6%), IIIB (62.5%) and IV (65.4%).

The patients' survival depending on the COX-2 expression has presented the following distribution:

- for the negative COX-2 carcinomas: 12 patients for one year, eight patients for 2 years, six patients for 3 years and five patients for 4 and 5 years;
- for the positive COX-2 carcinomas: 11 patients for one year, six patients for 2 and 3 years, five patients for 4 and 5 years.

The final 5-year survival rate was of 14.3% for the patients with COX-2 positive carcinomas, slightly lower than the 19.2% rate for the patients with COX-2 negative carcinomas ($p>0.05$ NS) (Figure 7).

Calculating in months the average survival, we obtained the same slight difference, statistically insignificant, for the two different groups of patients (20.4 months for the patients with negative COX-2 carcinomas and 15.2 months for the patients with positive COX-2 carcinomas) ($p>0.05$ NS) (Figure 8).

In order to evaluate a relation between the tumor angiogenesis and the immunohistochemical expression of COX-2 we have tracked the microvascular density (MVD) and the VEGF expression in the two gastric carcinomas groups: negative COX-2 (26 cases) and positive COX-2 (35 cases) (Table 5).

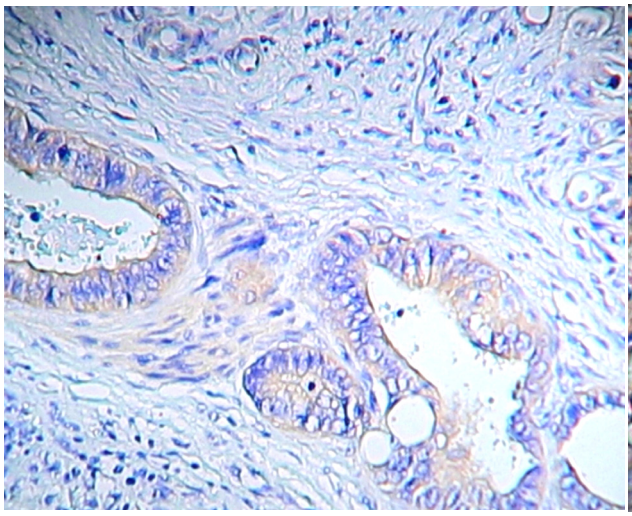


Figure 1 – ***Gastric adenocarcinoma with weakly positive COX-2 immunoreaction. DAB***

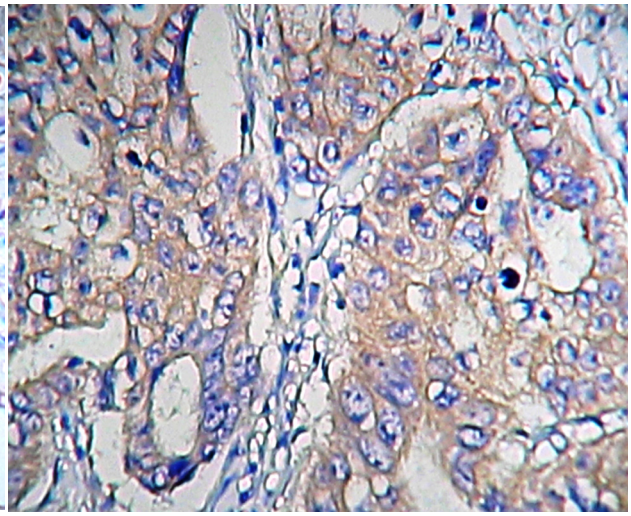


Figure 2 – ***Gastric adenocarcinoma with strongly positive COX-2 immunoreaction. DAB***

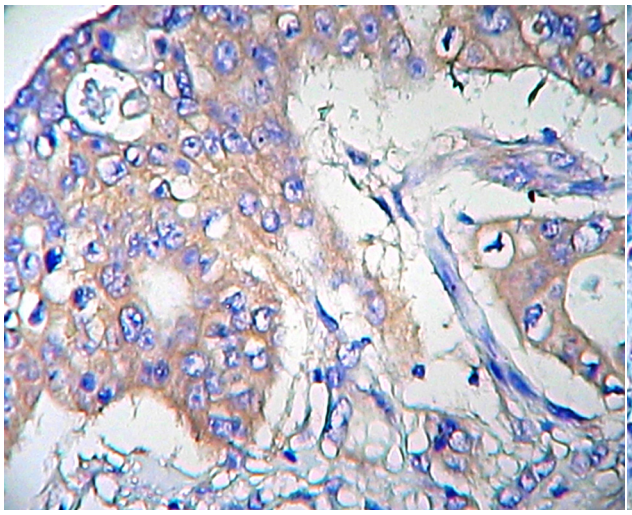


Figure 3 – ***COX-2 immunostaining with diffuse cytoplasmatic pattern. DAB***

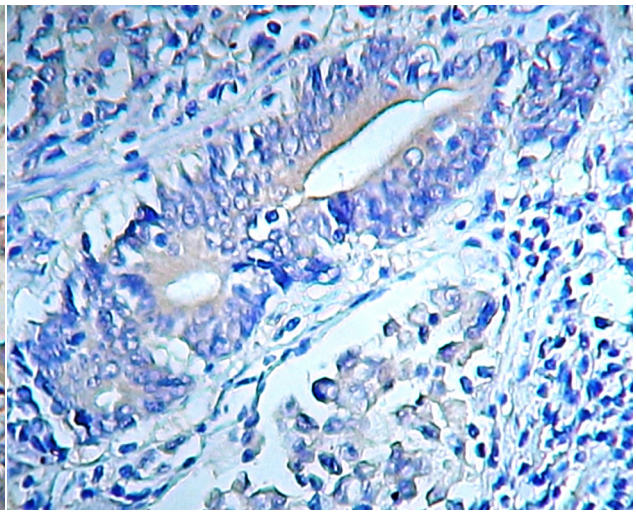


Figure 4 – ***Positive COX-2 immunoreaction in the dysplastic glands. DAB***

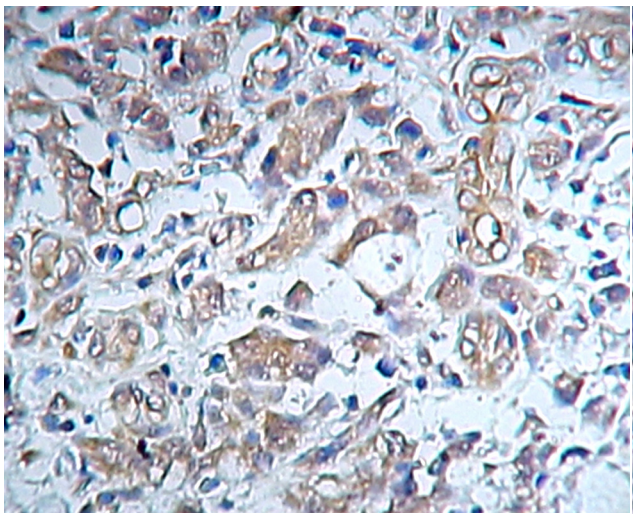


Figure 5 – ***Gastric carcinoma of diffuse type. COX-2 immunoreaction, DAB***

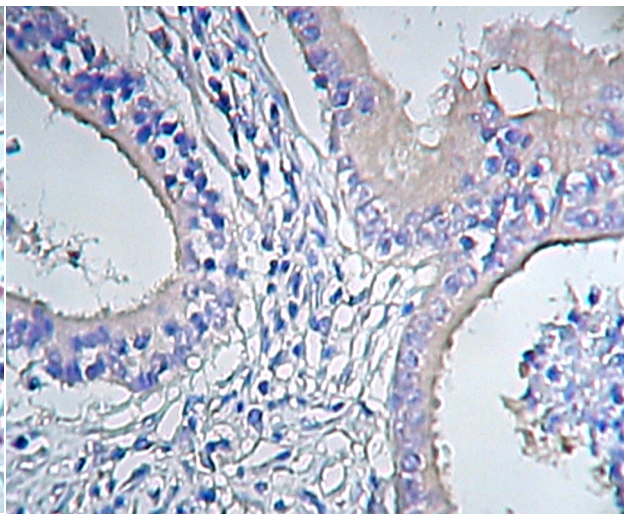
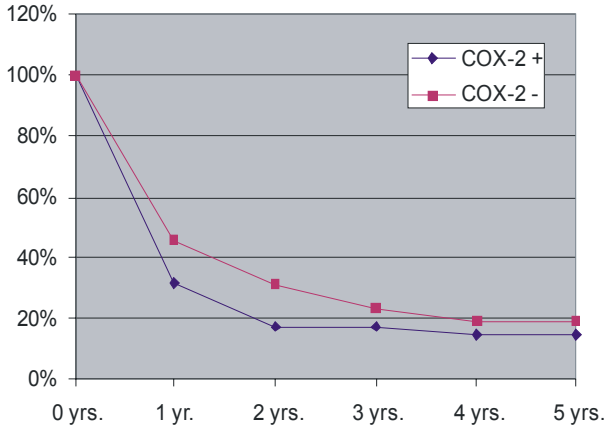


Figure 6 – ***Positive COX-2 immunoreaction in strongly differentiated adenocarcinoma. DAB***



	1 year	2 years	3 years	4 years	5 years
COX-2 +	31.4%	17.1%	17.1%	14.3%	14.3%
COX-2 -	46.2%	30.8%	23.1%	19.2%	19.2%

Figure 7– ***The 5-year survival depending on the COX-2 immunohistochemical expression***

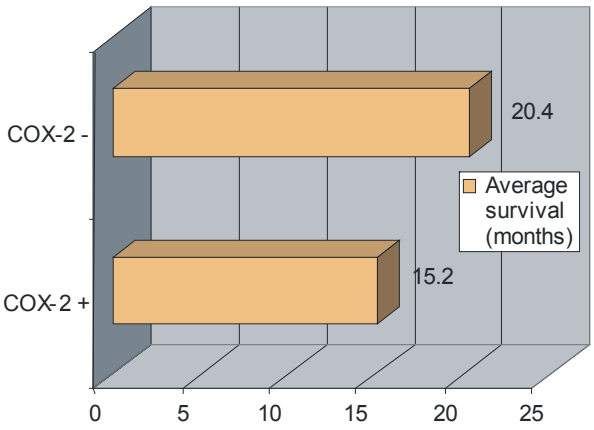


Figure 8 – ***Average patients' survival (in months) depending on the COX-2 expression***

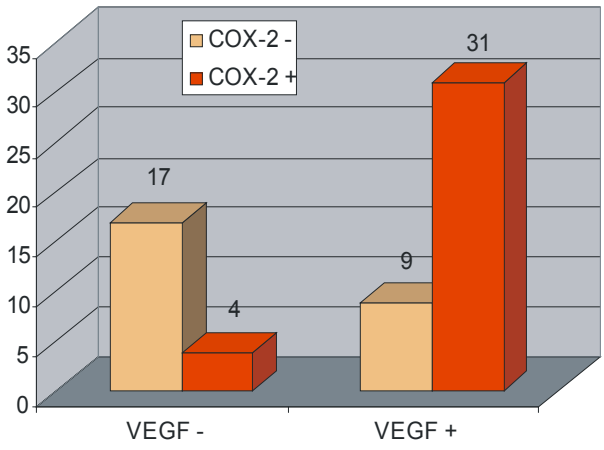


Figure 9 – ***The relation between VEGF and COX-2 in gastric carcinomas***

Table 5 – ***The relation between COX-2, VEGF and MVD in gastric carcinomas***

Parameter		COX-2 expression		P
		- (n = 26)	*+~++ (%) (n = 35)*	
Average DMV		31.5	39.4	<0.001 ES
VEGF	- (n = 21)	17	4 (19%)	
	+~++ (n = 40)	9	31 (77.5%)	

The average MVD value was significantly higher in the COX-2 positive carcinomas (39.4), suggesting an intensive angiogenesis activity within this group of tumors. For the negative COX-2 carcinomas, we have registered an average MVD value of 31.5.

Our results show a direct tight correlation ($r = 0.562$, $p<0.001$ ES) between the immunohistochemical expressions of COX-2 and VEGF in the gastric carcinomas (Figure 9). The VEGF negative carcinomas were positive for COX-2 only in 19% of the cases. Unlike these, the VEGF positive carcinomas have associated immunoreactivity for COX-2 in 77.5% of the cases.

Discussion

COX represents a key enzyme involved in the arahidonic acid conversion in prostaglandins, here being identified two isoforms COX-1 and COX-2. COX-1 is expressed in different tissues and is considered involved in various physiological functions, while COX-2 is induced by pathologic stimuli like the inflammation, various growth factors and cytokines produced by the tumor cells [1–5].

The COX-1 gene is therefore considered the gene responsible with the synthesis of the prostanoids involved in the protection of the gastrointestinal mucosa and in the production of the pro-aggregative thromboxane by the thrombocytes. Apart from this, the role of COX-2 is connected with the inflammation, reproduction, angiogenesis and carcinogenesis [6–9].

The contribution of COX-2 at the tumor angiogenesis includes the growth of the VEGF expression, the production of E prostaglandin (PGE)2 and I prostaglandin (PGI)2, which may stimulate directly the migration of the endothelial cells and the angiogenesis induced by the growth factors, as well as the endothelial cells' inhibition by Bcl-2 or Akt stimulation [10, 11].

Numerous studies were conducted in order to establish the relation between COX-2 and the tumor angiogenesis, as well as the development and the progression of the gastric cancer.

The normal gastric mucosa expresses COX-1, but the COX-2 value is under the detection limit. The studies showed that the COX-2 expression is increased in the gastric adenocarcinomas, in comparison with the non-neoplastic mucosa [11]. The data from literature have shown a positive immunoreaction of COX-2 on the histological sections of gastric cancer in a percentage of 43–100% (62% in average) of the cases [12–22].

Some authors have detected a positive reaction for COX-2 exclusively in the neoplastic epithelial cells, while others have detected an intense immunoreactivity also at the level of stromal cells, these differences probably due to the different manners of antibodies' preparation [16, 18, 20, 23, 24].

The COX-2 immunoreactions have been observed significantly more frequent in the gastric carcinomas included in the study (35 cases – 57.4%) and in the epithelial dysplasia close to the carcinomas of intestinal

type (35.5% of the cases), than in the normal peritumoral mucosa (three cases – 4.9%). In three cases (8.8%), we have encountered weakly positive and focal immunoreactions at level of the metaplastic glands associated with gastric carcinomas of intestinal type.

Sun WH *et al.* did not emphasize a significant correlation between the COX-2 expression and the age, sex and tumor localization, the histological type and the tumor differentiation type [25]. Our results do not show a correlation between the sex and the age of the patients and the COX-2 expression. Depending on the tumors' location, we have obtained positive immunoreactions in 54.8% of the antral carcinomas, 53.3% of the corporeal carcinomas, 60% of the pangastric carcinomas and 66.7% of the carcinomas developed on the gastric remnant. We would like to mention that both carcinomas developed in the proximal region of the stomach have turned positive for COX-2.

Most of the studies show a predominant expression of COX-2 in the gastric cancer of intestinal type and in its forerunning lesions [11, 19, 26]. The immunohistochemical expression of the COX-2 protein was accentuated in 58% of the intestinal type carcinomas, 44% of the dysplasia and only in 6% of the carcinomas of diffuse type [27, 28]. By using the RT–PCR technique, the COX-2 ARNm was increased in the intestinal metaplasia, in comparison with the normal tissues. By applying accurate immunohistochemical techniques, in the normal tissues have been detected comparable levels of the COX-2 protein expression, respectively with intestinal metaplasia. The protein's expression was significantly higher in the dysplastic tissue vs. the normal mucosa. These data suggest that, even the COX-2 transcription levels are already increased in the intestinal metaplasia stage, only the neoplastic cells (the dysplasia and the invasive carcinoma) express high levels of COX-2 protein. Therefore, the COX-2 expression seems to represent a rather precocious event in the gastric cancer of intestinal type's carcinogenesis sequence, because it appears even in the non-invasive stage of the tumorigenesis [22, 29, 30].

The immunoreactions for COX-2 have become positive in our study much more frequently in the gastric carcinomas of intestinal type (68.4%), in comparison with the carcinomas of diffuse type (29.4%). The obtained data are suggestive for the predominant expression of COX-2 in the carcinomas of intestinal type and in their forerunning lesions (the epithelial dysplasia and in smaller amount, the intestinal metaplasia). The COX-2 immunopositivation appears in our study as a precocious event in the sequence involved in the gastric carcinoma of intestinal type's development.

From the histological forms, we have obtained positive reactions in 75% of the tubular adenocarcinomas, 60% of the papillary adenocarcinomas and 62.5% of the mucinous adenocarcinomas. The COX-2 expression was more rarely in the signet-ring cell carcinoma (29.4%) and in the anaplastic carcinoma (33.3%). The COX-2 immunoreactivity was significantly higher in the well-differentiated (100%) and moderately differentiated carcinomas (70%), in comparison with the weakly differentiated carcinomas (48.7%). The obtained results do not emphasize a relation between the COX-2 expression and the lymphovascular invasion. Numerous studies prove a correlation between the COX-2 expression and the invasion's level, the presence of lymph node metastases and the advanced stage; some authors also suggest a correlation with the great tumor's dimension. As a result, the overexpression of COX-2 may induce an aggressive biological behavior of the neoplasm, involved in the invasion and metastasis process [16, 18, 20, 23, 31].

The COX-2 expression in the studied cases is significantly correlated with the level of invasion. We have noted positive immunoreactions in 25% of the pT1 carcinomas, 55.6% pf the pT2 carcinomas, 58.8% of the pT3 carcinomas and 63.3% of the pT4 carcinomas. In addition, the COX-2 expression is significantly correlated with the presence of the metastases in the regional lymph nodes (38.9% of the pN0 carcinomas, 56.3% of the pN1 carcinomas, 69.6% of the pN2 carcinomas and 75% of the pN3 carcinomas). The presence of distant metastases does not influence the COX-2 expression (positive immunoreactions in 57.4% of the pM0 carcinomas and 57.1% of the pM1 carcinomas).

Some studies have emphasized a COX-2 expression significantly higher in patients in the III and IV stage or with lymph node metastases, comparatively to the patients in I and II stage or without lymph node metastases [32]. The positive COX-2 immunoreactions have been more frequently encountered, in the case of our group, in advanced pTNM stages: IIIA (63.6%), IIIB (62.5%) and IV (65.4%).

A series of studies has shown that the COX-2 expression is correlated with clinicopathologic variables in the gastric cancer, as the level of invasion, the tumor's dimension, the lymph node metastases, the tumor's stage and MVD [15, 16, 20, 28, 33–37], but the association between COX-2 and the survival is controversial [14, 18]. Unlike these, in oesophageal and colorectal adenocarcinomas, some studies have shown a weak or even absent correlation between the COX-2 expression and the gastric cancer patient's prognosis [15, 17]. These observations suggest that the prognosis value of COX-2 is restrained at a certain sub-group of patients with gastric cancer, or that the role of COX-2 is different at the gastric level, in comparison with the adenocarcinomas developed in other regions of the gastrointestinal tract. The existing data show that in the case of carcinomas of the cardia, COX-2 does not predict the survival, but there can be noticed a survival reduction tendency in precocious carcinomas with an elevated COX-2 expression [38]. Other studies describe a correlation between COX-2 and survival, the expression of this protein being considered as an independent prognostic factor in patients with gastric cancer (Mrena J *et al.*; Shi H *et al.*) [32, 36]. In the Shi H *et al.* study, in which there were not included tumors exceeding the serosa, the 5-year survival in COX-2 positive patients was of 67.9% *vs.* 91.4% for the COX-2 negative patients [36].

The final 5-year survival rate for the studied cases was of 14.3% for the COX-2 positive carcinoma patients, slightly lower than the rate of 19.2% for the COX-2 negative carcinoma patients. Calculating in months the average survival, we have obtained the same reduced difference, statistically insignificant for the two groups of patients (20.4 months for the COX-2 negative carcinomas patients versus 15.2 months for the COX-2 positive carcinoma patients).

The literature data show a significant association between the COX-2 expression and VEGF. The average MVD value is significantly higher in COX-2 and VEGF positive tumors, in comparison with the COX-2 and VEGF negative tumors. These data suggest that VEGF and COX-2 are involved in the angiogenesis in the development of the gastric cancer and that VEGF plays a main role in the COX-2 stimulated angiogenesis [39].

In order to evaluate a relation between the tumor angiogenesis and the COX-2 immunohistochemical expression, we have tracked the microvascular density (MVD) and the VEGF expression in the two groups of gastric carcinomas: negative COX-2 (26 cases) and positive COX-2 (35 cases). The average MVD value was significantly higher in the positive COX-2 carcinomas (39.4), suggesting an intense angiogenesis activity within this group of tumors. For the negative COX-2 carcinomas, we have registered an average MVD value of 31.5. Our results show a tight correlation between the immunohistochemical expressions of COX-2 and VEGF in gastric carcinomas. The VEGF negative carcinomas turned positive for COX-2 only in 19 percent. Differently from these, the positive VEGF carcinomas have associated COX-2 immunoreactivity in 77.5% of the cases.

The epidemiologic studies suggest that the gastric cancer development risk is reduced in association with the use of aspirin, the majority of the data suggesting a protective effect of aspirin. To be remarked that generally has been noticed a reduction of the risk of development of gastric cancer of intestinal type, without a clear influence upon the gastric cancer of diffuse type. Moreover, a protective effect of AINS was noticed only in *Helicobacter pylori* positive patients, but not in the case of the ones not infected. These data suggest that the population subgroup, which would benefit by the use of AINS with the purpose of reduction of the gastric cancer apparition, includes the subjects with risk regarding gastric cancer of intestinal type, associated to the *H.p.* infection. In order to obtain a statistically significant protective effect, the aspirin has to be administered for a long period. However, severe adverse gastrointestinal effects [39–41] may accompany this administration. For this reason, the studies have concentrated on the selective COX-2 inhibitors. The experiments where, by the inhibition of COX-2 activity, in APC mice is reduced the polyps' growth are well known, as also those by which Sulindac and Celecoxib determine the regression of the colorectal adenomas in patients with familial adenomatous polyposis (FAP) [42, 43]. An experimental study of gastric carcinogenesis on mice with deficit of „trefoil factor-1" has shown that Celecoxib has suppressed the tumors' development. Recently, was shown that the transgenic expression of COX-2 and of the PGE's microsomal synthetase induces the development of hyperplasic tumors in mice and also that Celecoxib reduces the gastric carcinogenesis chemically induced in rats [44].

Clinically, it is important to determine if the selective COX-2 inhibitors present on one hand less adverse effects, and on the other hand, antineoplastic properties, at least as efficient comparatively to unselective AINS. Moreover, the premalignant lesions or invasive neoplasm's types must be recognized (based on histology, stage and/ore genotype), that are sensible to this therapeutic agents [45].

The performing of new studies is necessary, in order to evaluate the efficiency of the COX-2 selective inhibitors, by their integration in existing therapeutic protocols, as neoadjuvant therapy in the gastric cancer's treatment. A new therapeutic option could be represented by the combination of AINS and the growth factors' receptors from the Erb/HER family [46].

In addition, the application of the COX-2 and VEGF immunostainings on the endobioptic fragments before the surgical treatment could be used in the prediction of the clinical evolution and the pre-surgical selection of the adjuvant therapy in gastric cancer patients. Regarding this, the COX-2 activity inhibition could have an important therapeutic effect in the control of the gastric neoplasm [47].

Conclusions

The COX-2 immunoreactions have been significantly more frequent noticed in the gastric carcinomas included in the study (57.4%) and in the epithelial dysplasia areas adjacent to the carcinomas of intestinal type (35.5% of the cases), than in the normal peritumoral mucosa (4.9%) ($p<0.001$ ES).

The COX-2 immunoreactions have turned positive more frequently in gastric carcinomas of intestinal type (68.4%), in comparison to the carcinomas of diffuse type (29.4%) ($p<0.001$ ES). The results obtained are suggestive for the predominant expression of COX-2 in the carcinomas of intestinal type and its precursory lesions (epithelial dysplasia and in a smaller amount, intestinal metaplasia). The immunopositivation of COX-2 appears in our study as a precocious event in the sequence involved in the gastric carcinoma of intestinal type development.

The COX-2 immunoreactivity was significantly higher in the well differentiated (100%) and moderately differentiated carcinomas (70%), in comparison to the poorly differentiated carcinomas (48.7%) ($p<0.001$ ES). The COX-2 expression was more rarely accentuated in the signet-ring cell carcinoma (29.4%) and the anaplastic carcinoma (33.3%) ($p = 0.004108$ FS).

The COX-2 expression is significantly correlated with the invasion level, the presence of the metastases in the regional lymph nodes and the pTNM stage, but without influencing the prognosis of the gastric cancer patients.

The MVD average value was significantly higher in

the positive COX-2 carcinomas (39.4), suggesting an intense angiogenesis activity in that group of tumors ($p<0.001$ ES).

Our results show a tight correlation between the immunohistochemical expressions of COX-2 and VEGF in gastric carcinomas ($r = 0.562$, $p<0.001$ ES). The negative VEGF carcinomas have turned positive for COX-2 only for 19% of the cases. Different from those, the positive VEGF carcinomas have associated COX-2 immunoreactivity in 77.5% of the cases.

References

[1] WILLIAMS C. S., DUBOIS R. N., *Prostaglandin endoperoxide synthase: why two isoforms?*, Am J Physiol, 1996, 270(3 Pt 1):G393–400.

[2] SHENG H., SHAO J., DIXON D. A., WILLIAMS C. S., PRESCOTT S. M., DUBOIS R. N., BEAUCHAMP R. D., *Transforming growth factor-β1 enhances Ha-ras-induced expression of cyclooxygenase-2 in intestinal epithelial cells via stabilization of mRNA*, J Biol Chem, 2000, 275(9):6628–6635.

[3] RAMSAY R. G., FRIEND A., VIZANTIOS Y., FREEMAN R., SICURELLA C., HAMMETT F., ARMES J., VENTER D., *Cyclooxygenase-2, a colorectal cancer nonsteroidal anti-inflammatory drug target, is regulated by c-MYB*, Cancer Res, 2000, 60(7):1805–1809.

[4] DE LORENZO M. S., YAMAGUCHI K., SUBBARAMAIAH K., DANNENBERG A. J., *Bryostatin-1 stimulates the transcription of cyclooxygenase-2: evidence for an activator protein-1-dependent mechanism*, Clin Cancer Res, 2003, 9(13):5036–5043.

[5] JUNG Y. J., ISAACS J. S., LEE S., TREPEL J., NECKERS L., *IL-1beta-mediated up-regulation of HIF-1alpha via an NFkappaB/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis*, FASEB J, 2003, 17(14):2115–2117.

[6] TAKETO M. M., *Cyclooxygenase-2 inhibitors in tumorigenesis (Part II)*, J Natl Cancer Inst, 1998, 90(21):1609–1620.

[7] DANNENBERG A. J., ALTORKI N. K., BOYLE J. O., DANG C., HOWE L. R., WEKSLER B. B., SUBBARAMAIAH K., *Cyclooxygenase-2: a pharmacological target for the prevention of cancer*, Lancet Oncol, 2001, 2(9):544–551.

[8] GUPTA R. A., DUBOIS R. N., *Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2*, Nat Rev Cancer, 2001, 1(1):11–21.

[9] VAN REES B. P., RISTIMÄKI A., *Cyclooxygenase-2 in carcinogenesis of the gastrointestinal tract*, Scand J Gastroenterol, 2001, 36(9):897–903.

[10] GATELY S., *The contributions of cyclooxygenase-2 to tumor angiogenesis*, Cancer Metastasis Rev, 2000, 19(1–2):19–27.

[11] SAUKKONEN K., RINTAHAKA J., SIVULA A., BUSKENS C. J., VAN REES B. P., RIO M. C., HAGLUND C., VAN LANSCHOT J. J, OFFERHAUS G. J., RISTIMÄKI A., *Cyclooxygenase-2 and gastric carcinogenesis*, APMIS, 2003, 111(10):915–925.

[12] RATNASINGHE D., TANGREA J. A., ROTH M. J., DAWSEY S. M., ANVER M., KASPRZAK B. A., HU N., WANG Q. H., TAYLOR P. R., *Expression of cyclooxygenase-2 in human adenocarcinomas of the gastric cardia and corpus*, Oncol Rep, 1999, 6(5):965–968.

[13] LIM H. Y., JOO H. J., CHOI J. H., YI J. W., YANG M. S., CHO D. Y., KIM H. S., NAM D. K., LEE K. B., KIM H. C., *Increased expression of cyclooxygenase-2 protein in human gastric carcinoma*, Clin Cancer Res, 2000, 6(2):519–525.

[14] SUNG J. J., LEUNG W. K., GO M. Y., TO K. F., CHENG A. S., NG E. K., CHAN F. K., *Cyclooxygenase-2 expression in Helicobacter pylori-associated premalignant and malignant gastric lesions*, Am J Pathol, 2000, 157(3):729–735.

[15] CHEN C. N., SUNG C. T., LIN M. T., LEE P. H., CHANG K. J., *Clinicopathologic association of cyclooxygenase 1 and cyclooxygenase 2 expression in gastric adenocarcinoma*, Ann Surg, 2001, 233(2):183–188.

[16] LEE T. L., LEUNG W. K., LAU J. Y., TONG J. H., NG E. K., CHAN F. K., CHUNG S. C., SUNG J. J., TO K. F., *Inverse association between cyclooxygenase-2 overexpression and microsatellite instability in gastric cancer*, Cancer Lett, 2001, 168(2):133–140.

[17] LEUNG W. K., TO K. F., NG Y. P., LEE T. L., LAU J. Y., CHAN F. K., NG E. K., CHUNG S. C., SUNG J. J., *Association between cyclooxygenase-2 overexpression and missense p53 mutations in gastric cancer*, Br J Cancer, 2001, 84(3):335–339.

[18] RAJNAKOVA A., MOOCHHALA S., GOH P. M., NGOI S., *Expression of nitric oxide synthase, cyclooxygenase and p53 in different stages of human gastric cancer*, Cancer Lett, 2001, 172(2):177–185.

[19] SAUKKONEN K., NIEMINEN O., VAN REES B., VILKKI S., HÄRKÖNEN M., JUHOLA M., MECKLIN J. P., SIPPONEN P., RISTIMÄKI A., *Expression of cyclooxygenase-2 in dysplasia of the stomach and in intestinal-type gastric adenocarcinoma*, Clin Cancer Res, 2001, 7(7):1923–1931.

[20] JOO Y. E., OH W. T., REW J. S., PARK C. S., CHOI S. K., KIM S. J., *Cyclooxygenase-2 expression is associated with well-differentiated and intestinal-type pathways in gastric carcinogenesis*, Digestion, 2002, 66(4):222–229.

[21] KAWABE A., SHIMADA Y., UCHIDA S., MAEDA M., YAMASAKI S., KATO M., HASHIMOTO Y., OHSHIO G., MATSUMOTO M., IMAMURA M., *Expression of cyclooxygenase-2 in primary and remnant gastric carcinoma: comparing it with p53 accumulation, Helicobacter pylori infection and vascular endothelial growth factor expression*, J Surg Oncol, 2002, 80(2):79–88.

[22] YAMAGATA R., SHIMOYAMA T., FUKUDA S., YOSHIMURA T., TANAKA M., MUNAKATA A., *Cyclooxygenase-2 expression is increased in early intestinal-type gastric cancer and gastric mucosa with intestinal metaplasia*, Eur J Gastroenterol Hepatol, 2002, 14(4):359–363.

[23] OHNO R., YOSHINAGA K., FUJITA T., HASEGAWA K., ISEKI H., TSUNOZAKI H., ICHIKAWA W., NIHEI Z., SUGIHARA K., *Depth of invasion parallels increased cyclooxygenase-2 levels in patients with gastric carcinoma*, Cancer, 2001, 91(10):1876–1881.

[24] UEFUJI K., ICHIKURA T., MOCHIZUKI H., SHINOMIYA N., *Expression of cyclooxygenase-2 protein in gastric adenocarcinoma*, J Surg Oncol, 1998, 69(3):168–172.

[25] SUN W. H., SUN Y. L., FANG R. N., SHAO Y., XU H. C., XUE Q. P., DING G. X., CHENG Y. L., *Expression of cyclooxygenase-2 and matrix metalloproteinase-9 in gastric carcinoma and its correlation with angiogenesis*, Jpn J Clin Oncol, 2005, 35(12):707–713.

[26] VAN REES B. P., SAUKKONEN K., RISTIMÄKI A., POLKOWSKI W, TYTGAT G. N., DRILLENBURG P., OFFERHAUS G. J., *Cyclooxygenase-2 expression during carcinogenesis in the human stomach*, J Pathol, 2002, 196(2):171–179.

[27] RISTIMÄKI A., HONKANEN N., JÄNKÄLÄ H., SIPPONEN P., HÄRKÖNEN M., *Expression of cyclooxygenase-2 in human gastric carcinoma*, Cancer Res, 1997, 57(7):1276–1280.

[28] MURATA H., KAWANO S., TSUJI S., TSUJI M., SAWAOKA H., KIMURA Y., SHIOZAKI H., HORI M., *Cyclooxygenase-2 overexpression enhances lymphatic invasion and metastasis in human gastric carcinoma*, Am J Gastroenterol, 1999, 94(2):451–455.

[29] XUE Y. W., ZHANG Q. F., ZHU Z. B., WANG Q., FU S. B., *Expression of cyclooxygenase-2 and clinicopathologic features in human gastric adenocarcinoma*, World J Gastroenterol, 2003, 9(2):250–253.

[30] LIM H. Y., JOO H. J., CHOI J. H., YI J. W., YANG M. S., CHO D. Y, KIM H. S, NAM D. K, LEE K. B, KIM H. C, *Increased expression of cyclooxygenase-2 protein in human gastric carcinoma*, Clin Cancer Res, 2000, 6(2):519–525.

[31] HAN S. L., TANG H. J., HUA Y. W., JI S. Q., LIN D. X., *Expression of COX-2 in stomach cancers and its relation to their biological features*, Dig Surg, 2003, 20(2):107–114.

[32] MRENA J., WIKSTEN J. P., THIEL A., KOKKOLA A., POHJOLA L., LUNDIN J., NORDLING S., RISTIMÄKI A., HAGLUND C., *Cyclooxygenase-2 is an independent prognostic factor in gastric cancer and its expression is regulated by the messenger RNA stability factor HuR*, Clin Cancer Res, 2005, 11(20):7362–7368.

[33] JOO Y. E., REW J. S., SEO Y. H., CHOI S. K., KIM Y. J., PARK C. S., KIM S. J., *Cyclooxygenase-2 overexpression correlates with vascular endothelial growth factor expression and tumor angiogenesis in gastric cancer*, J Clin Gastroenterol, 2003, 37(1):28–33.
[34] LI H. X., CHANG X. M., SONG Z. J., HE S. X., *Correlation between expression of cyclooxygenase-2 and angiogenesis in human gastric adenocarcinoma*, World J Gastroenterol, 2003, 9(4):674–677.
[35] YU H. G., LI J. Y., YANG Y. N., LUO H. S., YU J. P., MEIER J. J., SCHRADER H., BASTIAN A., SCHMIDT W. E., SCHMITZ F., *Increased abundance of cyclooxygenase-2 correlates with vascular endothelial growth factor-A abundance and tumor angiogenesis in gastric cancer*, Cancer Lett, 2003, 195(1):43–51.
[36] SHI H., XU J. M., HU N. Z., XIE H. J., *Prognostic significance of expression of cyclooxygenase-2 and vascular endothelial growth factor in human gastric carcinoma*, World J Gastroenterol, 2003, 9(7):1421–1426.
[37] TATSUGUCHI A., MATSUI K., SHINJI Y., GUDIS K., TSUKUI T., KISHIDA T., FUKUDA Y., SUGISAKI Y., TOKUNAGA A., TAJIRI T., SAKAMOTO C., *Cyclooxygenase-2 expression correlates with angiogenesis and apoptosis in gastric cancer tissue*, Hum Pathol, 2004, 35(4):488–495.
[38] BUSKENS C. J., SIVULA A., VAN REES B. P., HAGLUND C., OFFERHAUS G. J. A., VAN LANSCHOT J. J. B., RISTIMÄKI A., *Comparison of cyclooxygenase 2 expression in adeno-carcinomas of the gastric cardia and distal oesophagus*, Gut, 2003, 52(12):1678–1683.
[39] FOSSLIEN E., *Review: molecular pathology of cyclooxygenase-2 in cancer-induced angiogenesis*, Ann Clin Lab Sci, 2001, 31(4):325–348.
[40] LANGMAN M. J. S., CHENG K. K., GILMAN E. A., LANCASHIRE R. J., *Effect of anti-inflammatory drugs on overall risk of common cancer: case-control study in general practice research database*, BMJ, 2000, 320(7250):1642–1646.
[41] AKRE K., EKSTRÖM A. M., SIGNORELLO L. B., HANSSON L. E., NYRÉN O., *Aspirin and risk of gastric cancer: a population-based case-control study in Sweden*, Br J Cancer, 2001, 84(7):965–968.
[42] MASFERRER J. L., LEAHY K. M., KOKI A. T., ZWEIFEL B. S., SETTLE S. L., WOERNER B. M., EDWARDS D. A., FLICKINGER A. G., MOORE R. J., SEIBERT K., *Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors*, Cancer Res, 2000, 60(5):1306–1311.
[43] JONES M. K., WANG H., PESKAR B. M., LEVIN E., ITANI R. M., SARFEH I. J., TARNAWSKI A. S., *Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing*, Nat Med, 1999, 5(12):1418–1423.
[44] SAUKKONEN K., TOMASETTO C., NARKO K., RIO M. C., RISTIMÄKI A., *Cyclooxygenase-2 expression and effect of celecoxib in gastric adenomas of trefoil factor 1-deficient mice*, Cancer Res, 2003, 63(12):3032–3036.
[45] TORRANCE C. J., JACKSON P. E., MONTGOMERY E., KINZLER K. W., VOGELSTEIN B., WISSNER A., NUNES M., FROST P., DISCAFANI C. M., *Combinatorial chemoprevention of intestinal neoplasia*, Nat Med, 2000, 6(9):1024–1028.
[46] MANN M., SHENG H., SHAO J., WILLIAMS C. S., PISACANE P. I., SLIWKOWSKI M. X., DUBOIS R. N., *Targeting cyclooxygenase 2 and HER-2/neu pathways inhibits colorectal carcinoma growth*, Gastroenterology, 2001, 120(7):1713–1719.
[47] FOSSLIEN E., *Biochemistry of cyclooxygenase (COX)-2 inhibitors and molecular pathology of COX-2 in neoplasia*, Crit Rev Clin Lab Sci, 2000, 37(5):431–502.

Corresponding author
Daniela Lazăr, Assistant, MD, PhD, Department of Gastroenterology, "Victor Babeş" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300 041 Timişoara, Romania; Phone +40722–961 955, E-mail: lazar_daniela@yahoo.com

Received: March 28th, 2008

Accepted: June 25th, 2008