

Aim of the study: Cyclooxygenase-2 (COX-2) expression has been observed in a substantial percentage of classical adenomas of the large bowel. The aim of the study was to assess and compare the expression of COX-2 in serrated polyps of the colon.

Material and methods: One hundred and nineteen serrated polyps were analyzed. There were 83 hyperplastic polyps (HP), 19 sessile serrated polyps (SSP) and 17 traditional serrated adenomas (TSA). COX-2 expression was assessed semi-quantitatively (0–2) and each lesion was fully characterized in terms of anatomical location, size, histology, age and sex of the patient. The general estimating equation (GEE) model with logit link was used in the statistical analysis.

Results: Epithelial expression of COX-2 was found in 85/119 serrated polyps (71.43%): 57/83 (68.67%) HP, 16/19 (84.21%) SSP, and 12/17 (70.59%) TSA. In HP and SSP it was predominantly of weak (49/83 HP, 12/19 SSP), whereas in TSA it was mainly of medium/strong intensity (8/17). The TSA category was associated with more frequent COX-2 expression (OR = 7.00, 95% CI: 1.49–32.88, $p = 0.014$) than HP, but such relation was not found for SSP vs. HP ($p > 0.1$). No associations between COX-2 expression and clinical parameters were found.

Conclusions: Immunohistochemical COX-2 expression cannot serve as a diagnostic adjunct to differentiate HP and SSP.

Key words: cyclooxygenase-2, hyperplastic polyp, sessile serrated polyp, traditional serrated adenoma, serrated neoplasia.

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Cyclooxygenase-2 immunohistochemical expression in serrated polyps of the colon

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Introduction

Colorectal cancer (CRC) is a significant cause of cancer-related death in many countries worldwide. Most sporadic CRCs develop from benign epithelial polyps, commonly via the adenoma-carcinoma or, less frequently, the serrated neoplasia pathway [1, 2]. Cyclooxygenases are key enzymes engaged in inflammation, which is involved in the pathogenesis of several cancers, including colorectal carcinoma [3]. The cyclooxygenase-2 (COX-2) overexpression has been observed in a substantial percentage of epithelial colonic polyps. In colorectal carcinomas it was shown to be overexpressed in approximately 70–80% of cases [4, 5]. Both COX isoforms – constitutive (COX-1) and inducible (COX-2) – catalyze the conversion of arachidonic acid to prostaglandin H₂ (PGH₂). It was demonstrated that the inducible form is engaged in acute inflammatory settings [6]. Though the role of COX-2 in the colorectal carcinogenesis has been intensely studied, the available data remain restricted, especially within the scope of serrated polyps [7]. A few authors have demonstrated that COX-2 is quite commonly overexpressed in traditional serrated adenomas, while in hyperplastic polyps it is an uncommon finding [8–10]. The aim of the study was to assess and compare the immunohistochemical expression of COX-2 in the serrated colorectal polyps and to determine whether COX-2 expression, along with other clinical and pathologic factors, could help in the differential diagnosis of such lesions.

Material and methods

One hundred and nineteen colorectal serrated polyps retrieved from 65 patients were analyzed. Polyps were obtained from 35 females (53.85%) and 30 males (46.15%), mean age 60.3 years (range 22–85), as a routine diagnostic procedure. Patients with inflammatory bowel diseases and polyposis syndromes were excluded from the study. The histology, anatomical location and size of 119 investigated polyps of the study group are presented in Table 1.

All polyps were diagnosed according to the WHO 2010 classification by pathologists with special expertise in pathology of the gastrointestinal tract [11]. In the case of divergent diagnoses the consensus was established at multi-head microscope sessions. The standard hematoxylin-eosin slides were used to reach the diagnosis. Then the COX-2 immunohistochemical expression was assessed. The analysis was based on the intensity of chromogene staining in the representative fields, and a three-degree semi-quantitative scale was applied (0 – no staining, 1 – weak, 2 – moderate/strong). In colorectal adeno-

Table 1. The anatomical distribution and size of the investigated serrated polyps

	Hyperplastic polyp (HP)	Sessile serrated polyp (SSP)	Traditional serrated adenoma (TSA)	Total
Proximal colon	12	11	4	27
Size				
< 5 mm	3	0	0	3
5–10 mm	9	7	2	18
≥ 10 mm	0	4	2	6
Distal colon	71	8	13	92
Size				
< 5 mm	56	5	2	63
5–10 mm	14	3	6	23
≥ 10 mm	1	0	5	6
Total				119

mas COX-2 was expressed in the cytoplasm as a diffuse or microgranular pattern. The adjacent normal mucosa did not exhibit COX-2 overexpression and it served as a negative control. The proximal part of the colon was defined due to its embryological origin and the topography of the mesocolon. In this designation the splenic flexure represented the boundary.

Immunohistochemistry

Tissue sections were cut from the paraffin blocks and mounted on the slides. The Cayman Chemical COX-2 monoclonal antibody was used (Clone CX-229). The sections were deparaffinized overnight in 68°C, rinsed with xylene (2 × 10 min) and dehydrated in graded alcohols. Antigen retrieval was performed by immersing the sections 3 times (7 + 2 × 5 min) in boiling citrate buffer (pH 6.0) under the microwave (700 W). After cooling for 30 minutes the sections were triply rinsed in double distilled water and then in phosphate buffer saline (PBS) (pH 7.4). The normal goat serum (Vector, cat. No. S-1000), avidin (Vector, cat. No. SP-2001) and biotin (Vector, cat. No. SP-2001) were used for 30, 15, 15 minutes, respectively. Incubation with primary antibody (Cayman Chemical COX-2, clone CX229) was conducted overnight (~18 h) at 4°C temperature (dilution 1 : 1000). After incubation, the sections were rinsed in PBS, and the biotin goat A-Mouse IgG (Immunotech, 1 : 1500; cat no 830) was applied for 30 minutes. The sections were rinsed again in PBS, then in 3% H₂O₂ in PBS (5 min), and rinsed in PBS again. Peroxidase conjugated streptavi-

din (Immunotech, 1 : 500; cat no 309) was applied for 45 min. Then the slides were rinsed in PBS (3 × 5 min), DAB (5 min) and double distilled water. After counter-staining with the Mayer's hematoxylin the slides were ready for analysis. Control immunohistochemistry for each set of the slides was performed.

Statistical analysis

The general estimating equation (GEE) model with logit link was used to assess the association between COX-2 expression and clinical and pathologic factors. The model included: age, sex, size of the polyp, anatomical location of each lesion (proximal vs. distal), and histology (HP, TSA, SSP). In additional analysis the diagnostic value of COX-2 for HP, SSP and TSA identification was evaluated. The *P* value of < 0.05 was considered statistically significant.

Results

One hundred and nineteen serrated polyps of the large bowel were analyzed. There were 83 (69.75%) hyperplastic polyps (HP), 19 (15.97%) sessile serrated polyps (SSP), and 17 (14.29%) traditional serrated adenomas (TSA). In general, 27 serrated polyps (22.69%) were located in the proximal, and 92 (77.31%) in the distal part of the colon. The epithelial expression of COX-2 was found in 85/119 serrated polyps (71.43%): 57/83 (68.67%) HP, 16/19 (84.21%) SSP, and 12/17 (70.59%) TSA. In HP and SSP it was predominantly of weak intensity (49/83 HP, 12/19 SSP), whereas in TSA it was mainly of medium/strong intensity (8/17). Dif-

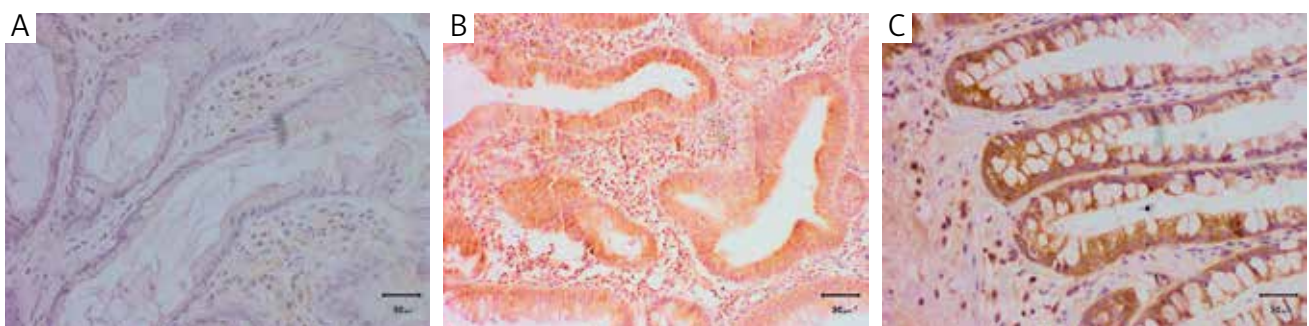


Fig. 1. **A)** The hyperplastic polyp epithelium with no COX-2 immunohistochemical expression; magnification 200×, **B)** The serrated adenoma epithelium exhibits mild COX-2 expression; magnification 100×, **C)** The sessile serrated polyp epithelium with strong COX-2 expression; magnification 200×

Table 2. The general estimating equation (GEE) model for COX-2 overexpression in serrated colorectal polyps

	Odds ratio [OR]	95% CI	p
Age (≥ 60 vs. < 60)	1.00	0.98–1.02	0.954
Sex (M vs. F)	0.70	0.20–2.41	0.575
Size			
≥ 5 vs < 5 mm	1.48	0.43–5.03	0.533
≥ 10 vs < 5 mm	0.85	0.13–5.52	0.862
Localization (proximal vs. distal)	0.58	0.14–2.44	0.458
Histology			
TSA vs. HP	7.00	1.49–32.88	0.014
SSP vs. HP	2.04	0.49–8.47	0.325

fuse and microgranular cytoplasmic reaction (Fig. 1) were observed, without marked heterogeneity.

The GEE model is presented in Table 2.

The TSA category was associated with statistically significant more frequent COX-2 overexpression (medium/strong chromogen intensity) (OR = 7.00, 95% CI: 1.49–32.88, $p = 0.014$) than HP, but there was no such relation for SSP vs. HP ($p > 0.1$). Neither age, sex, size nor anatomical location was significantly associated with COX-2 overexpression.

The GEE model to determine the diagnostic value of COX-2 overexpression for HP, SSP and TSA categories is presented in Table 3. In HP and TSA categories bigger size of the lesion was associated with the diagnosis. Bigger lesions (≥ 5 mm and ≥ 10 mm) were seldom diagnosed as HP (OR = 0.18 and 0.01, respectively), whereas among the larger polyps the TSA lesions were diagnosed much more frequently. The results were highly significant. The SSP lesions were located more frequently in the proximal portion of the colon (OR = 3.79; $p = 0.042$). Polyps diagnosed as HP seldom showed moderate or strong COX-2 expression (OR = 0.21; $p = 0.046$).

Discussion

Serrated neoplasia pathways account for about 25% of CRC cases and serrated polyps are quite commonly found during screening colonoscopies [12, 13]. Similarly to the well-known adenoma-carcinoma sequence, serrated neoplasia involves several genetic and epigenetic alterations, which may finally lead to malignant transformation of benign serrated polyps [14, 15]. The appreciation of the serrated pathway(s) during the last decades has led to a paradigm shift in the understanding of the molecular foundation of CRC. Special emphasis is put on the role of CpG island methylator phenotype (CIMP) and BRAF oncogene mutations [16–19]. One must also note that serrated polyps constitute a heterogeneous group. The serrated morphology refers to the hyperplastic polyps, traditional serrated adenomas as well as sessile serrated polyps. Traditional serrated adenomas show a mixture of classical adenoma features (dysplastic epithelium at all levels of the polyp, including the surface) and “saw-tooth pattern” morphology, in contrast to sessile serrated polyps (SSP), showing no or only little dysplastic change [20]. Recog-

nition of the malignant potential of traditional serrated adenomas and sessile serrated polyps has led to the inclusion of patients with such lesions in post-polypectomy surveillance guidelines [21]. As the risk seems to be similar to conventional tubular adenomas, the standard conventional adenoma-type surveillance has been recommended by the National Comprehensive Cancer Network (NCCN) in such cases (www.nccn.org).

Hyperplastic polyps, formerly believed to be harmless, have become suspicious. They are believed to be endowed with a small potential for carcinogenetic change – especially in light of their potential link with cancer in hyperplastic polyposis syndrome as well as cases of cancer arising in hyperplastic tissue [22, 23]. Several morphological subtypes of hyperplastic polyps are recognized – namely microvesicular, goblet cell rich and mucin poor. Little is known about the clinical implications of such a classification, thus in our study we do not distinguish such subtypes.

COX-2 pharmacological suppression by non-steroidal anti-inflammatory agents (e.g. aspirin, coxibs) reduces recurrence of adenomas and incidence of advanced adenomas in individuals with an increased risk of CRC and probably the CRC incidence [24, 25]. Whether COX-2 overexpression is a common pathogenetic mechanism acting both in classical and serrated pathways remains however unclear. Some authors suggest that the inflammation could play an even more considerable role in the serrated neoplasia pathway, when compared to the classical adenoma-carcinoma sequence [26]. According to Kawasaki *et al.* COX-2 overexpression is common in serrated and non-serrated colorectal adenomas, but uncommon in hyperplastic polyps as well as in sessile serrated polyps [27]. It should be noted that only seven sessile serrated polyps were analyzed in their study. In contrast, in our study COX-2 overexpression was demonstrated in most of the SSP lesions. COX-2 expression appears in a substantial number of benign epithelial polyps as well as CRCs [28, 29]. In the CRC the expression of COX-2 has been reported to alter the cohesiveness and apoptotic resistance of the neoplastic cells, thus accelerating tumor growth and infiltration [30].

Serrated lesions of the colon remain a significant issue in everyday practice of pathologists. The morphology is still debated and the concordance between pathologists

Table 3. The general estimating equation (GEE) model presents the relationship of COX-2 immunohistochemical expression and other clinical and pathological factors and the diagnosis of HP, SSP and TSA lesions

	HP			SSP			TSA		
	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>
Age (≥ 60 vs. < 60)	0.99	0.97–1.01	0.326	1.00	0.98–1.01	0.863	1.01	0.99–1.04	0.236
Sex (M vs. F)	1.82	0.55–6.08	0.329	0.30	0.07–1.25	0.098	1.04	0.23–4.70	0.958
Size									
≥ 5 vs < 5 mm	0.18	0.06–0.58	0.004	2.69	0.65–11.10	0.171	7.42	1.42–38.64	0.017
≥ 10 vs < 5 mm	0.01	0.001–0.13	0.0001	3.56	0.60–20.97	0.161	69.11	8.50–561.74	0.0001
Localization (proximal vs. distal)	0.55	0.15–2.06	0.378	3.79	1.05–13.70	0.042	0.37	0.07–1.90	0.231
COX-2 expression									
mild	0.91	0.25–3.31	0.882	2.13	0.46–9.94	0.335	0.37	0.07–2.10	0.262
moderate/strong	0.21	0.04–0.97	0.046	2.53	0.40–15.97	0.322	2.87	0.53–15.70	0.223

is low. The carcinogenetic potential of serrated polyps underlines the importance of proper classification within this group. While the diagnostic criteria for traditional serrated adenomas are well established, the differential diagnosis between hyperplastic and sessile serrated polyps may be still a challenge. Proper, perpendicular orientation of the tissue sample is of crucial importance and the basic hematoxylin-eosin staining remains the gold standard.

In our study we tried to determine, whether COX-2 expression differs among certain categories of benign serrated colonic polyps and whether COX-2 expression and other clinical and pathologic factors could serve as potential diagnostic adjuncts in distinguishing problematic serrated lesions – especially HP and SSP. The WHO 2010 criteria were applied consistently to minimize the risk of misdiagnosis.

The analysis of 119 serrated polyps of the colon have confirmed the higher predilection of SSP to the proximal portion of the colon. Polyps diagnosed as HP seldom showed moderate or strong COX-2 expression (OR = 0.21, *p* = 0.046), while in the SSP category the results were not significant. It seems conceivable, however, that including a larger group of SSP lesions could give some new insight into the serrated neoplasia pathobiology. Several authors suggest a pathogenetic link between HP (especially microvesicular subtype) and SSP. Kwon *et al.* even suggest the existence of “intermediate serrated polyp” as a staging category in terms of both morphological and molecular aspects [31]. A gradual increase of the COX-2 expression could possibly support such a link, if precise diagnostic criteria could be applied. Still, COX-2 expression was not investigated as to this issue. While the differential diagnosis between HP and TSA is not a challenge due to well-established diagnostic criteria, SSP polyps remain a problematic issue in everyday practice. SSP are easily misdiagnosed as HP. Whether the presence of COX-2 overexpression could point away from the diagnosis of HP in equivocal cases is still to be established in bigger groups of serrated colorectal polyps.

In summary, COX-2 overexpression was more common in traditional serrated adenomas than in sessile serrated and hyperplastic polyps. Though HP seldom show moderate/strong expression of COX-2 and no such associations

exist in SSP, the immunohistochemical COX-2 expression cannot serve as a reliable diagnostic adjunct in such cases.

The authors declare no conflict of interest.

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