

## KI-67 AND P53 EXPRESSION IN ILEAL POUCHES

JAKUB ŻURAWSKI<sup>1</sup>, KATARZYNA IWANIK<sup>2</sup>, PAWEŁ BURCHARDT<sup>3</sup>, PIOTR RZYMSKI<sup>1</sup>, KRZYSZTOF WIKTOROWICZ<sup>1</sup>, VIOLETTA RATAJCZAK<sup>1</sup>, TOMASZ BANASIEWICZ<sup>1</sup>, MARCIN GROCHOWALSKI<sup>4</sup>, WIESŁAWA SALWA-ŻURAWSKA<sup>5</sup>

<sup>1</sup>Chair and Department of Biology and Environmental Protection, Poznan University of Medical Sciences, Poland

<sup>2</sup>Chair and Department of Clinical Pathomorphology, Poznan University of Medical Sciences, Poland

<sup>3</sup>Chair and Division of Cardiology-Intensive Therapy, Poznan University of Medical Sciences, Poland

<sup>4</sup>Chair and Department of General, Gastroenterological and Endocrine Surgery, Poznan University of Medical Sciences, Poland

<sup>5</sup>Chair and Department of General and Colorectal Surgery, Poznan University of Medical Sciences, Poland

Development of dysplastic lesions that may potentially lead to cancer is sometimes reported within the ileal pouches. Dysplasia is in turn associated with increased expression of proliferation indices. The goal of this study was to evaluate the mitotic activity and possible expression of p53 in the epithelium within the ileal pouches in patients with chronic ulcerative colitis.

The study involved archive material consisting of ileal pouches surgically removed from 17 patients diagnosed with ulcerative colitis. Several specimens were collected from each pouch. The immunohistochemistry (Ki-67 and p53 protein) control group (14 cases) consisted of the resection line specimens of colons removed due to colorectal adenocarcinoma. Intensity of the expression of the markers under study within the inflammatory infiltrates was assessed using a 5-point scale proposed by Bernstein *et al.* Ki-67 expression was observed in all studied patients with marked intensity (Bernstein scale score +3, +4). Protein p53 expression was observed only in eight patients, and was mostly of low intensity (Bernstein scale score +1, +2).

Immunohistochemical results confirmed the histopathological results that revealed dysplastic lesions, which are often an indication for radical procedures in ulcerative colitis patients.

Our results suggest the usefulness of these examinations, also in the ileal pouch material.

**Key words:** ileal pouches, pathology, immunohistochemistry.

### Introduction

Expression of proliferation factors and p53 protein in various colon pathologies continues to be the subject of interest for pathomorphologists and gastroenterologists. This is due to the prognostic value of these markers in the assessment of potential risks of cancer development. Histopathological assessment of epithelial dysplasia is obviously of primary importance; however, immunohistochemical examinations have

a significant accessory value. Their usefulness was demonstrated in studies conducted in patients with adenocarcinoma [1, 2]. Immunohistochemical examinations are also of a similar value in cases of ulcerative colitis as the long-term course of this disease is quite often associated with cancer development. Both epithelial dysplasia and the early stage of cancer are difficult to detect in the endoscopic examination differentiate from inflammatory and regenerative lesions of intestinal epithelium. Histological evaluation of epithelial dyspla-

sia is also dubious in some cases. Then, determination of the expression of Ki-67 and p53 proteins is sometimes helpful [3-6]. Convincing results were obtained by Radović *et al.* [7]. They assessed the expression of p53, bcl-2 and Ki-67 proteins in inflamed mucosa and dysplastic lesions of different intensity of dysplasia. They assessed the cellular location and intensity of reactions and counted cells that expressed the positive reaction. Both groups of lesions were compared to the results obtained for unchanged mucosa.

Cioffi *et al.* [8] determined the expression of the p53 protein in the serum of ulcerative colitis patients and compared the results to these obtained in healthy subjects.

The results of serum p53 determinations were consistent with the results of immunohistochemical examinations in both groups.

Sometimes, dysplastic lesions are also observed in the epithelium lining the ileal pouches. However, reports of immunochemical studies conducted in such cases are scarce [9] whereas these investigations might have a significant value due to possibility of carcinoma development within the ileal pouches [10-13].

## Objective

The objective of the study was to assess the mitotic activity and expression of p53 in the epithelium within the ileal pouches in patients with chronic ulcerative colitis.

## Material and methods

The study involved the archive material consisting of ileal pouches surgically removed from 17 patients undergoing surgeries due to ulcerative colitis. The study population included 8 women aged 25 to 85 and 9 men aged 45 to 75. Patients were diagnosed and treated in the Faculty and Clinic of General, Gastroenterological and Endocrinological Surgery of the Poznan University of Medical Sciences. Morphological diagnoses were made by the Biopsy Diagnostic Lab of the Faculty of Clinical Pathomorphology. Immunohistochemical studies were performed in the Faculty of Biology and Environmental Protection. Several specimens were collected from each pouch (including the resection lines). The severity of the inflammatory process was evaluated according to the modified criteria of Moskowitz [14, 15] where the following morphological features were considered.

Acute inflammation:

- polymorphonuclear leukocyte infiltration: a) mild – 1, b) moderate + crypt abscess – 2, c) severe + crypt abscess – 3;
- ulceration per low-power field: a) <25% – 1, b) 25-50% – 2, c) >50 – 3.

Chronic inflammation:

- chronic inflammatory infiltration: a) mild – 1, b) moderate – 2, c) severe – 3.
- villous atrophy: a) partial – 1, b) subtotal – 2, c) total – 3 [16].

The immunohistochemistry control group (14 cases) consisted of the resection line specimens of colons removed due to colorectal adenocarcinoma. Paraffin blocks containing specimens with most characteristic lesions were selected for further immunohistochemical assays based on HE staining. The streptavidin-biotin-peroxidase method and DakoCytomation LSAB kit (K0675) were used for detection of expression of markers under study, i.e. Ki-67 and p53. In order to facilitate the antigen-antibody reaction, antigens were retrieved in a microwave oven at 96°C in pH 6.0 citrate buffer over 30 min. Endogenous peroxidase activity was blocked using 3% H<sub>2</sub>O<sub>2</sub>. Next, the specimens were incubated for 24 hours at 4°C with anti-Ki-67 (Novocastra RTU-Ki67-MM1) or anti-p53 (DakoCytomation N1581) antibodies. Subsequent 30-minute incubation was performed with biotinylated antibodies, which were in turn incubated with peroxidase-streptavidin complex. Between incubations, specimens were rinsed with pH 7.6 TBS buffer. The antigen was located with the chromogen DAB-3,3 (Sigma Aldrich D5637), which is a peroxidase substrate. Next, specimens were stained with hematoxylin, dehydrated and covered with cover glasses. Reaction performed in the absence of the primary antibody was used as negative control for immunocytochemical assays.

The intensity of the expression of the markers under study within the inflammatory infiltrates was assessed using a 5-point scale proposed by Bernstein *et al.* [16], where individual scores correspond to the following:

- 0 – no expression in infiltrate cells,
- 1 – less than half of the 10 fields studied contain infiltrates with the reaction products (poor expression),
- 2 – expression visible in 1-10 cells per field (in all fields),
- 3 – expression visible in 11-30 cells per field (in all fields),
- 4 – expression visible in more than 30 cells per field (in all 10 fields).

For 2 patients with carcinoma development within ileal pouches, immunohistochemical investigations were also performed.

## Results

Inflammatory lesions of different intensity were found within the walls of all studied pouches. In four cases, inflammation included the excision line. In three cases, moderate epithelial dysplasia was detected. In all cases chronic inflammatory markers were found.

Tables I and II list the results of histochemical and immunohistochemical examinations.

**Table I.** Histological lesions and the intensity of expression of Ki-67 and p53 in ileal pouches in the female group

PATIENT	AGE	INFLAMMATORY L. SEC. MOSCOWITZ	DYSPLASIA	KI-67 EXPRESSION	p53 EXPRESSION
S.Z.	25	inflammatory infiltration 2 villous atrophy 1	–	1+	1+
D.A.	29	inflammatory infiltration 3 also within the pouch wall and along the excision line villous atrophy 1	–	1+, 2+, 4+	–
O.M.	31	inflammatory infiltration 2 villous atrophy 1	–	4+	2+
Sz.K.	34	inflammatory infiltration 3 villous atrophy 2	moderate	2+, 3+, 4+	1+
D.E.	38	inflammatory infiltration 3 villous atrophy 2	–	3+	1+
B.E.	60	inflammatory infiltration 3 villous atrophy 2	–	1+, 3+, 3+	–
K.M.	68	inflammatory infiltration 3 villous atrophy 2	–	3+	–
K.A.	85	inflammatory infiltration 2 affecting the entire thickness of the ileal pouch wall villous atrophy 1	–	3+	–

**Table II.** Histological lesions and the intensity of expression of Ki-67 and p53 in ileal pouches in the male group

PATIENT	AGE	INFLAMMATORY L. SEC. MOSCOWITZ	DYSPLASIA	KI-67 EXPRESSION	p53 EXPRESSION
P.W.	45	inflammatory infiltration 2 affecting the entire thickness of the ileal pouch wall villous atrophy 1	–	1+, 2+, 2+	–
P.M.	49	inflammatory infiltration 3 including the excision lines villous atrophy 1	–	3+, 3+, 4+	–
K.K.	50	inflammatory infiltration 2 along the distal excision line villous atrophy 1	moderate	2+	1+
S.R.	54	inflammatory infiltration 3 including the excision lines villous atrophy 2	–	2+, 2+, 3+, 3+	–
W.Z.	55	inflammatory infiltration 2 villous atrophy 1	–	1+	1+
M.S.	59	inflammatory infiltration 2 villous atrophy 1	moderate	2+, 3+, 4+	–
H.W.	61	inflammatory infiltration 3 including the distal excision line villous atrophy 2	–	2+	–
Sz.Z.	67	inflammatory infiltration 2 including the vascular walls villous atrophy 1	–	1+, 4+	1+
B.M.	76	inflammatory infiltration 2 focal inflammatory lesions, including the vascular walls villous atrophy 1	–	3+	1+

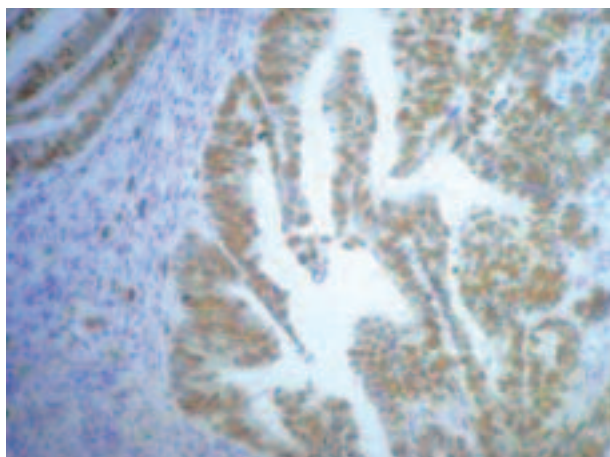


Fig. 1. A section of adenocarcinoma. Ki-67 expression (+4 Bernstein scale score). Magnification 480×

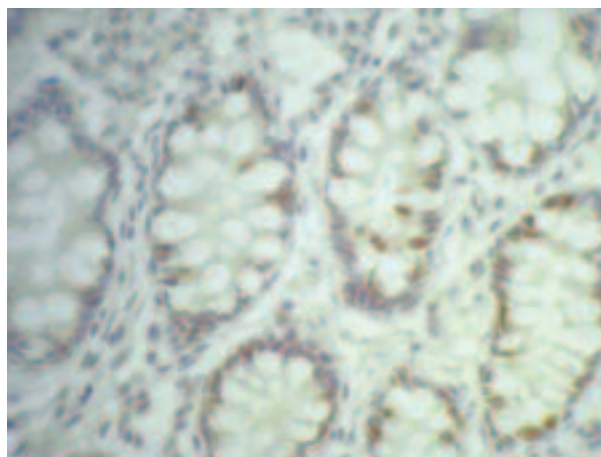


Fig. 2. Mucosal membrane of the ileal pouch Ki-67 expression (+1 Bernstein scale score). Magnification 600×

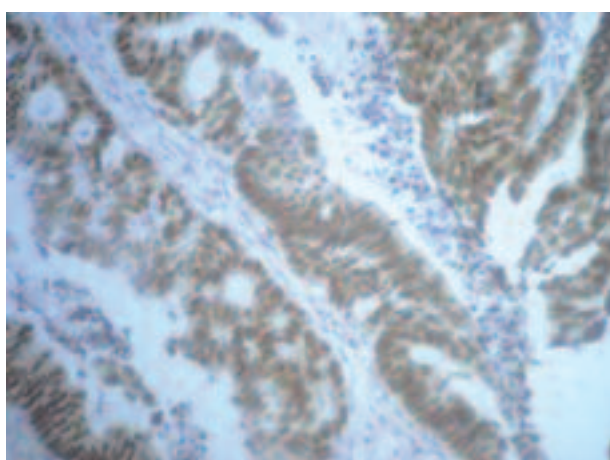


Fig. 3. A section of adenocarcinoma. p53 expression (+4 Bernstein scale score). Magnification 480×

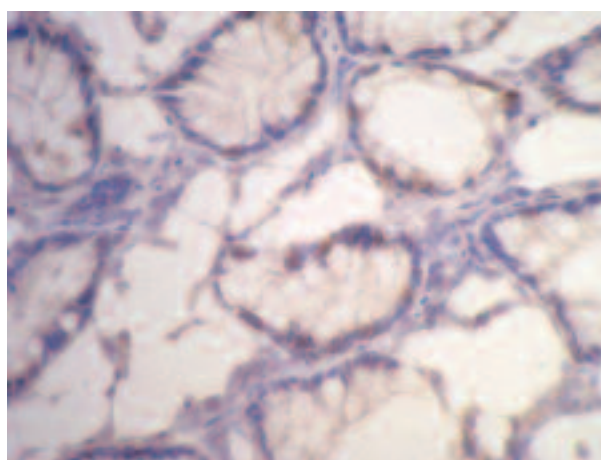


Fig. 4. Mucosal membrane of the ileal pouch p53 expression (+1 Bernstein scale score). Magnification 600×

In most cases, significant intensification of Ki-67 expression was observed; in 8 cases, the intensity of the expression was different in sections collected from different areas of the ileal pouches (for this reason, two or three sections are presented in tables). The expression was of significant intensity (Bernstein scale score 3+, 4+) in 11 pouches, moderate (2+) in 4 pouches and mild (1+) in 2 pouches.

p53 expression was detected in 8 patients. It was of mild intensity (1+) in 7 patients and of moderate intensity (2+) in 1 patient. In half of the cases, expression of p53 was accompanied by expression of Ki-67 (3+, 4+), moderate (2+) in 1 case and mild (1+) in 2 cases. In two pouches, expression of p53 accompanied moderate dysplasia.

Three cases in which p53 expression was detected (2+ in one female patient, 1+ in two patients) together with high intensity (4+, 3+) expression of Ki-67 are of note. No determinants of dysplasia were found.

Expression of Ki-67 was demonstrated in all cases. The average Bernstein scale score for Ki-67 expression was  $>2$  (SD = 1.03, SE = 0.17).

No significant age-dependence was demonstrated for Ki-67 expression ( $R_s = 0.24$ ,  $p > 0.05$ ), which means that similar levels of expression may be observed in both young, middle-aged and elderly individuals. No relationships between the expression of Ki-67 and the expression of p53 were identified. No significant age differences were observed for individuals with and without expression (mean 47 years, SD = 18.1 and mean 56 years, SD = 15.5, respectively).

## Discussion

Despite the increasingly used proctocolectomy with ileal pouch-anal anastomosis, its pathomorphological aspects are rarely discussed in the Polish literature [10, 17].

Proctocolectomy with ileal pouch formation significantly reduced the risk of cancer in patients with ulcerative colitis [18, 19] as well as in patients with familial adenomatosis coli [20]. However, in some (luckily not numerous) patients, inflammatory lesions develop within the ileal pouches, leading to prolifer-

ative lesions and dysplasia, thus posing a danger of cancer development [9, 18, 19, 21]. For this reason, systematic monitoring of patients with ileal pouches gains increased attention.

To date, histopathological examinations of ileal pouch mucosa have been limited to histological examinations. As mentioned in the introduction, immunohistochemical assessments are very rare in these cases [9]. Yet, it is known from numerous observations that proliferation factors and p53 protein may be helpful in cancer risk detection. That is why we considered it substantiated to present the preliminary results of our observations. The obtained results are not uniform in the studied patient population. However, they make it possible to draw some cautious conclusions. As shown by our studies, chronic inflammation within the pouch walls usually leads to expression of a proliferation factor such as Ki-67, and the intensity of the expression most commonly reflects the intensity of inflammation. Some conformity of results may be observed in this respect. However, such conformity could not be demonstrated with regard to the presence of dysplastic lesions and the expression of p53. Regardless of the above, one may conclude that carrying out immunohistochemical examinations in cases of inflammatory processes taking place within ileal pouch walls is substantiated.

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## Address for correspondence

Jakub Żurawski  
 Katedra Biologii i Ochrony Środowiska  
 ul. Długa 1/2  
 61-848 Poznań  
 tel. +48 61 853 05 71, +48 61 854 91 78  
 mail: zurawski@ump.edu.pl