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## Stricture of ileo-anal J pouch anastomosis increased the intensity of pouchitis in an experimental rat model

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### Summary

Pouchitis appears to be the most common complication after restorative proctocolectomy.

**Background:**  
**Material/Methods:**

In experimental models we investigated the correlation between the width of anastomosis and the frequency of pouchitis. Twenty-three Wistar rats underwent restorative proctocolectomy under pentobarbital anesthesia. Normal width anastomosis was performed in 11 animals (Group I). In the remaining 12 animals (Group II) the diameter of anastomosis was reduced by 50%. All animals were sacrificed and the pouch mucosa was histologically (Moskowitz score) and immunohistochemically (IL-1, IL-6, IL-10, IL-12 expression) examined.

**Results:**

Morphological assessment of pouchitis symptoms based on Moskowitz scale revealed considerably more severe inflammation ( $p=0.0079$ ) in the animals from Group II than in the rats from Group I. The expressions of investigated cytokines, assessed qualitatively in histopathological examination, were higher in rats with narrow anastomosis in comparison with animals with normal anastomosis.

**Conclusions:**

The stricture of anastomosis increases the intensity of pouchitis and stimulates the production of interleukins. It seems that anastomotic stricture plays an important role in the development of pouchitis.

**key words:**

proctocolectomy • experimental model • anastomotic stricture • pouchitis

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## BACKGROUND

Restorative proctocolectomy involves the removal of the large intestine with the preservation of the anal sphincter and the performance of ileoanal anastomosis. One of the aims of this procedure is to create an ileal reservoir. Small intestinal mucosa, which forms the intestinal pouch, undergoes several adaptive processes aimed at taking over some of the functions of the removed large intestine. Apart from adaptive changes, a significant number of patients may experience a certain type of inflammation. Its frequency is estimated at 23–60% of patients who underwent restorative proctocolectomy [1,2]. However, the data vary considerably depending on the source. Several potential etiopathological causes of pouchitis might be listed, including static fecal residue in the pouch in patients and experimental models [3]. This phenomenon has also been explained by the overgrowth of bacteria, but the latest research does not confirm this theory [3]. Patients with pouchitis symptoms have been found to develop anastomotic stricture [4,5]. It has been observed that endoscopic dilatation of the stricture decreases the clinical symptoms and improves comfort [6]. Whether the anastomotic stricture is the cause of pouchitis or the result of an inflammatory condition which induces fibrosis is an issue that still raises many doubts and is widely discussed.

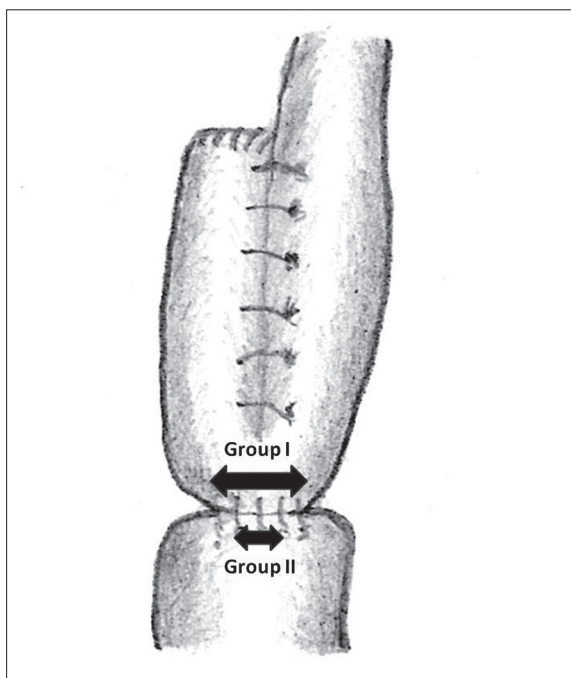
## MATERIAL AND METHODS

All animal studies were approved by the Local Ethics Committee. All surgical procedures were conducted by a qualified surgeon in accordance with the guidelines of the European Community Council directives 86/609/EEC and with the agreements of the Local Ethics Committee (42/2006).

### Surgical treatment

Adult male Wistar rats in good general condition (300–350 gms) were housed individually in stainless steel metabolic cages under controlled temperature (21–23°C) on 12: 12-h light-dark cycle and fed with standard laboratory diet and water ad libitum.

In all examined animals, feeding was stopped 24 hours before surgery. After an intraperitoneal injection of pentobarbital anesthesia (pentobarbital sodium, 50 mg/kg body wt) the skin of the abdomen was sterilized and draped with gauze and a 3–4 cm midline laparotomy was performed. The total proctocolectomy was performed by resecting the colon and ligating the mesentery with 4-0 silk. The intestinal segment was excised from 0.1 cm proximal to the ileocecal junction. The rectum was resected at the level of the pelvic floor, with a 0.5 cm rectal stump. Small intestinal contents were washed with 0.9% warm saline and a 2 cm ileal J-pouch was created by the duplication of the distal end of the small intestine by a single-layer interrupted 6-0 Prolene suture. The pouch anal anastomosis was performed with a single layer interrupted 6-0 Prolene suture. The sufficiency of the anastomosis was controlled by injection of 3–5 ml of 0.9% warm saline through the anus. Two different techniques of anastomosis were applied. In Group I (11 cases) “normal” width anastomosis was performed, assuming 75% of the diameter of the distal part of small intestine to be “normal”.



**Figure 1.** Diameter of anastomosis in Group I (normal width) and Group II (50% of normal width anastomosis).

In Group II (12 cases) the diameter of anastomosis was reduced by 50% in comparison to Group I (Figure 1).

The peritoneal cavity was irrigated with 0.9% warm saline. The abdominal incision was closed with a running 2-0 silk suture and the skin with a single-layer interrupted 3-0 silk suture. Intraoperative mortality was not observed and the median time of surgery was 75 minutes. Water was provided immediately after surgery and feeding was restarted 24 hours later.

### Collection of the specimens

After 9 weeks of postoperative follow-up, all animals were sacrificed by decapitation. There were no signs of other diseases or malignancy in the abdominal cavity. The intestinal reservoir was resected, opened and rinsed free of intestinal contents. The internal diameter of anastomosis in both groups was measured. The external muscle layer was removed by blunt dissection. The mucosa specimens with an area of 0.5 cm<sup>2</sup> were fixed in formalin for further histological and immunohistochemical evaluations.

### Histological assessment

Each time the histopathological examination was performed by 2 independent histopathologists and every time the examined section was found to be inflamed according to Moskowitz scale and positive for villi atrophy according to Laumonier scale. Histological classification of inflammation suggested by Moskowitz objectively presents the microscopic assessment of acute and prolonged inflammation and ascribes certain points, which we sum up, to particular features. The inflammation is diagnosed when the coefficient of inflammation amounts to  $\geq 4$  [7]. Villi atrophy development was assessed in accordance to Laumonier 4-stage scale [8].

**Table 1.** Assessment of pouchitis (Moskowitz scale), villi atrophy (Laumonier scale) and expression of IL-1, IL-6, IL-10, IL-12 (immunohistochemistry) in rats with normal anastomosis (Group I) and narrow anastomosis (Group II).

	Mean diameter of anastomosis – millimeters	Histological examination		Immunohistochemical examination				
		Moskowitz	Laumonier	IL-1	IL-6	IL-10	IL-12	
Group I wide anastomosis (n=9)	4.2	Mean	2.7	1.4	0.9	1.1	1.4	1.2
		Med.	2	1	1	1	1	1
		Min	2	1	0	0	1	0
		Max	5	3	1	2	2	3
Group II narrow anastomosis (n=7)	2.6	Mean	4.6	1.7	1.7	1.7	2.4	2.1
		Med.	5	1	1	1	2	2
		Min	3	1	1	0	2	1
		Max	6	3	3	3	3	3
p value	0.0086*	0.0079*	0.6806**	0.1142**	0.407**	0.0115*	0.0712**	

\*Significance, \*\* non-significance.

### Immunohistochemistry

Based on hematoxylin & eosin preparations, paraffin blocks containing sections with the most characteristic changes were selected for further immunohistochemical tests. To demonstrate the expression of the investigated markers (IL-1 alpha, IL-6, IL-10, IL-12), the streptavidin-biotin-peroxidase method was employed using an LSAB kit manufactured by DAKO. The antigen was located using DAB-3.3 (DAKO) as a chromogen, which is a substrate for peroxidase. The preparations were then stained with hematoxylin and – following dehydration – closed with a glass cover. Appropriate positive and negative controls were used.

### Statistical analysis

Values are expressed as ranges and medians. The differences in the anastomosis diameter, intensity of pouchitis and interleukin expression between the 2 selected groups of animals were calculated with the use of the Mann-Whitney U test. The level of significance was set at  $p < 0.05$ .

### RESULTS

Higher postoperative mortality (5 cases) was found in Group II as compared with Group I (2 cases), but the difference did not reach the level of significance. The mean diameter of anastomosis in Group I was 4.2 mm and in Group II was 2.3 mm; the differences were statistically significant ( $p = 0.0086$ ). Morphological assessment of pouchitis symptoms based on Moskowitz scale revealed considerably more severe inflammation ( $p = 0.0079$ ) in the rats from Group II than in the rats from Group I. No significant differences related to intestinal villi atrophy were observed. The expressions of investigated cytokines, assessed qualitatively in histopathological examination, were higher in rats with narrow anastomosis in comparison with animals with normal anastomosis. The differences in IL-10 were statistically significant ( $p = 0.0115$ ) and differences in IL-1, IL-6, IL-12 showed

no statistical significance. Detailed data related to all investigated animals are presented in Table 1.

### DISCUSSION

Clinical research on pouchitis is limited by the number of patients, their biological and clinical differences, and their willingness to participate in the follow-up examinations, which are often invasive and have to be performed with particular time periods. Thus, appropriate experimental models are needed in order to enable the better understanding of mechanisms of pouchitis and, consequently, its more effective treatment. It is also believed that pouchitis is a natural model of IBD, with a defined onset time with in mucosa [9].

Pig models have long been used in research on restorative proctocolectomy for the assessment of inflammatory manifestations, bacteria colonization or post-operative peristalsis in the digestive tract [10,11]. These models allow for the reconstruction of surgical procedures in humans but they are relatively rarely used due to their sizes and related costs, as well as other restrictions. Rat models are used much more frequently [12,13], in which either idiopathic pouchitis is assessed [14] or it is chemically induced, most frequently with the use of 5% dextran sulfate sodium (DSS) [15], iodoacetamide or dinitrobenzene sulfonic acid (DNBS) [16]. The rat experimental models of pouchitis pose a vast array of surgical difficulties related to the necessity of the performance of a low anastomosis. Some trials aimed at technical modifications have already been described, which allow for simplification of surgical procedures and the avoidance of a low anastomosis. One of them is to create a U-shaped ileal pouch (analogous to a classical proctocolectomy) and anastomose it to the intestine anterior to the ileocecal valve (without colectomy being performed) [17].

Clinical observations reveal that anastomotic stricture is one of the most significant problems of restorative

proctocolectomy patients, and its endoscopic dilatation decreases the clinical symptoms as well as improving comfort [6]. Presently, more accurate data is available that allows for more precise definition of anastomotic stricture. It is now assumed that an 8 mm diameter is a critical anastomotic stricture after restorative proctocolectomy [5]. There is still a lack of data about the correlations between the diameter of the anastomosis and the occurrence and the severity of pouchitis. Therefore, the model of anastomotic stricture, which is described in our research, was regarded as provisional and based on the team's previous experiences. The starting point was a standard performance of anastomosis, whose diameter corresponded to 75% of the diameter of the distal part of the small intestine in the part slightly above the pouch. In analogy to clinical data, the stricture was assumed to have an anastomotic diameter equal to 50% or less of the normal diameter.

On the basis of our research it has been concluded that the group of patients with narrow anastomosis presented more severe pouchitis assessed in accordance to the Moskowitz histological classification. Anastomotic stricture leads to static fecal residue, which is one of the potential causes of pouchitis in clinical [18] and experimental [3] models. In the examined sections, no divergences related to the atrophy of intestinal villi, in accordance with Laumonier classification, were observed. Atrophy of intestinal villi is a long-term process indicating the continuing restructuring of the mucosa of the small intestinal reservoir. In restorative proctocolectomy patients this process may last several years [19]. Thus, in our research the time for observation might have been insufficient to note the increased atrophy of intestinal villi and potential differences among the groups.

In the present study we also evaluated the expression of several interleukins in the mucosa of the intestinal reservoir. Interleukins known for their pro-inflammatory properties, such as IL-1 [20,21], IL-6 [22] and IL-12 [23], as well as anti-inflammatory IL-10 [24,25], were assessed. The expression of assessed cytokines was higher in rats with narrow anastomosis. However, in cases of pro-inflammatory cytokines (IL-1, IL-6, IL-12), these differences were statistically insignificant. Obviously, the procedure itself was the factor that greatly influenced the activity of assessed interleukins. The phenomenon of increased activity of pro-inflammatory (IL-6) and anti-inflammatory (IL-10) interleukins was observed in peritoneal cavity exudates in patients with anastomotic leakage after partial resection of the large intestine [26]. The increased level of IL-6 and IL-10 was noted even in cases of non-complicated large intestine partial resection.

The increased level of IL-10 is of particular interest. Despite its known anti-inflammatory properties, the role of this interleukin in the development of pouchitis has not been thoroughly examined. There is some evidence supporting its anti-inflammatory activity in pouchitis [27], yet it has also been noted that its level was similar in patients with pouchitis and healthy subjects [28]. It seems that significantly higher levels of IL-10 activity in rats with narrow anastomosis may be related to higher inflammatory reaction, which is a non-specific stimulus to IL-10 and, to a smaller extent, to pro-inflammatory interleukins.

## CONCLUSIONS

Narrow anastomosis significantly influences the intensity of inflammatory changes within the pouch mucosa and stimulates the production of interleukins, in particular the anti-inflammatory IL-10. Obtained results suggest that recommendations in pouchitis should take into consideration the assessment of the anastomosis width and its dilatation in justified cases.

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