Ileal Pouch Morphology and Microbiology in Ulcerative Colitis Patients

1 First Department and Clinic of General, Gastroenterological and Endocrinological Surgery, Wroclaw Medical University, Poland
2 Division of Pathomorphology, Department of Pathomorphology, Wroclaw Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article; G – other

Abstract

Background. Ideal pouch created during restorative proctocolectomy is a new gastrointestinal organ – “neorectum”. Although it is made from the ileum, it takes over function of the removed rectum. This new function results in significant morphological changes in pouch’s mucous membrane, which becomes similar to the large bowel mucosa. The most common pathology of the ileal pouch is its inflammation – pouchitis. One of the suspected causes of pouchitis is bacterial flora disturbance.

Objectives. The aim of the study was to analyze the morphological and microbiological changes in ileal pouches in different time periods after ileostomy closure and to evaluate the influence of certain bacterial strains on the degree of inflammation.

Material and Methods. The study involved 47 patients who had been treated surgically; they were investigated before and at different stages after ileostomy closure. They underwent repeated rectoscopies with biopsies of pouch mucosa and swabs for microbiological examination. In total 89 rectoscopies were performed, which provided 70 histopathological results according to the Heidelberg Pouchitis Activity Score and 87 microbiological test results.

Results. The assessment of the morphology of intestinal pouches showed increased signs of chronic inflammation as the length of time after the closure of a protective ileostomy increased. There was no correlation between the signs of acute inflammation and the length of time after surgery; there were more signs of acute inflammation in cases of pouchitis. The composition of the bacterial flora of intestinal pouches changed as the length of time after ileostomy closure increased, with significant increases in the number of enterobacteriaceae species. The presence of Staphylococcus aureus significantly correlates with a higher degree of chronic inflammation; this bacterium may be a potential infectious factor in pouchitis.

Conclusions. Microbiological analysis of intestinal pouch lumen is a useful tool that can be used in routine follow-up assessment of intestinal pouches as well as in diagnosing pouchitis (Adv Clin Exp Med 2015, 24, 2, 267–274).

Key words: ileal pouch, ulcerative colitis, restorative proctocolectomy, pouchitis.
(pouchitis, cuffitis), as well as features of dysplasia or even malignant transformation [3–7]. The most common pathology is inflammation of the ileal pouch (pouchitis), which develops in up to 50% of patients at different stages after the closure of a protective ileostomy and the restoration of the natural intestinal passage. Good results in the treatment of pouchitis can be achieved using empiric antibiotic therapy [5, 8]. There is also a growing number of publications confirming the beneficial influence of probiotics on the treatment and prevention of pouchitis [5, 9, 10]. These findings strongly suggest that bacterial infection may be a significant factor in the pathology of pouchitis. The aim of this study was to assess the morphology and bacterial flora of ileal pouches at different stages after the closure of the protective ileostomy and restoration of the normal passage of intestinal contents in patients who had undergone surgery for ulcerative colitis. Possible correlations were investigated between microbiological factors and the degree of inflammation assessed by histological, clinical and endoscopic examinations. Another important aim of the study was answering the practical question of whether microbiological examination may be a useful tool in diagnosing pouchitis.

Material and Methods

The study group comprised of 47 patients (26 female and 21 male) chosen from a group of 148 patients who underwent restorative proctocolectomies at the First Department of General, Gastroenterological and Endocrinological Surgery of Wroclaw Medical University (Wroclaw, Poland). These were patients who had accepted invitations to participate in the research program. All of them provided written consent for participation in the study.

In all the examined patients, the indication for restorative proctocolectomy was prolonged ulcerative colitis with an unsatisfactory response to conservative treatment. The surgery was divided into 2 or 3 stages. J-shaped ileal pouches with a capacity of 180–250 mL were constructed, and pouch-anal anastomosis was performed using the Knight-Griffen technique, with a one-layer stapled suture 1 to 1.5 cm above the dentate line without a distal rectal mucosectomy. In each case the pouch-anal anastomosis was protected by means of a loop ileostomy. The closure of the ileostomy was done 3 months after the construction of the ileal pouch.

The patients were examined shortly before and at different stages (3 months to 10 years) after the closure of the protective ileostomy. The endoscopic examination of the ileal pouch before the ileostomy closure was done in the hospital less than 48 h preoperatively. The rigid rectoscopy tube was inserted under direct visual guidance into the ileal pouch.

The morphology of the ileal pouch mucosa was established and, when necessary, signs of inflammation were assessed using the Heidelberg Pouchitis Activity Score (HPAS), which was proposed by Heuschen from Heidelberg University [11] (Table 1).

Using biopsy forceps 3 samples were collected for histological examination and the material was fixed in 8% formaldehyde solution. All the histological examinations were done by one pathologist with significant experience in GI histology. After the collection of the biopsy specimens a microbiology swab sample was taken from the ileal pouch lumen. Material for the evaluation of aerobic, anaerobic and fungi cultures was placed on an agar medium.

The clinical, endoscopic and histological signs of ileal pouch inflammation were assessed in all the patients according to HPAS criteria. The numeric values obtained from each patient were added up. The results obtained were divided into 6 subgroups based on the time interval since the closure of ileostomy. Separate subgroups were created for the analysis of the relationship between microbiological composition and inflammation.

Quantitative variables were expressed as mean and range, while qualitative data were expressed as percentages. The homogeneity of variance was examined by Levene’s test. A one-way ANOVA analysis with a least significant difference (LSD) post-hoc test were used to test the differences between the study parameters in the tested groups, using STATISTICA 5.1 software (StatSoft, Tulsa, OK, USA). P values less than 0.05 were considered statistically significant.

Results

The study examinations were performed on 47 patients; a total number of 87 microbiology results and 70 histology reports were obtained.

Clinical and Endoscopic Picture

In terms of the clinical picture as well as the endoscopic findings, pouchitis was diagnosed in 11 patients (23%) following ileostomy closure. In 6 patients (12.77%) pouchitis was diagnosed more than once; overall 19 (31.15%) out of the 61 rectoscopies performed after ileostomy closure showed pouchitis. In addition, 3 patients (6.38%) with no signs of pouch inflammation at the time of the study examination had at least one episode of pouchitis in their history. Thus, the total number of patients suffering from pouchitis at various stages after ileostomy closure was 14 (29.79% of all the patients). Inflammation of the remaining cuff of rectal mucosa
Changes in Ileal Pouch Mucosa

Changes in Ileal Pouch Mucosa

(‘so-called cuffitis’) was diagnosed in 6 different patients (12.77%). In 3 cases (6.38%), signs of pouchitis and cuffitis were present at the same time. The most common complaints and rectoscopic pathological findings are summarized in Table 2. The most common clinical symptoms were a sudden urgency to pass stools and diarrhea; and in the endoscopic picture the most common symptoms were contact bleeding and hyperemia of the mucosa.

**Histopathology**

Using the HPAS scores allowed the histological features of both acute and chronic inflammation to be assessed. The overall rate and degree of acute and chronic inflammation at different stages after ileostomy closure are presented in Table 3.

There was a statistically significant difference in the mean degree of acute inflammation between groups 3 and 5 only (p = 0.036). The mean degree of chronic inflammation differed significantly between groups 2 and 4 only (p = 0.046), while the degree of villous atrophy was significantly different between groups 1 and 2 (p = 0.030), 2 and 4 (p = 0.030) and 2 and 5 (p = 0.045). There were no statistically significant differences between these features in other groups.

**Microbiological Assessment**

Another part of the study was the microbiological assessment of intestinal pouches. The cultures showed the presence of 1 to 4 species of bacteria or fungi in the lumen of each intestinal
pouch. Aerobic cultures were positive for 97.7% of the samples; both aerobic and anaerobic cultures were positive in 1.15% of the samples; only anaerobic cultures were positive in 1.15% of the samples; and fungi were observed in 20.69% of the pouches. The mean number of species present inside the pouch did not change significantly as the length of time after ileostomy closure increased and intestinal passage through the pouch was restored (p > 0.05). The composition of the bacterial flora in the intestinal pouches in different groups is presented in Fig. 1.

At the next stage of analysis, possible correlations between bacterial flora (a potential trigger of inflammatory response) and the degree of inflammation were investigated. Table 4 presents the degree of acute and chronic inflammation in pouches colonized by different species of bacteria and fungi.

There were no statistical differences in the degree of acute inflammation according to the HAPS scale. The least significant difference test showed statistically significant differences in the degree of chronic inflammation between groups 3 and 6 (p = 0.048), 4 and 6 (p = 0.036) and 6 and 7 (p = 0.025).

*Escherichia coli* and *Enterobacter* species that are part of normal intestinal bacterial flora were the only components of pouch flora found in 5 of the pouches (26.32%) diagnosed pouchitis. The presence of *Staphylococcus* species was associated with the highest degree of acute and chronic inflammation, and this bacteria was found in 9 of the pouches (47.37%) diagnosed with pouchitis.

Table 2. Clinical symptoms and rectoscopic findings in 61 clinical studies after restorative proctocolectomy

<table>
<thead>
<tr>
<th>Clinical symptom</th>
<th>Examinations N (%)</th>
<th>Rectoscopic pathology</th>
<th>Examinations N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudden urgency to pass stool</td>
<td>20 (32.26)</td>
<td>contact bleeding</td>
<td>37 (59.68)</td>
</tr>
<tr>
<td>Higher number of stools</td>
<td>18 (29.03)</td>
<td>hyperemia</td>
<td>31 (50.06)</td>
</tr>
<tr>
<td>Rectal bleeding</td>
<td>16 (25.81)</td>
<td>granulation</td>
<td>21 (33.87)</td>
</tr>
<tr>
<td>Abdominal cramps</td>
<td>7 (11.29)</td>
<td>loss of vascularity</td>
<td>16 (25.81)</td>
</tr>
<tr>
<td>Fecal incontinence</td>
<td>3 (4.84)</td>
<td>mucous exudates</td>
<td>14 (22.58)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>edema</td>
<td>13 (20.97)</td>
</tr>
<tr>
<td>Fever</td>
<td>3 (4.84)</td>
<td>flattening of mucous folds</td>
<td>9 (14.52)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>erosions/ulcerations</td>
<td>4 (6.45)</td>
</tr>
</tbody>
</table>

Table 3. Degree of acute and chronic inflammation as well as villous atrophy assessed according to the HPAS at different stages after ileostomy closure

<table>
<thead>
<tr>
<th>Time since closure of ileostomy</th>
<th>Degree of acute inflammation according to HPAS (0–6)</th>
<th>Mean</th>
<th>Degree of chronic inflammation according to HPAS (0–6)</th>
<th>Mean</th>
<th>Degree of villous atrophy according to HPAS (0–3)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: before closure (9 rectoscopies)</td>
<td>0–5</td>
<td>2.00</td>
<td>3–6</td>
<td>4.67</td>
<td>1–3</td>
<td>2.00</td>
</tr>
<tr>
<td>Group 2: 3 to 6 months after closure (13 rectoscopies)</td>
<td>1–4</td>
<td>1.62</td>
<td>3–6</td>
<td>3.92</td>
<td>1–3</td>
<td>1.23</td>
</tr>
<tr>
<td>Group 3: 7 to 18 months after closure (13 rectoscopies)</td>
<td>0–2</td>
<td>0.92</td>
<td>2–6</td>
<td>4.15</td>
<td>0–3</td>
<td>1.31</td>
</tr>
<tr>
<td>Group 4: 2 to 3 years after closure (17 rectoscopies)</td>
<td>0–4</td>
<td>1.53</td>
<td>3–6</td>
<td>4.71</td>
<td>0–3</td>
<td>1.88</td>
</tr>
<tr>
<td>Group 5: 4 to 5 years after closure (7 rectoscopies)</td>
<td>1–4</td>
<td>2.29</td>
<td>4–6</td>
<td>4.86</td>
<td>2–2</td>
<td>2.00</td>
</tr>
<tr>
<td>Group 6: more than 5 years after closure (11 rectoscopies)</td>
<td>0–4</td>
<td>1.73</td>
<td>2–6</td>
<td>4.36</td>
<td>1–3</td>
<td>1.73</td>
</tr>
</tbody>
</table>
Changes in Ileal Pouch Mucosa

Discussion

Intestinal pouches are constructed from the ileum to replace large bowel that must be removed in patients undergoing surgery for ulcerative colitis, familial adenomatous polyposis, multifocal colorectal cancer or some other rare indications. The intestinal pouch must take on the function of the rectum in the formation, storage and controlled excretion of stools. These new activities of the small bowel result in significant functional and morphological changes in its mucous membranes.

Many different morphological changes that develop gradually after the restoration of the intestinal passage through the intestinal pouch have been described. These include villous atrophy, increased numbers of intestinal crypts, deepening of intestinal crypts, increased numbers of goblet cells and the development of inflammatory infiltrates of mono- and polynuclear cells [1, 2]. These changes in the architecture of the intestinal pouch mucosa were also observed in the present study. They reflect a dynamic process which eventually leads to the ileal mucous membrane becoming strikingly similar to colonic mucosa. This phenomenon has been described in literature as “colonic metaplasia” or “colonization”. In the present study, changes in the morphology of the participants’ intestinal pouches were assessed by analyzing atrophic and inflammatory changes, both acute and chronic.

Villous atrophy may result from permanent interaction between mucous membranes and feces stored in the intestinal pouch, from the multiplication of aerobic and anaerobic bacteria or from an increase in the concentration of potentially toxic bile acids. Kuisima et al. observed increased atrophic changes in pouches with coexisting inflammation [12]. Inflammatory and atrophic changes have also been described in an animal model – i.e., in intestinal pouches in dogs evaluated between 2 and 8 weeks after their construction [13]. The presence and degree of atrophic and inflammatory changes correlated well with the multiplication of anaerobic and aerobic flora [12] or with anaerobic flora only [13]. The use of the HPAS scale in the present study permitted precise assessment of different degrees of villous atrophy in relation to the length of time that had passed since the restoration of the intestinal passage. The majority of authors describe only mild villous atrophy before ileostomy closure in comparison with the significant atrophy present

![Fig. 1. The most common bacterial species colonizing intestinal pouches in relation to the time since ileostomy closure](image)

<table>
<thead>
<tr>
<th>Composition of bacterial flora</th>
<th>Degree of acute inflammation according to HPAS scale</th>
<th>Degree of chronic inflammation according to HPAS scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>range</td>
<td>mean</td>
</tr>
<tr>
<td>Group 1: only E. coli (8 samples)</td>
<td>0–4</td>
<td>1.38</td>
</tr>
<tr>
<td>Group 2: &gt; 1 species enterobacteriaceae (22 samples)</td>
<td>0–4</td>
<td>1.5</td>
</tr>
<tr>
<td>Group 3: presence of Proteus sp (5 samples)</td>
<td>1–3</td>
<td>1.67</td>
</tr>
<tr>
<td>Group 4: presence of Klebsiella sp (7 samples)</td>
<td>0–5</td>
<td>1.71</td>
</tr>
<tr>
<td>Group 5: presence of Candida (12 samples)</td>
<td>0–4</td>
<td>1.42</td>
</tr>
<tr>
<td>Group 6: Staphylococcus aureus (9 samples)</td>
<td>0–6</td>
<td>2.22</td>
</tr>
<tr>
<td>Group 7: coagulase-negative Staphylococcus (12 samples)</td>
<td>0–5</td>
<td>2</td>
</tr>
<tr>
<td>Group 6 + 7: presence of any Staphylococcus (21 samples)</td>
<td>0–4</td>
<td>2.14</td>
</tr>
</tbody>
</table>
in functioning pouches [1, 14, 15]. The observations made in the present study are different: before ileostomy closure normal intestinal villi were not observed. In the majority of the patients in the current study (over 77%) first-degree atrophy was observed, but in more than 22% of them atrophy of the second or even third degree was found. The mean degree of villous atrophy before the closure of the protective ileostomy was significantly higher than in pouches assessed between 3 and 18 months of the protective ileostomy was significantly higher than in pouches assessed between 2 and 5 years after ileostomy closure. It seems rather unrealistic that this significant villous atrophy should reflect a process of healing after surgical manipulations [16] or chronic irritation of the ileal mucosa by different substances present in feces, which is believed to be the main reason for atrophy after the restoration of the intestinal passage [1, 15]. Campbell et al. believe that the development of some adaptive changes in the intestinal pouch before ileostomy closure is triggered by different factors, for example by discrete changes in the intestinal flora [17]. It also seems possible that villous atrophy may result from a lack of contact with digested food, leading to inadequate nutrition of enterocytes, which is largely based on the diffusion of nutritional agents from the intestinal lumen. Only a limited number of reports describe the morphology of intestinal pouches before ileostomy closure, and their analysis does not lead to any clear conclusions explaining why in some patients the highest degree of atrophy is seen before or shortly after ileostomy closure, whereas in other cases only mild atrophy is present even many years after the restoration of the intestinal passage through the intestinal pouch. Moreover, there are some reports describing different degrees of atrophy in specimens collected from different sites of the same intestinal pouch [1, 18].

However, Silva et al. proved that even in cases of complete villous atrophy and significant colonic metaplasia, some biochemical features typical of the small bowel persist. They confirmed the presence of the sucrose-isomaltase enzyme, which is characteristic of small bowel mucosa, in all the intestinal pouches they assessed [19]. It seems that despite significant adaptation of the intestinal pouch to its new function and significant changes in its mucosa, the process of “colonic metaplasia” is never complete.

Changes described in the literature as “colonic metaplasia” are present in all intestinal pouches regardless of the original pathology that was the indication for restorative proctocolectomy. However, inflammatory changes are mainly seen in patients with ulcerative colitis. Inflammation of the intestinal pouch – “pouchitis” – is the most common late complication of restorative proctocolectomy and its prevalence increases with the passage of time after ileostomy closure. It is currently believed that about 50% of patients operated on for ulcerative colitis will experience at least one episode of pouchitis [20]. Chronic inflammation is seen in 10–15% of intestinal pouches [21]. In the current study symptoms of pouchitis, which was later confirmed by endoscopic and histological examinations, were present in almost 30% of the cases. In other reports this rate oscillated between 23% and 46% [3, 22]. Recurrent episodes of pouchitis were observed in 6 of the patients examined in the current study (12.77%).

The most popular present theory explains pouchitis as a recurrence of ulcerative colitis [20]. This is supported by the fact that in the 2 conditions the changes in the intestinal mucosa have a similar endoscopic and microscopic picture, as well as similar immunology, with the activation of the same pro-inflammatory cytokines and a similar response to antibiotic treatment. The gradual changes of the pouch mucosa into mucosa typical of the large bowel with its characteristic antigens may be responsible for turning the intestinal pouch into a new target organ for autoimmune pathology, to which the whole organism is somehow predisposed. The lack of pouchitis in patients operated on for familial adenomatous polyposis seems to support this theory. Moreover, an obvious risk factor for pouchitis is inflammation of the distal part of the ileum – “backwash ileitis”.

In the present study, the degree of acute inflammation did not correlate with the length of time since ileostomy closure. However, when the degree of chronic inflammation was high before ileostomy closure, it lowered significantly during the first months after the restoration of the intestinal passage and then started to increase again after more than 18 months after ileostomy closure. Similar observations were reported by Merret [14]. In cases with pouchitis the degree of chronic inflammation was only slightly higher than in cases without pouchitis (4.39 vs 4.27 on the HPAS), while more obvious differences (however still without statistical significance) were observed in the degree of acute inflammation (1.78 vs 1.22 on the HPAS). These findings were also confirmed in earlier studies by Moskowitz et al., who showed a correlation between the presence of pouchitis and a higher degree of chronic inflammation, and a lack of any significant relationship between pouchitis and chronic inflammation of the intestinal pouch [23].

Collecting biopsy specimens from randomly chosen sites in intestinal pouch as well as from sites with macroscopic abnormalities allows
Changes in Ileal Pouch Mucosa

malignant transformation or dysplasia to be diagnosed. Only a limited number of cases of adenocarcinoma in an intestinal pouch have been described so far [24–26], while dysplasia of different degrees can be seen in 4.4% of intestinal pouches [6]. In the present study no cases of dysplasia were diagnosed. In 3 cases single polyps less than 5 mm in diameter were observed, and in all 3 cases they were removed by a diathermy loop. In all 3 cases histological examinations revealed inflammatory polyps with no signs of dysplasia. Despite the fact that the risk of malignant transformation in an intestinal pouch is described as relatively low [6], persistent pouchitis, especially in its atrophic form, may predispose to dysplasia, thus promoting the development of cancer [27]. It is commonly believed that a history of colorectal cancer, the presence of “backwash ileitis” and the co-existence of primary sclerosing cholangitis are significant independent risk factors for malignant transformation in the “neorectum” [24]. Some authors suggest that a malignancy developing in an intestinal pouch may in fact be cancer of ileal-anal anastomosis originating from the remaining “cuff” of rectal mucosa [28, 29]. Jiang et al. describe the features of 12 cases of pouch and peripouch adenocarcinoma, and there was just 1 in the pouch only; 8 in the anal transitional zone (ATZ, defined as the part of the rectum between the dentate line and anastomosis); 2 in the ATZ and pouch; and 1 in a nonspecific peripouch region [26].

In the present study, in addition to the clinical, endoscopic and histological evaluation of intestinal pouches, a microbiological assessment of material collected from pouch lumen was performed. The main components of the bacterial flora of the intestinal pouches were aerobic species. The most common were enterobacteriaceae strains, which were present in more than 90% of the pouches before and up to 6 months after closure of the protective ileostomy. Later they were present in 100% of the pouches. Staphylococcus species were also a significant component of pouch flora, present in 31.03% of all the cases; these species were mainly present before ileostomy closure and 3 to 18 months after it. A report by Onderdonk et al. also confirmed the common presence of Staphylococcus species (in more than 50% of the cases) [30]. A significant finding is the common presence of Staphylococcus species in pouches with diagnosed pouchitis; there was also a relatively high rate of acute inflammation accompanying Staphylococcal colonization (but without statistical significance). There was also a statistically significant correlation between the presence of Staphylococcus aureus and a high degree of chronic inflammation. It seems possible that Staphylococcus aureus may be the pathogen possibly responsible for the development of chronic pouchitis.

The authors concluded that the assessment of the morphology of intestinal pouches showed increased signs of chronic inflammation (especially atrophy) as the length of time after the closure of a protective ileostomy increased. There was no correlation between the signs of acute inflammation and the length of time after surgery; there were more signs of acute inflammation in cases of pouchitis, but the increase was not statistically significant. The composition of the bacterial flora of intestinal pouches changes with time after ileostomy closure, with a significant increase in the number of enterobacteriaceae species. The presence of Staphylococcus aureus significantly correlates with a higher degree of chronic inflammation. This bacterium may be a potential infectious factor in pouchitis. Microbiological analysis of intestinal pouch lumen is a useful tool that can be used in routine follow-up assessment of intestinal pouches as well as in diagnosing pouchitis.

References


Address for correspondence:

Dorota Pawełka
First Department and Clinic of General Gastroenterological and Endocrinological Surgery
Wroclaw Medical University
Curie-Skłodowskiej 66
50-369 Wroclaw
Poland
Tel.: +48 71 784 21 62
E-mail: dorotapawelka@wp.pl

Received: 5.12.2013
Revised: 18.06.2014
Accepted: 19.03.2015