Dysplasia and DNA Aneuploidy in a Pelvic Pouch

Report of a Case

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A patient with an 18-year history of ulcerative colitis was operated on with colectomy, mucosal proctectomy, ileoanal anastomosis, and an S-type pelvic pouch due to intractable chronic continuous disease. The patient was followed by endoscopic controls and biopsy sampling from the pouch at regular intervals. A gradual development of severe atrophy in the ileal mucosa was followed by the development of low grade dysplasia. At the most recent endoscopic control, 4 years after the construction of the pouch, biopsies were sampled also for flow cytometric DNA analyses. DNA aneuploidy was detected in a biopsy from the center of the pouch, and a biopsy taken immediately adjacent showed low grade dysplasia. These findings underline the importance of endoscopic follow-up after construction of a pelvic pouch and focus attention to the potential of malignant transformation of the mucosa. [Key words: Pelvic pouch; Ulcerative colitis; Dysplasia; DNA aneuploidy]

Colectomy, mucosal proctectomy, and construction of a pelvic pouch with ileoanal anastomosis have been developed as a new surgical treatment modality for ulcerative colitis during the last decade.[1–6] The advantages of preserved bowel continuity and sphincter continence have made this approach an attractive alternative to conventional proctocolectomy and ileostomy, particularly in younger patients. Provided that all mucosal tissue in the rectum is removed, the main advantage of the new procedure over the simpler ileorectal anastomosis is the elimination of the risk of recurrent disease and subsequent malignant transformation of the rectal mucosa.

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There are no reports on the development of dysplasia or carcinoma in a pelvic pouch or in a Kock's pouch. However, adenocarcinoma complicating conventional ileostomies following colectomy for ulcerative colitis have been described.[7,8] A recent morphometric analysis of the pouch mucosa by our team revealed two types of mucosal adaption during the first 2 years of function; type A showing stable slight atrophy without dysplasia and type B comprising progressive, eventually severe atrophy.[9] In two patients with type B response, indefinite epithelial changes or low grade dysplasia developed. The present report describes the occurrence of low grade dysplasia accompanied by DNA aneuploidy in the pouch mucosa in one of these patients.

REPORT OF A CASE

A 36-year-old male from Sri Lanka had the diagnosis of ulcerative colitis established in 1972. Colonoscopy performed in 1981 and in 1985 revealed a severely inflamed mucosa extending from the rectum proximally to the cecum. Due to chronic continuous disease activity, unresponsive to treatment with corticosteroids and sulfasalazine, curative surgery was performed in 1985. Colectomy, mucosal proctectomy, and construction of an S-type pelvic pouch with ileoanal anastomosis were carried out in September 1985. The temporary loop-ileostomy was closed 3 months later. There were no surgical complications. Histologic examination of the resected bowel revealed an atrophic mucosa with some structural villous changes and varying degree of inflammatory changes including crypt abscesses. Indefinite changes, probably negative for dysplasia (probably inflammatory), were recorded. At the preceding colonoscopy, flow cy-

tometry showed a diploid DNA content in the mucosal biopsies.

After restoration of bowel continuity, the frequency of bowel movements were six to seven during the day and two to three at night. Endoscopy performed with a pediatric, rigid sigmoidoscope 1 month after closure of the loop-ileostomy, revealed an inflamed mucosa in the pouch with granularity, edema, and friability. No vascular pattern was visible. A clinical and endoscopic diagnosis of pouchitis was made and treatment with metronidazole 200 mg three times daily was initiated. Although some improvement in terms of decreased frequency of bowel movements as well as of the endoscopic picture was achieved, the inflammatory changes in the pouch reappeared as soon as the treatment with metronidazole ceased. The patient was thereafter treated with metronidazole on an almost continuous basis with doses ranging from 200 to 600 mg a day depending on the degree of symptomatic improvement.

Biopsy Sampling

Biopsies were sampled at two or three levels in the pouch at endoscopic examinations carried out at 0, 6, 12, 24, 36, and 48 months following closure of the loop ileostomy. After fixation in 4 percent phosphate buffered formaldehyde overnight, the biopsies were embedded in glycolmethacrylate. Sections of 2 μm thickness were cut and stained with H&E, PAS, high iron diamine/alcian blue, and according to the KOH/alcian blue 1.0/PAPS technique. The specimens were evaluated for dysplasia, mucosal atrophy, grade of inflammation, quantity of goblet cells and mitoses as previously reported.[9] Epithelial dysplasia was assessed according to the classification adopted by Riddell *et al.*[10] Epithelial changes were scored from 0 to 3 (0 = negative for dysplasia, 1 = indefinite changes, probably positive, 2 = low grade dysplasia and 3 = high grade dysplasia). Mucosal atrophy was determined by measurement of villous surface density (Sv) as described previously,[11] and the range of Sv-values was divided into four categories (0–3) corresponding to normal, slight, moderate, and severe atrophy. Figure 1 shows the development of atrophy as well as dysplasia during the 4-year follow-up.

At the time of the latest examination, 48 months after closure of the loop-ileostomy, biopsies were sampled from three different levels in the pouch.

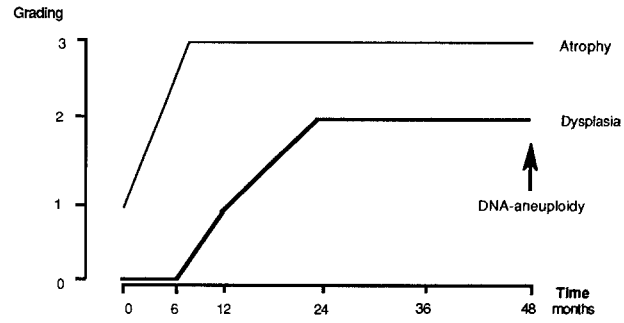

Fig 1. Grading of mucosal dysplasia (thick line) and atrophy (thin line) in biopsies from the pelvic pouch during 48 months of follow-up from the time of closure of the loop ileostomy.

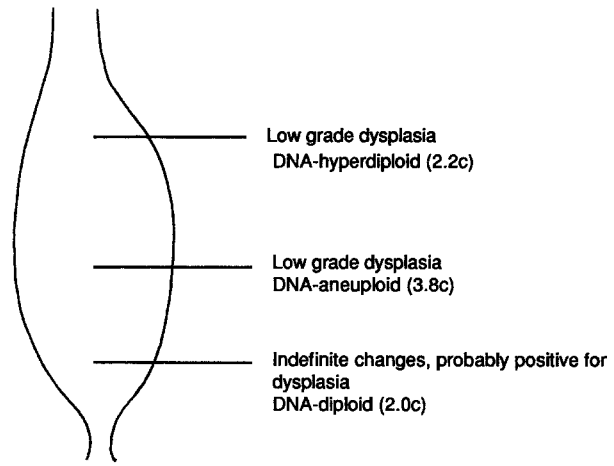

Fig. 2. Findings of dysplasia and DNA aneuploidy at three levels in the pelvic pouch.

From each level two biopsies were taken 0–2 mm apart. One of the biopsies from each level was assessed histologically, and one biopsy was also analyzed by flow cytometry according to a method previously described in detail[12] for determination of DNA content. Marked abnormal, aneuploid DNA content at 3.8c (DNA relative value) was detected in the biopsy taken from the center portion of the pouch (Figs. 2 and 3) in addition to a normal, diploid background. The aneuploid cells comprised 55 percent of the specimen. The biopsy from the most proximal part of the pouch also displayed a disturbance in the DNA content with a hyperdiploid additional peak at 2.2c. The DNA analysis from the distal biopsy showed a diploid DNA content. Histology showed severe atrophy in all three biopsies (Sv value = 51–84 cm^{-1}; normal value > 350 cm^{-1}). Low grade dysplasia was recorded in the proximal and center biopsies (Fig. 4), whereas the biopsy from the distal part of the

pouch showed indefinite changes, probably positive for dysplasia.

DISCUSSION

This case of ulcerative colitis operated on with colectomy, mucosal proctectomy, and construction of a pelvic pouch, and subsequently developing marked atrophy and low grade dysplasia in the ileal mucosa, is to our knowledge the first one reported of its kind. The malignant potential of the changes is confirmed by the unequivocal finding of DNA-aneuploidy at the latest examination. These findings are intriguing as they raise the question whether the potential of malignant transformation of the mucosa in the colon and rectum in ulcerative colitis also exists in the ileal mucosa of a pelvic pouch under adaptation to a new function and environment. There is a strong correlation between findings of dysplasia or carcinoma and DNA-aneuploidy in longstanding ulcerative colitis,[12,13] and the risk of an impending pouch-carcinoma in our patient must be considered.

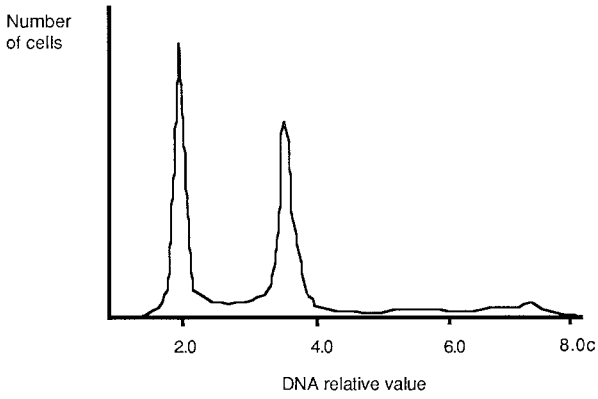

Fig. 3. Histogram from flow cytometric DNA analysis of a biopsy in the midportion of the pelvic pouch showing a distinct aneuploid peak at 3.8c.

Small bowel adenocarcinoma is normally very rare, and the question arises why the mucosa in an ileal pouch should be more prone to malignant transformation. No case of carcinoma complicating of Kock's pouch has been reported so far.[14] This restorative procedure has been practiced in a great number of patients during the last two decades. To some extent, the conditions in a Kock's pouch are similar to those in a pelvic pouch; the same segment of ileum is used for the restorative operation

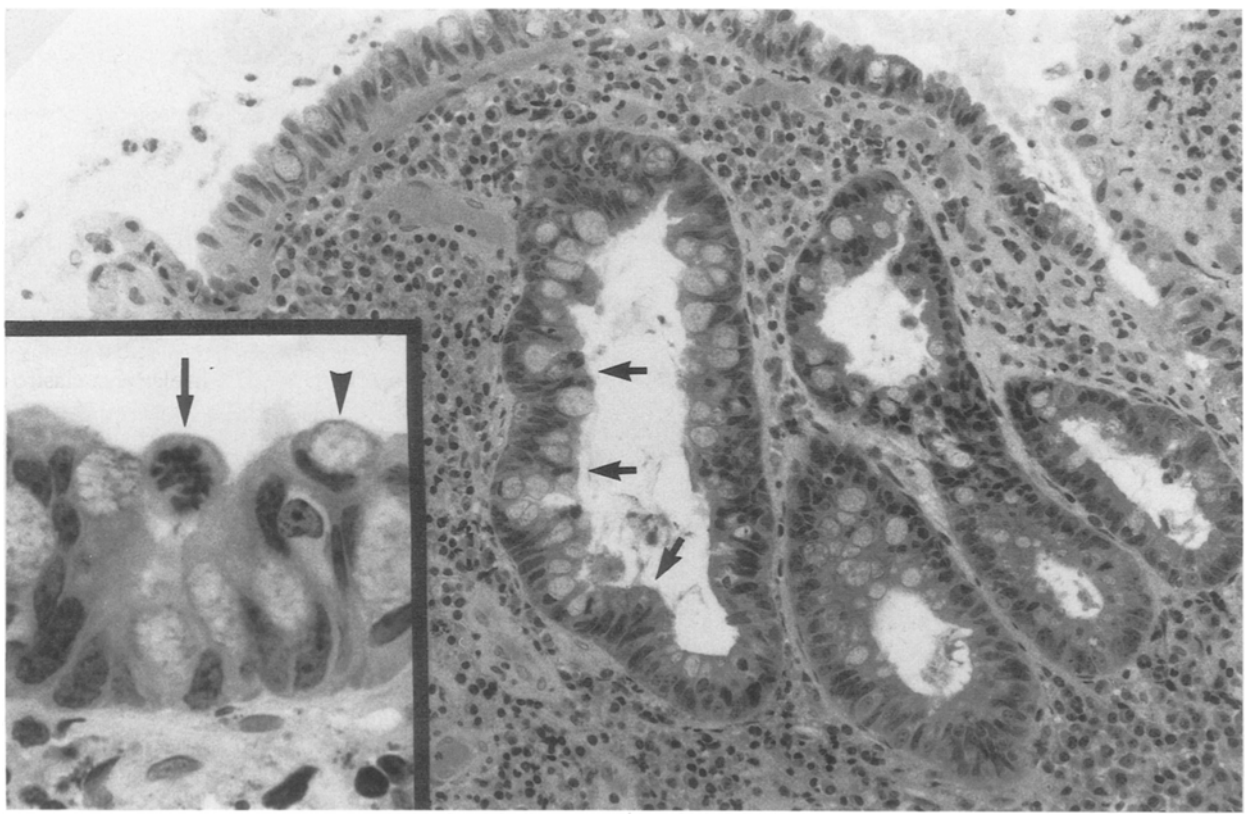

Fig. 4 Mucosal biopsy from midportion of the pouch showing severe atrophy and low grade dysplasia with disorderly arranged absorptive epithelial cells and goblet cells (arrows). Inset: mitotic figure at the top of the epithelium (arrow) and dysplastic goblet cell (arrowhead). Hematoxylin and eosin, × 240, inset × 730.

and the time of mucosal exposure to fecal content is probably similar. As implicated by the patients, albeit few, who developed adenocarcinoma in a conventional ileostomy,[7,8] other factors may play a role in the malignant transformation in individual patients. Rather than the inflammatory process per se, luminal factors or differences in the composition of the fecal contents, such as the amount and proportion of bile and fatty acids,[15] may be of importance in this respect.

In a small series from Huddinge of consecutive pelvic pouch patients, two types of mucosal adaptation, A and B, were delineated.[9] A response with stable, slight atrophy and no signs of dysplasia was recorded in type A (five patients). A progressive, eventually severe atrophy developed in type B (five patients), accompanied by sometimes severe inflammation. In the latter group two patients also developed indefinite changes, probably positive for dysplasia, and one patient (the one here reported) developed low grade dysplasia. The validity of the previous histologic assessment of dysplasia is confirmed by the results from the present DNA analyses.

It is obvious that some patients receiving a pelvic pouch have an abnormal adaptation of the ileal mucosa in the pouch, and this group of patients should be carefully surveyed with endoscopy and biopsy sampling at regular intervals. The risk of malignant transformation should not be underestimated, and centers performing pouch surgery in long-standing ulcerative colitis should keep track of their patients in order to identify this subgroup of patients.

Long-term follow-up with frequent biopsy sampling in patients displaying dysplastic changes and severe atrophy in the pouch is needed to determine if the changes are reversible or progressive. The use of flow cytometry to detect DNA aneuploidy may be of additional value in this follow-up.

ACKNOWLEDGMENT

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Editorial Comment

The authors report a patient with a pelvic S-pouch, in whom a chronic, continuous type of pouchitis developed, necessitating long-term use of Flagyl. Morphologic changes of severe atrophy developed in the pouch, with one portion of the

pouch showing histologic evidence of low-grade dysplasia and DNA aneuploidy. The aneuploidy documented on flow cytometry was detected in a biopsy from the center of the pouch and a biopsy immediately adjacent to this showed low-grade dysplasia. The authors suggest that the malignant potential of the pelvic pouch—particularly those that develop severe atrophic changes—should not be underestimated and that continuing follow-up is necessary.

The current major controversy in pelvic pouch surgery centers around stapling techniques and the risk of carcinoma developing in residual anorectal mucosa. The histologic changes of mild and/or severe atrophy occurring in an ileal reservoir have been well documented from the studies done originally on the Kock pouch and confirmed in the pelvic pouch. Despite hundreds of ileal reservoirs being performed over a 20-year period, there have been no reported cases of adenocarcinoma arising in an ileal reservoir. Inflammatory changes are quite common in the reservoir, as are atrophic changes. It would seem logical to assume that areas of chronic continuous inflammation might be predisposed to the development of carcinoma, and it is surprising that a carcinoma has not been documented in an ileal reservoir. In this particular case of documented pouchitis, it would have been interesting to know from the authors as to the state of the inflammatory changes, at the time of biopsy. Although it is mentioned in the biopsy sampling that the biopsies were assessed for the grade of inflammation, this information was not made available. If the inflammatory process was moderate to severe, it would make the interpretation of low-grade dysplasia more difficult and less meaningful. It is also well known that low-grade dysplasia does not necessarily proceed to severe dysplasia and may, in fact, regress to a more normal state. It is also known, histologically, that some of the ileal reservoirs develop a colonic type of epithelium and, therefore, it might not be surprising that dysplasia could develop with this type of epithelium following chronic inflammation.

In view of the numerous ileal reservoirs that have been performed and the long follow-up, the risk of malignant transformation thus far has been surprisingly low. The authors should bear this in mind before making the statement that the risk of malignant transformation should not be underestimated.

In summary, I would agree with the authors that those patients with pouches showing severe atrophy and chronic inflammation should be followed more closely for the possible development of dysplastic changes. A long-term follow-up of all of our pelvic pouch patients is certainly indicated and further documentation of colonization of epithelium, chronic inflammation, dysplasia, and potential carcinoma awaits further correlation.

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Author's Response

We appreciate the points raised by Dr. Cohen and agree that careful long-term follow-up is indicated after pouch surgery for ulcerative colitis. Concerning the question of inflammatory changes in the pouch of the patient described in the case report, we may add that only a chronic inflammatory cell infiltrate, composed of predominantly plasma cells, was present in the stroma at the time of the last occasions for biopsy sampling. As no neutrophil attack on the epithelium was present, we consider that the interpretation of dysplasia is correct, in line with previous reports.

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