Brush cytology of the colon and rectum in ulcerative colitis: an aid to cancer diagnosis

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SUMMARY In a prospective study of 100 patients with ulcerative colitis, 82 of whom had extensive colitis, carcinoma and dysplasia were distinguished cytologically from reactive hyperplasia. Six patients had carcinoma complicating colitis and satisfactory samples were obtained from five; the cytological appearances were interpreted as carcinoma in three and as dysplasia in two. Seventy eight patients had not developed carcinoma or dysplasia; the cytological appearances were interpreted as negative for dysplasia in 75 and indefinite for dysplasia in three. In patients who had developed dysplasia the changes seemed to be more widespread on cytological rather than on histological examination. Brush cytology may complement histological assessment in patients with ulcerative colitis who have developed strictures or in whom there is a high suspicion of neoplastic change.

Visual examination of the colon by colonoscopy or sigmoidoscopy is an accurate method of diagnosing most colorectal neoplasia. In patients with long standing ulcerative colitis, however, early carcinomas are difficult to identify visually particularly when the mucosa is very inflamed or deformed. In surveillance programmes for patients with extensive ulcerative colitis mucosal biopsy specimens are taken in addition to visual examination. These specimens may be difficult to interpret, especially in the presence of active inflammation, and they sample only a very small area of the colorectal mucosa.

Brush cytology through the colonoscope has been shown to have a high accuracy in the diagnosis of colorectal cancer and has been recommended in the diagnosis of colonic strictures. This method of examination has not been used routinely in the diagnosis of carcinoma in ulcerative colitis because of reports that the inflammatory changes of ulcerative colitis were difficult to distinguish from neoplastic changes. These reports, however, were based on cytological preparations collected by the irrigation-suction method, by colonic irrigation, or by using a specially designed perspex tool. When brush cytology through the colonoscope became possible a study was carried out to assess its accuracy. Of 57 patients with ulcerative colitis, the four who developed colorectal cancer were correctly identified. No attempt, however, was made to analyse the extent or duration of the colitis in these patients, nor to determine whether inflammation produced difficulties in cytological diagnosis.

The aims of this prospective cytological study of patients with long standing extensive ulcerative colitis were threefold: firstly, to provide an accurate description of the cytological changes in long standing ulcerative colitis; and secondly to determine whether dysplasia or malignancy in ulcerative colitis could be reliably detected by cytological examination. A third aim was to determine whether cytological examination added any extra information to that already provided by histological examination.

Patients and methods

Patients without ulcerative colitis
Specimens were taken from 34 patients undergoing surgery, colonoscopy, or sigmoidoscopy at St Mark’s hospital as part of the diagnosis or treatment of their condition. Thirteen patients had carcinoma, three had adenomas, two had Crohn’s disease, seven patients were undergoing screening for colorectal cancer, six patients had irritable bowel syndrome, two patients were constipated, and one had diverticular disease. In
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15 cases the samples were collected by brushing surgical specimens before fixation; in 12 cases the samples were collected at colonoscopy; and in seven cases the samples were collected at sigmoidoscopy.

PATIENTS WITH ULCERATIVE COLITIS
Specimens were collected from 100 patients with ulcerative colitis on 113 different occasions. The patients’ mean age was 50 years (range 21–79), their colitis was extensive (extending up to or beyond the hepatic flexure on barium enema) in 82. Among the 82 patients with extensive colitis the mean duration of disease was 21 years (range four to 44). In 92 patients specimens for cytological examination were collected only once; in a further eight patients samples were collected on at least two separate occasions. Five of the samples were collected from operative specimens; in 55 cases they were collected at colonoscopy; and in 53 cases they were collected through the rigid sigmoidoscope.

COLLECTION OF SAMPLES
Cytology brushings were taken during colonoscopy either with a standard sheathed 10 mm × 3 mm reusable colonoscopy brush, or in some of the early cases, with disposable brushes. The cytology brush was rinsed in tap water and wiped with a gauze swab between brushings, when multiple brushings were taken from a given patient. This procedure was shown, by making smears immediately after cleaning the brush, not to carry over cells from one sample to another. Between examinations on different patients the reusable brushes were always carefully cleaned with detergent and then disinfected by immersing in 4% alkaline glutaraldehyde solution. All patients undergoing colonoscopy were given a standard 48 hour bowel preparation with two sachets of Picolax (Nordic Limited) and one of X-prep (NAPP Limited).

The brushings taken through the rigid sigmoidoscope were made with a specially designed brush (Keymed Ltd). Initial studies had shown that a straight, rigid 10 mm × 3 mm nylon brush on the end of a 40 cm steel shaft did not give satisfactory preparations. When the eyepiece of the sigmoidoscope was removed the bowel deflated and the mucosa fell across the end of the scope preventing brushing with a cylindrical brush. A brush with a rounded end was designed which could be easily rotated on the mucosa and which brushed an area of about 3 cm². As no bowel preparation was given before sigmoidoscopy it was occasionally necessary to wipe the mucosa clean with swabs before brushing it. Brushings from surgical specimens were made within an hour of their excision using the proctosigmoidoscope cytology brush. Biopsy specimens were taken for histological assessment from mucosa adjacent to the area brushed.

Sigmoidoscopic specimens were taken with the Brock biopsy forceps, and colonoscopic specimens were taken with a standard Olympus colonoscopic biopsy forceps.

FIXATION AND STAINING OF SAMPLES
After brushing, samples were smeared directly on to slides and then immediately fixed by immersion within five to 10 seconds in a solution containing 95% ethyl alcohol and 3% acetic acid. The slides were frosted at one end and they were marked with a pencil so that the side on which the smear had been made could be easily identified during staining. The slides were stored in the fixative before staining with haematoxylin and eosin. For the first 30 samples taken, a duplicate set were stained by Papanicolaou’s method. The results of this staining, however, did not give any additional diagnostic information, and so all subsequent staining was done with haematoxylin and eosin alone; the analysis of the results is based only on smears stained with haematoxylin and eosin.

CYTOLOGICAL CRITERIA FOR DYSPLASIA AND MALIGNANCY
The cytological criteria for dysplasia and malignancy were developed in a study of cytological preparations from colorectal carcinomas and normal tissue of patients without ulcerative colitis, as well as from 20 patients with ulcerative colitis. Having evolved criteria for diagnosing malignancy in this way, the slides from a further 80 patients with ulcerative colitis were examined by two pathologists (PIR and NAS) who were unaware of the clinical details, the histological findings, or each other’s grading. They also reread the original 20 slides. The slides were graded as inadequate, normal, inflamed, indefinite for dysplasia, dysplastic, or malignant. The two gradings for each slide were compared and a measure made of interobserver agreement. In the few cases where the two pathologists differed, the worst grading given was used for comparison with the histological results. This cytological grading was not reported to the clinicians looking after the patients at the time.

The “cytological grading” was then compared with the histological report for biopsy specimens which had been taken at the same time as the brushings from adjacent mucosa. Histological results were reported by a senior registrar and two consultant histopathologists of St Mark’s Hospital who had access to both clinical findings and reports from previous biopsy specimens. The histological slides were graded as no dysplasia, indefinite for dysplasia, definite, low, or high grade dysplasia, and carcinoma in accordance with the criteria of Riddell et al.¹⁶
RESULTS

CLINICAL OUTCOME AND HISTOLOGICAL RESULTS

For samples taken from patients on more than one date the worst cytological assessment was compared with the worst histological result for that patient. Where the final clinical outcome for the patient was known, this was compared with the cytological and histological assessments; where no operative specimen was available the final histological grading was taken as the correct one.

Results

UNSATISFACTORY SAMPLES

There were 11 non-colitic samples and 13 colitic samples which were considered to be inadequate for analysis (total = 24 of 137, or 18%). In the case of the sigmoidoscope samples this was due to inadequate samples being obtained during the development of the prototype brush described above. The inadequate colonoscopic samples were generally gathered with the disposable type of brushes which had insufficient bristles to obtain brush samples in this study. Faecal contamination was surprisingly not generally a problem apart from in one patient with carcinoma palpable through the anus, in whom the cancer was difficult to visualise, and in whom no prior cleaning with swabs was attempted.

CYTOLOGICAL CRITERIA FOR MALIGNANCY

Low power assessment

Overall cellularity: This was plentiful in normal mucosa or dysplastic lesions but strikingly higher in smears from carcinomas in which there was often a "dirty background" to the clumps of epithelial cells.

Architecture: The brush usually removed recognisable crypts from normal tissue which could be seen either in transverse or longitudinal section (fig 1). Strips and sheets of cells from the surface epithelium were also often present (fig 2). In dysplastic tissue against this normal background there were occasional clumps of cells which were disorganised, showing both pseudostratification and loss of polarity (figs 3 and 4). Smears from carcinomas often showed complete architectural disruption with loss of coherence and wide dispersion of the cells (figs 5 and 6).

Inflammatory cells: These were present in most of the smears. They consisted mainly of polymorphs, although collections of lymphocytes, plasma cells, eosinophils and histiocytes were also found.

High power assessment

Normal cells: These were well polarised and contained small rounded nuclei, with condensed regular chromatin; some appeared pyknotic. There were few mitotic figures and moderate amounts of eosinophilic cytoplasm. In samples from regenerating epithelium some cells showed minor or moderate nuclear enlargement, but in such cells a regular chromatin pattern was retained and the nuclear membrane was thin and regular.

Cells from areas of dysplasia: These were characterised by enlarged nuclei with irregular angular borders. They sometimes contained up to four prominent nucleoli. The nuclear chromatin showed a dispersed,
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Fig 3 Cytological preparation from patient with long standing ulcerative colitis. A sheet of normal epithelial cells (above) together with a separate fragment of dysplastic cells (below) showing nuclear enlargement, atypical chromatin pattern, and irregular nuclear membrane. The nuclear polarity is disturbed.

Fig 4 Cells from a patient with long standing ulcerative colitis and biopsy specimens showing high grade dysplasia (case 2). There is prepared nuclear pleomorphism with an abnormal chromatin pattern and small central nucleoli.

spotty pattern. There were also frequent mitotic figures. Cells from carcinomas were similar but showed more pleomorphism than dysplastic ones with very large irregular nuclei, and more prominent nucleoli (fig 6). Bizarre mitotic figures were also present.

Fig 5 Cytological assessment of carcinoma arising in a patient with ulcerative colitis (case 1). There is loss of polarity, nuclear pleomorphism, and an abnormal chromatin pattern. A mitotic figure is present.

INTEROBSERVER AGREEMENT
For the 10 smears graded as showing carcinoma by an observer, the second observer agreed in eight cases and graded the other two as dysplastic. Eleven smears were graded by one observer as showing dysplasia; the second observer graded eight of these as dysplastic, two as indefinite for dysplasia, and one as normal.

Six patients developed a carcinoma following long standing ulcerative colitis. Only one of these patients had been in a regular surveillance programme but three had had biopsy specimens showing dysplasia before the diagnosis of carcinoma. Four patients had also had biopsy specimens that had shown definitive high or low grade dysplasia, but they had not yet come to surgery.

CORRELATION OF CYTOLOGICAL ANALYSIS WITH CLINICAL OUTCOME AND HISTOLOGICAL RESULTS

Non-ulcerative colitis
Using the above cytological criteria, smears from all 13 cancers and three adenomas were differentiated in a blind assessment from the seven normal controls. Carcinomas were also distinguished from adenomas using architectural features in addition to the cytological ones (table).

Ulcerative colitis
Adequate smears were obtained from five of the six patients with carcinoma. Three of these were diagnosed as carcinomas by cytological assessment; one was graded as dysplasia, and one was graded as
indefinite for dysplasia. Three of these carcinomas had been diagnosed preoperatively by histological assessment, although dysplasia had been recognised in one of the other cases.

In all four patients who had had a biopsy specimen showing dysplasia (but who had not yet undergone surgery) at least one cytology brushing had also shown dysplastic change. Where multiple brushings were taken at colonoscopy in such patients more cytological smears than histological biopsy specimens showed dysplastic changes. This was not simply due to carriage of cells on the colonoscopic brush, because rectal brushings through the rigid sigmoidoscope were positive when dysplastic lesions had been shown by biopsy on the right side of the colon and the corresponding rectal biopsy specimens were normal.

One further patient had dysplasia diagnosed on cytological assessment but the biopsy specimens were graded as indefinite for dysplasia.

In three patients (excluding those who had dysplasia or carcinoma) cytological findings were reported as indefinite for dysplasia; in one the histological sample was inadequate, in one case the subsequent cytology returned to normal, and in a third case of a patient undergoing colectomy for acute colitis, there was no evidence of dysplasia or carcinoma in the specimen.

In the other 74 patients who had adequate samples taken for assessment the cytological and histological findings showed neither dysplasia nor carcinoma. Active inflammation was a feature of about half the malignant, dysplastic, and normal specimens and did not hinder the cytological grading.

**CASE REPORTS**

The history of two of the above patients are given in slightly more detail to illustrate the value of cytology as a complement to histology.

**Case 1** A 41 year old man with extensive ulcerative colitis of 21 years' duration attended the hospital for colonoscopy. He had previously refused prophylactic proctocolectomy and had been an irregular attender at the hospital. He was colonoscoped and multiple histological biopsy specimens were taken which showed active inflammation but no evidence of dysplasia. He was admitted a few days later because of persisting abdominal pain. A barium enema showed a stricture of the ascending colon and it was realised that the first colonoscopy had reached the hepatic flexure only; a second colonoscopy was performed with brush cytology as well as histological biopsy. Again the histological results showed no dysplasia, but the cytological results were clearly malignant (fig 5). Carcinoma of the hepatic flexure metastatic to the liver was confirmed at laparotomy and the patient died three months later.

**Case 2** A 45 year old man with extensive ulcerative colitis of 17 years' duration attended regularly as part of an extensive colitis surveillance programme. Over the previous two years biopsy specimens from the ascending colon had shown both low and high grade dysplasia; other biopsy specimens had been normal. In addition to showing abnormal cytological findings from this region (fig 3) dysplasia was found in two different smears taken from the rectum through the sigmoidoscope on separate occasions to the colonoscopic examination; corresponding rectal biopsy specimens showed no dysplasia. As a result of the repeated finding of dysplasia proctocolectomy was performed. Detailed histological examination of the surgical specimen showed high grade dysplasia in the ascending and transverse colon as well as low grade dysplasia in the descending colon and rectum.
**Discussion**

**IS IT POSSIBLE TO DISTINGUISH BETWEEN NEOPLASTIC AND INFLAMMATORY CHANGES BY BRUSH CYTOLOGY?**

The cytological criteria presented in this report for identifying dysplasia and cancer in colitis are reproducible; there were very few false negative and no false positive results. Although cytological changes were noted in cases with regeneration and inflammation, these were easily identified. The brush cytology specimens contained many of the architectural features of normal colonic crypts which greatly aided interpretation.

We did not find that the cytological changes of active inflammation in ulcerative colitis mimicked malignant change as had previously been described.\(^\text{13}^\text{15}\) This difference is most likely to be related to the method of preparation of the samples. In the Chicago study describing "large bland cells" and "active cells" the samples were obtained by the irrigation-suction method or by colonic lavage and were fixed in less than 50% alcohol.\(^\text{13}\) In this study brushings were fixed in 95% alcohol plus 3% acetic acid. This is also in keeping with the previous report that, "lavage produces considerably more reactive and extraneous cells and fewer malignant cells" than brushing.\(^\text{17}\) Furthermore, the cytological appearances of dysplasia and carcinoma in patients with ulcerative colitis were identical with those identified in non-colitic colorectal cancers and were not present in patients who showed only inflammatory changes.

The differences between carcinoma and dysplasia were difficult to make cytologically. This is in keeping with the fact that the distinction between carcinoma and dysplasia is made by the identification of invasion which cannot be detected cytologically. There were cytological features, however, that were strongly suggestive of malignancy—namely, pronounced nuclear enlargement, gross nuclear pleomorphism, and very large nucleoli.

**WHAT ARE THE PRACTICAL PROBLEMS IN THE USE OF COLONOSCOPIC CYTOLOGY AS AN AID TO SCREENING FOR CARCINOMA IN ULCERATIVE COLITIS?**

Nearly a fifth of the cytological samples taken in this study were inadequate, and this figure is clearly unacceptable, but, these occurred during the development of the technique and brushes; the disposable cytology brushes tested initially in this study were not very robust and removed only small numbers of epithelial cells. Nearly all samples taken can be satisfactory with the reusable colonoscopy brushes and with the proctosigmoidoscope cytology brush developed in this study.

A single cytological specimen can be taken rapidly as sigmoidoscopy. When multiple cytological and biopsy specimens are taken through the colonoscope, the brushings add about 10 minutes to the total time taken for the procedure, though this extra time can be minimised by using separate cytology brushes to take brushings rapidly while the histological biopsy specimens are being orientated on cellulose acetate membrane filters.

**WHAT ADDITIONAL INFORMATION DOES CYTOLOGY PROVIDE AND FOR WHICH CASES SHOULD IT BE USED?**

The cytology brush covers a larger area (about 3 cm\(^2\)) than a biopsy specimen (0.1–0.2 cm\(^2\)), and selectively samples the mucosa. This may increase the sensitivity of the technique compared with biopsy as illustrated by the second case report. The first case report emphasises the value of cytological analysis in the diagnosis of strictured areas.\(^\text{7}\) Cytological assessment should be used in cases of ulcerative colitis complicated by stricture formation, and should also be taken in cases where neoplastic change is suspected on visual, clinical, or histological grounds.

The histological identification of dysplasia itself is not an infallible marker of malignant change.\(^\text{18}\) On the basis of this study, cytology appears to have a similar predictive value for malignant change. This is only a pilot study, however, and cytology will need to establish its accuracy in clinical use before its results can be relied on.

The cytological changes of neoplasia in ulcerative colitis can be distinguished from those due to acute inflammation. Neoplastic change can be accurately identified, although cancer and dysplasia are, on occasion, difficult to differentiate cytologically. The technique seems to provide additional information to that obtained by histology and therefore should be considered to improve sampling in patients where neoplastic change is suspected or where the visual appearances at colonoscopy indicate that dysplasia could be present.

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