Carcinoma and DNA aneuploidy in Crohn's colitis – a histological and flow cytometric study

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Abstract

Twenty four patients with longstanding colonic Crohn's disease were examined prospectively with colonoscopy and multiple biopsy sampling in order to detect histological dysplasia or abnormal aneuploid DNA content, or both. Biopsy specimens were taken from 10 predetermined locations in the colon and rectum. No patient had definite dysplasia but three displayed DNA aneuploidy (12.5%), and one of these subsequently developed a carcinoma (Dukes' C at operation) in the ascending colon. No concomitant dysplasia was detected but the carcinoma as well as other parts of the mucosa were DNA aneuploid. It is concluded that dysplasia is rare in patients with Crohn's colitis, but findings of DNA aneuploidy warrant vigilance in follow up as this may indicate impending carcinoma. Further prospective studies are needed before the predictive value of DNA aneuploidy can be determined and before general recommendations of colonoscopic surveillance, as in longstanding ulcerative colitis, can be made.

Carcinoma development in the colon and rectum complicating longstanding colonic Crohn's disease has been reported in several studies¹⁻⁴ of patients from large referral centres. Their risk may be significantly increased compared with the general population, but epidemiological data do not support this.^{5,6} Only a limited number of patients with Crohn's related colonic carcinoma has been reported so far. In retrospective case reports based on surgical specimens, a varying association between colorectal carcinoma and epithelial dysplasia in Crohn's disease has been reported.⁷⁻¹⁰ The need for colonoscopic surveillance programmes, similar to those in longstanding ulcerative colitis, has been suggested.⁹ However, there are no prospective studies supporting a dysplasia-carcinoma sequence in Crohn's disease. Recently, a case of Crohn's colitis was reported, where the finding of high grade dysplasia in a macroscopic lesion was associated with an abnormal, aneuploid DNA content.¹¹ DNA aneuploidy has also been shown in longstanding extensive ulcerative colitis,¹²⁻¹⁴ a condition with a verified increase in colorectal carcinoma incidence.¹⁵

In this prospective study, 24 patients with longstanding Crohn's colitis underwent colonoscopy and multiple biopsy sampling according to the surveillance scheme used in longstanding ulcerative colitis.^{13,16} The purpose of this study was to determine the frequency and grade of dysplasia or carcinoma in colonic Crohn's disease. Furthermore, the usefulness of DNA

aneuploidy, detected by flow cytometry, as a marker of malignancy was investigated.

Methods

PATIENTS

Twenty four patients with a definite diagnosis of Crohn's disease who had had symptoms for more than seven years underwent colonoscopy. Criteria for a definite diagnosis were either: (1) typical histological changes including epithelioid granuloma found at the histopathological examination of biopsy specimen or (2) typical endoscopy findings according to Pera *et al.*,¹⁷ including segmental inflammation, rectal sparing and aphthoid or large shallow or deep ulcers, or both. Four patients had ileocolitis and in 20 patients the disease was confined to the large bowel only. Twenty two patients (92%) had extensive colonic involvement (greater than two thirds of the colon) and two had only partial involvement (less than one-third) as judged by the endoscopic appearance. Median duration of disease was 16.5 years (range 7-44). Mean follow up with colonoscopy was 1.9 years.

ENDOSCOPY

Colonoscopy was performed with an Olympus CF-1T10L instrument, and, in four instances, with a flexible sigmoidoscope (CF-P10S). If needed, fluoroscopy was used to determine the position of the tip of the colonoscope.

BIOPSY SPECIMENS

Biopsy specimens were routinely sampled from the large bowel at 10 predetermined locations (1=caecum, 2=ascending colon, 3=hepatic flexure, 4=proximal and 5=distal transverse colon, 6=splenic flexure, 7=proximal and 8=distal descending colon, 9=sigmoid colon, and 10=rectum). One or two specimens were taken from each location for histopathology and one specimen immediately adjacent (0-2 mm apart) was analysed by flow cytometry. The biopsy specimens for DNA-analysis were pooled in three fractions (A=1-3, B=4-6, and C=7-10) for screening of DNA aneuploidy.^{13,16} If DNA aneuploidy or dysplasia was detected at one examination, all specimens sampled for flow cytometry were analysed separately when the next colonoscopy was carried out. In one patient who had previously been operated on with subtotal colectomy and ileosigmoid anastomosis, biopsy specimens were also taken from the terminal ileum.

Additional specimens were taken if macro-

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TABLE I Three patients with longstanding colonic Crohn's disease with DNA aneuploidy detected at colonoscopy with multiple biopsy specimens

<i>Patient no</i>	<i>Age (yrs)</i>	<i>Duration since onset (yrs)</i>	<i>Extent of disease</i>	<i>Year of colonoscopy</i>	<i>Dysplasia</i>	<i>DNA aneuploidy</i>	<i>Biopsy location</i>	<i>Remarks</i>
1	31	14	Total	1986 1988 1989	Negative Negative Carcinoma	3·2c* ? (3·3c, 3·8c, 4·0*)	Ascending Ascending	DNA analyses deteriorated (Dukes' C at op). No dysplasia. DNA analyses made on surgical specimen. Ileosigmoid anastomosis 1982
2	44	27	Ileocolonic	1988	Indefinite (probably +ve)	2·6c*	Terminal	
3	19	13	Total	1989 1986 1988	Negative Negative Negative	Diploid 1·8c* Diploid	Transverse ileum	

*DNA relative value of aneuploid peak.

scopic polypoid or raised lesions other than endoscopically typical inflammatory polyps were detected.

DYSPLASIA

Biopsy specimens for histopathology were fixed in 10% formaline, embedded in paraffin, and stained with haematoxylin and eosin. All specimens were assessed by one of the authors (ÅÖ) without knowledge of the results of the DNA analyses. Dysplasia was classified according to Riddell *et al*¹⁸ and graded as (1) negative, (2) indefinite, probably negative (inflammatory), (3) indefinite, probably positive, (4) low grade, or (5) high grade.

DNA FLOW CYTOMETRY

Preparation for DNA flow cytometry was done according to the procedure previously reported^{12 13 16} and is described briefly below. The fresh specimens were placed in saline and gently pressed through a nylon grid (40–50 mesh counts/cm). The cells were then fixed in 96% ethanol. The RNA was eliminated using 1 mg/ml RNase in an isotonic Tris EDTA buffer, pH 7.5. After pepsin treatment (0.5%, pH 2.0) for 10 minutes, suspensions of single cell nuclei were obtained. The DNA was stained with 2.5 × 10⁻⁵ mol/l ethidium bromide in Tris EDTA buffer with a molarity of 395 mOsm.

Suspensions of 5000–20 000 cells were analysed in a rapid cytofluorometer (ICP 11, Phywe,

Figure 1: (A) Flow cytometric DNA analyses of biopsy specimens from the proximal third of the colon in a patient with longstanding colonic Crohn's disease. An aneuploid peak at 3.2c comprising 31% of the analysed cells is seen. (B) Three years after the finding of DNA aneuploidy depicted in (A), a carcinoma was detected in the ascending colon. The carcinoma was staged as Dukes' C at surgery and a hemicolectomy according to the drawing was performed. Flow cytometric DNA analyses were performed on eight samples (I–VIII) from the surgical specimen. I: diploid, II: aneuploidy at 3.3c (8% of the cells), III: diploid, IV: aneuploidy at 4.0c (33%), V: aneuploidy at 3.8c (38%), VI: diploid, VII: diploid, VIII: diploid. No dysplasia was found. (C) Histologic detail of the carcinoma showing signet ring cell pattern. (Haematoxylin and eosin, original magnification ×200.) (D) Flow cytometric DNA histogram from sample V (within the carcinoma) showing a diploid background and an additional aneuploid peak at 3.8c.

Figure 2: (A) Colonoscopic biopsy specimen from the terminal ileum of a patient with ileocolonic Crohn's disease previously operated on with colectomy and ileosigmoid anastomosis. A flattened, irregular small intestinal mucosa is seen with indefinite changes, probably positive for dysplasia. (B) DNA histogram from a specimen taken immediately adjacent to (A) showing a distinct aneuploid peak at 2.6c together with a normal, diploid DNA content.

Germany) equipped with a xenon lamp. The excitation and emission wavelengths were 455–490 nm and 590–630 nm respectively. A 256 channel analyser was used to sort the output and the result was presented as a histogram of the cell population.

The DNA values of the analysed nuclei were calculated in relation to the DNA content of normal human lymphocytes in order to get a diploid reference point (2c). The coefficient or variation of the lymphocyte standard diploid peak was below 3%. Cell suspensions with a single G₁ peak deviating less than 10% from the standard lymphocytes were considered to be

diploid. An aneuploid cell population was considered to be present when, in addition to the diploid peak, another distinct peak in the DNA histogram was observed.

Results

A total of 39 complete colonoscopies and four flexible sigmoidoscopies were carried out in 24 patients (1.8 per patient). Biopsy specimens for DNA analyses were sampled at all examinations except two.

No patient had findings of definite dysplasia but DNA aneuploidy was detected in three patients (Table I). Thus, the crude incidence rate of aneuploidy was 12.5%. The first patient (Fig 1A–D) had DNA aneuploidy detected in the proximal third of the colon at the initial colonoscopy. At colonoscopy two years later neither macroscopic lesions nor histologic dysplasia were detected but unfortunately all 10 biopsy specimens for DNA analyses deteriorated through technical problems. One year later, colonoscopy showed a carcinoma in the ascending colon. At laparotomy, metastatic growth in the lymph nodes was encountered and a right side hemicolectomy with an end to end ileotransversostomy was performed. The carcinoma was staged as Duke's C. Dysplasia was neither found adjacent to nor distant from the carcinoma when the surgical specimen was examined, but the carcinoma as well as the mucosa proximal to the tumour showed DNA aneuploidy. The ploidy level of one of the aneuploid specimens was similar to that detected three years earlier, although there was a notable heterogenicity of the carcinoma with several areas having a normal diploid DNA content. The carcinoma was mainly poorly differentiated with small areas of signet ring cell carcinoma and also with areas with well differentiated colloid adenocarcinoma. The patient was in a quiescent phase of the inflammatory bowel disease with very limited clinical activity. Histological assessment of the colectomy specimen showed moderate inflammatory activity in the ascending colon with a few giant cells and inconspicuous granuloma.

In one other patient (patient 2), who had previously had colectomy and ileosigmoid anastomosis, DNA aneuploidy was detected once in the terminal ileum just proximal to the anastomosis. In the same part of the ileum, changes classified as indefinite, probably positive for dysplasia, were found as well (Fig 2A and B). However, no abnormal finding was made at a renewed endoscopy one year later. The third patient (Table I) had a hypodiploid DNA content in one fraction at the initial colonoscopy but follow up examination two years later showed a normal diploid DNA content. No dysplasia was found. One of the other 21 patients had isolated findings of indefinite, probably negative (probably inflammatory) changes in flat mucosa, but no aneuploidy was detected. In the remaining 20 patients neither dysplasia nor DNA aneuploidy was found (Table II).

Discussion

The patients in this study all had a history of

TABLE II Histological changes and results of flow cytometric DNA analyses in 24 patients with colonic Crohn's disease

DNA-analyses:	Histological changes			
	Carcinoma	Indefinite, probably +ve for dysplasia	Indefinite, probably -ve for dysplasia	Negative
Aneuploid	1	1	0	1
Diploid	0	0	1	20

Crohn's colitis exceeding seven years. Five patients were originally classified as having ulcerative colitis and had already taken part in a surveillance programme,¹⁶ but their diagnosis was changed to one of Crohn's disease because of the subsequent histological and endoscopic findings. This highlights the problem of applying the correct diagnosis in some patients with chronic inflammatory bowel disease and the difficulties in selecting patients for surveillance programmes. However, none of the patients who had their diagnosis reclassified developed dysplasia or DNA aneuploidy in the present study. Only patients with a definite diagnosis of Crohn's disease according to the criteria were selected for this surveillance, and we did not include patients with a 'working diagnosis' of Crohn's colitis or with indeterminate colitis. Nevertheless, those latter patients have also been examined with repeated colonoscopies after eight to 10 years of disease duration, albeit without developing either dysplasia nor DNA aneuploidy. By applying strict criteria for patient selection in various surveillance programmes, we may possibly be able to define the patients with colonic inflammatory bowel disease who are at the highest risk of developing dysplasia and carcinoma.

In retrospective studies of colorectal carcinomas in Crohn's disease,^{8–10} the duration from onset of symptoms of the disease to cancer diagnosis is around 20 years. The median disease duration in our group was more than 15 years, and eight patients had a duration exceeding 20 years. Furthermore, most patients had extensive disease. If the same risk factors for colorectal carcinoma in ulcerative colitis – that is, extensive disease and long duration¹⁵ – also apply for colonic Crohn's disease, then the patients in this series would definitely be in the 'risk zone'.

DYSPLASIA FINDINGS

This study did not find any patient with definite epithelial dysplasia among those with longstanding, extensive colonic Crohn's disease. Only two patients had indefinite changes. This result is in accordance with data previously reported by Warren and Barwick,¹⁹ who found dysplasia (classified as mild) in only 2% of a total of 100 consecutive resected specimens from patients with ileocolonic Crohn's disease. Farmer *et al.*,²⁰ in a retrospective study from the Cleveland Clinic, did not find any dysplasia in 615 patients with Crohn's disease (427 with colonic involvement) observed over more than 13 years, although one patient developed carcinoma in the colon and one in the jejunum. However, Petras *et al.* from the same centre¹⁰ evaluated 3500 patients with Crohn's disease between 1975 and 1984 and found dysplasia in five of seven patients with colonic carcinoma, although they concluded that dysplasia distant from the tumour was not as common as in ulcerative colitis related carcinoma. In a series of 10 patients with colonic Crohn's disease and colorectal carcinoma described in detail,⁹ nine had high grade dysplasia close to the carcinoma but only five had high grade dysplasia in flat mucosa distant from the tumour. The low yield of dysplasia findings in

the present study could be due to the short follow up or to the limited number of patients being followed. However, dysplasia in colonic Crohn's disease seems to be an infrequent finding in large patient series and may be a late phenomenon in patients developing colorectal carcinoma.

DNA ANEUPLOIDY AND CARCINOMA

The crude incidence of DNA aneuploidy (12.5%) in the present series of patients with colonic Crohn's disease is rather high, but still lower compared with the figures found in substantially larger retrospective and prospective series of patients with longstanding ulcerative colitis.^{14,16} In these studies, however, a positive correlation was found between aneuploidy and dysplasia in most patients with an abnormal DNA content. The correlation between the presence of aneuploidy and neoplastic development is strong, and aneuploidy has been shown in gastrointestinal malignancies such as adenocarcinoma in Barrett's oesophagus and gastric and colorectal carcinoma. Apart from ulcerative colitis, aneuploidy has been shown in other premalignant conditions – for example, Barrett's oesophagus²¹ and colorectal adenomas²² – although the predictive value of an aneuploid finding in respect of future development of a manifest carcinoma in these conditions still remains to be determined.

As changes in the DNA content are likely to take place before any histopathological dysplastic changes occur,^{16,23} the detection of aneuploidy in mucosal biopsy specimens indicates an increased risk of eventual histological malignant change. The time sequence between the development of aneuploidy and eventual dysplasia or of carcinoma, or both, is unknown, but may be substantial (several years) as illustrated by the first patient in this study. The finding of DNA aneuploidy in this patient was 'predictive' of the subsequent development of a carcinoma three years later. There was neither preceding nor concomitant dysplasia, and as dysplasia seems to be rather rare in colonic Crohn's disease, screening for mucosal DNA aneuploidy may be a more fruitful way to identify high risk patients prone to malignant changes. We still do not know if *all* patients with DNA aneuploidy will eventually develop a manifest carcinoma.

IS SURVEILLANCE NEEDED IN COLONIC CROHN'S DISEASE?

In longstanding extensive ulcerative colitis there is strong evidence of a dysplasia-carcinoma sequence, and the incidence of dysplasia is comparatively high.^{16,24} A dysplasia-carcinoma sequence is not clearly evident in Crohn's disease affecting the large bowel, and the incidence of dysplastic findings seems to be low in large patient groups. These two factors do not favour a surveillance programme based on detection of dysplasia in colonic Crohn's disease. The only marginally increased incidence of colonic carcinoma so far reported is another factor against surveillance. The yield in such a programme would probably be small. However, since the number of patients with longstanding Crohn's

colitis may increase during the coming years because of more effective medical treatment and a decreased colectomy rate, a higher proportion of patients with extensive Crohn's colitis may therefore evolve. Thus, the number of patients with an intact colon and long disease duration may become quite substantial. These factors may increase the incidence of colorectal carcinoma in longstanding Crohn's disease, which accordingly will affect the requirements of surveillance programmes. In fact, some authors have already indicated a similar incidence of carcinoma in ulcerative colitis and Crohn's disease.²⁵

In conclusion, dysplasia is rare in colonic Crohn's disease but findings of DNA aneuploidy in individual patients warrant careful consideration of colectomy. Further prospective trials are needed before any recommendations of surveillance should be issued.

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