

DNA aneuploidy in ulcerative colitis

J B J FOZARD, P QUIRKE, M F DIXON, G R GILES AND C C BIRD

From the University Department of Surgery, St. James's Hospital, Leeds and University Department of Pathology, Leeds

SUMMARY The prevalence of deoxyribonucleic acid (DNA) aneuploidy in 297 samples from 38 patients with ulcerative colitis of varying duration was investigated by flow cytometry. In 12 patients colitis was complicated by the development of colorectal carcinoma: one had three synchronous carcinomas. Only four of 14 carcinomas were DNA aneuploid. Deoxyribonucleic acid aneuploidy occurred focally in the colorectal mucosa in the presence and absence of carcinoma: rates of aneuploidy (67% in cancer patients and 42% in non-cancer patients), were not significantly different ($\chi^2=1.0962$, $p=0.295$). A higher rate of DNA aneuploidy was found in dysplastic tissues (21%) compared with non-dysplastic tissues (15%), but again these differences did not reach statistical significance ($\chi^2=1.0747$, $p=0.299$). Deoxyribonucleic acid aneuploidy and dysplastic change occurred more often with increasing duration of ulcerative colitis ($p<0.001$, $p<0.005$ respectively). We conclude that flow cytometric analysis of cellular DNA content should not replace present morphological methods of assessment of premalignancy in ulcerative colitis, but may be a useful adjunct in the identification of abnormal mucosa.

The risk of carcinoma complicating ulcerative colitis was previously held to be substantial.¹⁻³ More recent work, however, suggests a lower incidence of malignant change in the overall colitic population.⁴ To avoid unwarranted surgery, colonoscopic surveillance for dysplastic change is of value,^{5,6} but the criteria for determining cancer risk remain unsatisfactory.⁷ Studies of mucin and lectin histochemistry,^{8,9} epithelial marker antigens¹⁰ and electron microscopic features¹¹ have shown changes associated with dysplasia and cancer, but have not been useful in defining a high risk population within ulcerative colitis patients.

Deoxyribonucleic acid aneuploidy as measured by flow cytometry is present in about 60% of colorectal adenocarcinomas and is related to survival.^{12,13} It has also been shown in 6-27% of colorectal adenomas^{14,15} and in chronic ulcerative colitis.¹⁶ The finding that DNA aneuploidy correlates with size and histopathological type of adenoma,¹⁵ both accepted criteria of malignant potential in the adenoma-carcinoma sequence, supports the hypothesis that flow cytometric DNA analysis may be of value in cancer surveillance in ulcerative colitis.

This study was undertaken to establish the prevalence of DNA aneuploidy in ulcerative colitis, and its relationship to the development of carcinoma.

Methods

PATIENTS

The departmental files were searched for patients with total ulcerative colitis who had undergone proctocolectomy. Three groups of patients were selected: group 1 with short duration ulcerative colitis, less than eight years ($n=14$); group 2 with long duration ulcerative colitis, greater than eight years ($n=12$); group 3 with cancer complicating ulcerative colitis ($n=12$), one patient having three synchronous carcinomas. Further details of age and duration of disease are given in Table 1. Paraffin embedded material was available from all areas of the colorectum in each patient, including carcinomas where present. A total of 297 tissue blocks were suitable for assessment.

Standard haematoxylin and eosin stained ($5\ \mu\text{m}$) sections were prepared and given random code numbers and designated by one observer to one of five diagnostic categories. Sections showing normal or typical hyperplastic appearances in the epithelium were allocated to quiescent or regular hyperplasia categories. Those sections showing 'atypical'

Address for correspondence: Mr J B J Fozard, Dept of Pathology, University of Leeds, Leeds LS2 9JT.

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Table 1 Groups of patients with ulcerative colitis; age and duration of disease (median and range), and prevalence of DNA aneuploidy in colorectal mucosa

	Patients (no)	Age (yr)	Duration (yr)	DNA aneuploidy (%)
Cancer in colitis	12	49 (33-71)	20 (4-31)	8/12 (67)
Longstanding	12	40 (26-64)	11 (8-20)	8/12 (67)
Short duration	14	32 (16-72)	3 (1m-6)	3/14 (21)

cytological and architectural features were allocated to one of four categories, namely reactive hyperplasia (if the changes were considered to be secondary to active inflammation), low grade dysplasia, high grade dysplasia, and carcinoma.

FLOW CYTOMETRY

Flow cytometric analysis of nuclear DNA was done by a modification of the method of Hedley.¹⁷ Thick sections (50 µm) were cut from the paraffin embedded tissue and plated on glass slides. Excess tissue was removed with a scalpel, preserving only the mucosal layer. After dewaxing in xylene and rehydration through graded alcohols to water, the tissue was scraped from the slide and digested with 0.5% pepsin (Sigma Chemical Company, UK) in 0.9% NaCl at pH 1.5 and temperature 37°C for 30 minutes. After washing and centrifugation, the pellet was resuspended for staining in a solution of (1 µg/ml) 4'-6-diamidino-2-phenylindole-dihydrochloride (DAPI) (Boehringer Mannheim, West Germany) in RPMI 1640 tissue culture medium at 20°C for 30 min. The cell suspension was filtered through four layers of muslin followed by syringing with a 23 gauge needle. An EPICS V flow cytometer (Coulter Electronics, Hialeh, Florida, USA) with a Coherent Innova-90 5W UV enhanced argon ion laser at 50 mW and wavelength 350 nm was used for DNA analysis. A 408 nm interference filter removed scattered incident ultraviolet light. Ten thousand nuclei were counted. Deoxyribonucleic acid aneuploidy was defined as the presence of more than one G₀/G₁ peak¹⁸ and internal standards were not included for reasons previously stated.^{17, 19} For DNA aneuploid samples a DNA index was calculated as the ratio of the abnormal G₀/G₁ peak modal

channel number to the diploid G₀/G₁ peak modal channel number. The mean half peak coefficient of variation, as calculated on a standard programme (Coulter Electronics, Hialeh, Florida, USA) was 8%.

Results

CANCER VS NON-CANCER

Only four of 14 (29%) carcinomas complicating ulcerative colitis showed DNA aneuploidy. Of this group, three patients had DNA aneuploid mucosa elsewhere in the colorectum. Such findings were not universal throughout the entire colorectum but appeared as discrete foci separated by DNA diploid mucosa. Four of the eight patients with DNA diploid carcinomas had DNA aneuploid mucosa distant to the carcinoma and this showed a similar pattern of discrete foci. In total eight of 12 (67%) cancer patients had a change to DNA aneuploidy at some point in the bowel (Table 1). Deoxyribonucleic acid aneuploidy also occurred in the absence of carcinoma being present in 11/26 (42%) non-cancer patients. No statistically significant difference in rates of aneuploidy exists between the cancer and non-cancer patients ($\chi^2=1.0962$, $p=0.295$).

PLOIDY AND DYSPLASIA

Deoxyribonucleic acid aneuploidy occurred in a proportion of all tissues irrespective of histological diagnosis (Table 2). Although there was a tendency for an increased rate of aneuploidy in tissues showing dysplastic features - 16/78 (21%) as opposed to 32/219 non-dysplastic tissues (15%), this difference did not reach statistical significance ($\chi^2=1.0747$, $p=0.299$). Dysplasia was found in both

Table 2 Ploidy in relation to histological diagnosis in colorectal mucosa (Numbers represent tissue samples)

	Quiescent/regular hyperplasia	Reactive hyperplasia	Low grade dysplasia	High grade dysplasia	Carcinoma
DNA diploid	47	140	31	10	21
DNA aneuploid	7	25	8	2	6
Aneuploid (%)	13	15	21	17	22

Table 3 Numbers of patients in the three groups (cancer, long and short duration) showing dysplastic change

		High grade dysplasia	Low grade dysplasia	No dysplasia
Cancer		4	4	4
Non-cancer	Long duration (>8 yr)	1	6	5
	Short duration (<8 yr)	0	4	10

long and short duration colitis as well as those complicated by carcinoma. The increased frequency of dysplastic change in patients with cancer, eight of 12 (67%) compared with those without 11/26 (42%) (Table 3) was not statistically significant ($\chi^2=1.0962$, $p=0.295$). Four of five patients; however, showing high grade dysplasia had a concurrent carcinoma.

DURATION OF DISEASE

When the presence or absence of DNA aneuploidy was related to the duration of disease (Fig. 1) a significantly higher rate of aneuploidy was shown to occur with increasing duration of disease ($p<0.001$, Mann-Whitney). The median duration of disease in

the aneuploid group was 12.5 years compared with 3.5 years in the diploid group. Deoxyribonucleic acid aneuploidy did not occur with less than a four year history of colitis. A similar relationship between the finding of dysplastic change and increasing duration of disease was noted ($p<0.005$, Mann-Whitney) (Fig. 2). Patients with no dysplastic change had a median duration of colitis of four years compared with 13 years for those with dysplasia.

ABNORMAL STEM LINES

Calculation of DNA indices for DNA aneuploid samples showed that 10/11 non-cancer colons containing DNA aneuploid stem lines appeared to have developed a single abnormal stem line. In contrast,

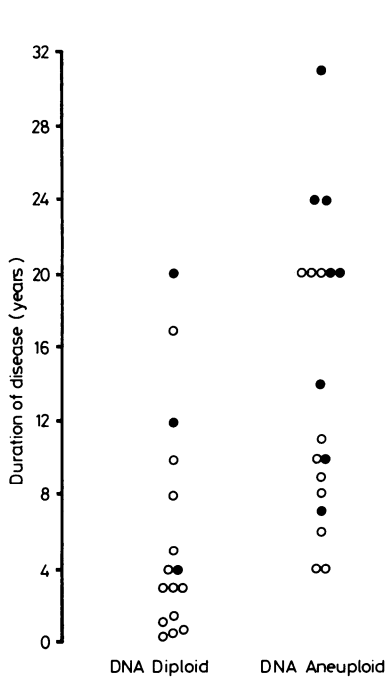


Fig. 1 Patients with DNA aneuploidy have a longer duration of disease ($p<0.001$, Mann-Whitney). ● cancer patients, ○ non-cancer patients.

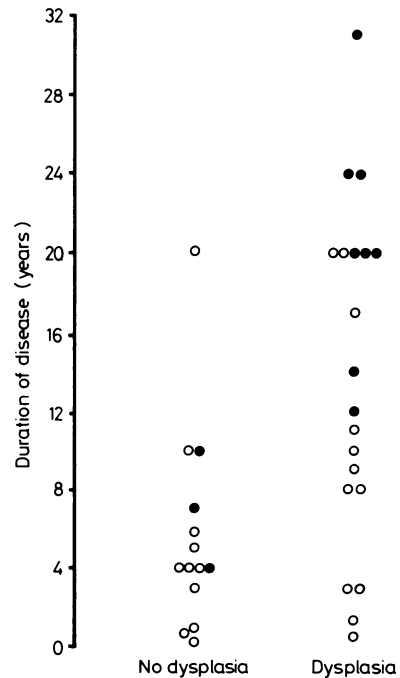


Fig. 2 Patients with dysplastic change have a longer duration of disease ($p<0.005$, Mann-Whitney). ● cancer patients, ○ non-cancer patients.

four of eight DNA aneuploid cancer colons showed more than one stem line and this was unrelated to the ploidy of the carcinoma. It is of note that the same or similar abnormal stem line (as defined by the same diploid index, $DI \pm 0.2$) appeared in widely separate areas of the colorectum in individual patients. This feature was apparent in 10/24 patients from both the cancer and long duration disease groups. Three carcinomas showed heterogeneity of DNA content.

Discussion

The assessment of 'pre-malignancy' and cancer risk in ulcerative colitis, using current criteria⁷ is highly subjective. Flow cytometry provides rapid, quantitative and reproducible measurements of DNA ploidy. A recent study has described DNA aneuploidy occurring in ulcerative colitis¹⁶ but the prospective nature and design of the investigation limit full assessment of its value in cancer surveillance. Using a technique that enables us to examine paraffin embedded tissue, we have been able to retrospectively select groups of patients with ulcerative colitis, who are fully documented histologically and who also have a known clinical outcome.

It is of interest that only 29% of carcinomas complicating ulcerative colitis showed DNA aneuploidy compared with 60% of colorectal carcinomas arising in the general population measured under the same conditions in parallel studies (Quirke, unpublished observations). Although the numbers are small, our finding of similar rates of DNA aneuploidy (33%) in 36 colorectal carcinomas associated with synchronous adenomas¹⁵ may suggest fundamental biological differences in these tumours over those generally seen.

In agreement with previous work¹⁶ we have also shown that DNA aneuploidy in ulcerative colitis is not restricted to patients with invasive carcinoma. Contrary to Hammarberg's findings on pooled biopsies, however, we have shown that the change to DNA aneuploidy is highly focal in nature and not a field change, although it may occur at several points in the mucosa of an individual colorectum. No significant distinction could be made between cancer and non-cancer patients with regard to rates of DNA aneuploidy, when matched for duration of ulcerative colitis.

In this study similar rates of dysplastic change were seen in both cancer and non-cancer patients. Of five patients showing high grade dysplastic change, however, four had a carcinoma. We were unable to show a significant association between DNA aneuploidy and dysplastic change within individual tissue samples and this mirrors previous

experience with colorectal adenomas.^{14 15} In contrast, Hammarberg¹⁶ has claimed that such an association exists. These conclusions were again based on the use of pooled biopsies and are invalidated by our observation that both dysplastic change and the finding of DNA aneuploid stem lines may be highly focal.

We have provided further evidence that the development of DNA aneuploidy is significantly related to the duration of ulcerative colitis and that there is a variable latent period of several years duration before the development of abnormal DNA. Similarly, we have shown that the development of dysplastic change is linked to the duration of disease. These findings fit with the epidemiological evidence of a negligible risk of developing carcinoma complicating ulcerative colitis in the first decade of disease, followed by increasing risk thereafter. Whether or not the development of DNA aneuploidy means that the tissue is inevitably committed to malignant change is open to question. It may be that the appearance of an abnormal stem line is but one of the steps in multistage carcinogenesis. With these concepts in mind, it is also interesting to note that similar, possibly identical, abnormal stem lines appeared at widely separate points in the colorectum and that the development of multiple abnormal stem lines was more common in cancer bearing patients.

The significance of DNA aneuploidy and its relationship to the development of cancer remains to be fully established. Certainly the finding of DNA aneuploidy in a colorectal biopsy would have no predictive value in deciding whether or not a patient has concurrent carcinoma elsewhere in the bowel. It may, however, be an indicator of future malignant change and this will be evaluated in our prospective series. Even if DNA aneuploidy is found to be predictive, however, the development of diploid carcinomas could not be anticipated by our present methods of flow cytometric DNA analysis. Although flow cytometric analysis may assist in the identification of abnormal mucosa, it would appear that the histological recognition of high grade dysplasia, even with its difficulties and limitations remains the most useful investigation in the routine surveillance of patients with ulcerative colitis.

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