

## Constipation, Anthranoid Laxatives, Melanosis Coli, and Colon Cancer: A Risk Assessment Using Aberrant Crypt Foci

Riccardo Nascimbeni,<sup>1</sup> Francesco Donato,  
Mauro Ghirardi, Pierpaolo Mariani, Vincenzo Villanacci,  
and Bruno Salerni

Cattedra di Chirurgia Generale [R. N., M. G., P. M., B. S.], Istituto di Igiene [F. D.], and Cattedra di Anatomia Patologica [V. V.], University of Brescia, 25124 Brescia, Italy

### Abstract

The associations between colorectal cancer (CRC) and constipation, anthranoid laxative use, and melanosis coli are controversial. Aberrant crypt foci (ACF) are microscopic lesions of the colonic mucosa suspected of being preneoplastic, and their investigation has been advocated to evaluate the cause-effect relationship between putative risk factors and CRC. To this aim, we investigated the relationship between sigmoid cancer (SC) and constipation, anthranoid laxative use, and melanosis coli using ACF analysis as an additional tool of investigation. Fifty-five surgical patients with SC, 41 surgical patients with diverticular disease (DD), and 96 age- and sex-matched subjects without intestinal disease (controls) were interviewed on their history of constipation and anthranoid laxative use. Melanosis coli and ACF characteristics were investigated on sigmoid mucosa in patients with SC or DD. Constipation and anthranoid laxative use were similar between patients with SC (30.9% and 32.7%, respectively) and those with DD (39% and 26.8%) but higher than among controls (18.8% and 8.3%). Melanosis coli was found in 38.2% of patients with SC and in 39% of those with DD. Mean ACF frequency was higher in patients with SC (0.24/cm<sup>2</sup>) than in those with DD (0.10/cm<sup>2</sup>;  $P < 0.0001$ ), and it did not vary according to constipation, laxative use, or melanosis coli in either group. This study confirms the association of ACF frequency with colon cancer and does not support the hypothesis of a cause-effect relationship of CRC with constipation, anthranoid laxative, use or melanosis coli.

### Introduction

An association between constipation and colon cancer has been found in several epidemiological studies, with a pooled OR of 1.5 from 14 case-control studies in a recent meta-analysis (1). The association could be either true, because of prolonged

contact between carcinogens in the lumen and intestinal mucosa, or spurious, because of factors associated with constipation, particularly a diet poor in fiber and vegetables, which has been found to increase the risk of CRC<sup>2</sup> (2, 3). Although some recent case-control studies confirmed the association, after checking for dietary factors and other risk factors for CRC (4–7), a large prospective study carried out among women found no association (8).

Laxative use, which is related to constipation, has been advocated as a possible cause of colon cancer by itself. Anthranoids, which are among the most commonly used laxatives, have been found to have mutagenic and carcinogenic effects by *in vitro* and animal studies (9–11). Melanosis coli is a brownish pigmentation of colonic mucosa which, since 1933 (12), has been associated with the chronic ingestion of anthranoid laxatives. However, melanosis coli has also been found in patients who do not use laxatives or suffer from constipation, possibly because of the apoptosis of epithelial cells and their subsequent phagocytosis by macrophages of lamina propria with accumulation of lipofuscin pigment (13, 14). Few studies have investigated a possible association of anthranoid laxative use and melanosis coli with CRC in humans, and the results are contradictory (15–19).

ACF are putative preneoplastic lesions of the colonic mucosa first described in the colon of mice treated with carcinogens (20, 21) and subsequently found in humans (22, 23). Their potential role of modulable risk markers for adenoma/carcinoma development has been demonstrated in rodents (24, 25), and in both human *ex vivo* (26) and *in vivo* models (27, 28). Accordingly, ACF are being used as intermediate end points for evaluation of potential carcinogens and chemopreventive agents in rodents (29). Their widespread use has also been advocated in the study of human colon carcinogenesis as they can “provide a quantitative approach to assess the disease process and molecular events as affected by cancer preventive or promoting agents” (30).

The present study aims to evaluate the risk of colon cancer by constipation, anthranoid laxative use, and melanosis coli using ACF frequency as an additional tool of investigation. To this end, we investigated history of constipation and of anthranoid laxative use, and the presence of melanosis coli and ACF in the sigmoid colon of patients with colon cancer or with DD undergoing surgery. We also investigated constipation and laxative use among patients without colon cancer or DD as controls.

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<sup>1</sup> To whom requests for reprints should be addressed, at Cattedra di Chirurgia Generale, University of Brescia, Via Valsabbina 19, 25124 Brescia, Italy. Phone: 39-030-3995614; Fax: 39-030-3700472.

<sup>2</sup> The abbreviations used are: CRC, colorectal cancer; ACF, Aberrant crypt foci; SC, sigmoid cancer; DD, diverticular disease; OR, odds ratio; CI, confidence interval.

## Materials and Methods

**Patients.** Subjects undergoing resection of the sigmoid colon for cancer (SC) or DD were prospectively enrolled in the study in 1997–1999. For accrual purposes, some of these patients had also been included in a previous study on ACF characteristics (31). Patients admitted to the same department because of minor trauma were also consecutively enrolled as controls. Each control was matched with 1 patient with either SC or DD by age, sex, and date of admission. Before surgery or at hospital admission, all of the patients underwent physical examination and were interviewed on their family cancer history, bowel habits, and anthranoid laxative use. Subjects with constipation because of intestinal obstruction, electrolyte unbalances, medications, or hypothyroidism were excluded. Patients with diagnosis of familial adenomatous polyposis or hereditary nonpolyposis CRC, and patients with both colon cancer and DD were also excluded. The project was approved by the Hospital Ethics Committee, and all of the subjects gave their informed consent to participate.

**Preoperative Questionnaire.** According to the Rome criteria (32), patients were classified as having constipation if they reported two or more of the following symptoms at least 25% of the time when not taking laxatives: straining at defecation, feeling of incomplete evacuation, or evacuation of hard or pellet stools. Furthermore, subjects who claimed two or fewer bowel movements per week were also classified as affected by constipation. Because colon cancer may be a cause of constipation by itself, only subjects who claimed to have had the above-mentioned symptoms or bowel movements for at least 3 years before the interview were classified as having constipation. Laxative use was investigated according to the type of drug, dosage, frequency of use, and duration of consumption. Anthranoid-containing laxatives were defined as herbal drugs containing senna, cascara, frangula, aloe, or rheum, or as laxative drugs containing danthron or purified sennosides. Patients consuming one or more of these medications at least twice a week for at least 3 years before the interview were defined as chronically using anthranoid laxatives. All of the subjects reporting chronic use of other types of laxative, such as phenolphthalein, mineral salts, or bisacodyl, were excluded from the study, to allow a comparison between people assuming anthranoid laxatives and those assuming no laxatives at all. Face-to-face interviews were all performed in the hospital setting by a physician from the Department of Surgery, who was not blind in regard to the patient disease but ignored the results of melanosis coli and ACF analyses. A structured questionnaire was used.

**Melanosis Coli Detection and Analysis of ACF.** Melanosis coli was defined by the microscopic identification of the typical brownish pigment in the lamina propria of the colonic specimen. Multiple samples of normal-appearing mucosa were selected from each colonic specimen for light microscopy. Histological studies were performed on 4- $\mu$ m paraffin sections of formalin-fixed tissue, stained with H&E and using the long Ziehl-Neelsen method.

The method used to detect and analyze ACF has been described previously (31). Briefly, after sampling for melanosis study, strips of normal-appearing mucosa were dissected from the underlying submucosa. The strips were fixed in 10% buffered formalin for 60 min and stained with 0.2% methylene blue for 20 min. Samples were subsequently placed luminal side up and observed at 40-fold magnification. ACF were identified as described previously by Bird (20). Mucosal strip area, total number, frequency (no. of foci/cm<sup>2</sup>), and multiplicity (no. of

crypts/focus) of ACF were recorded in each colonic specimen. Because foci <10 crypts were difficult to dissect and foci >110 crypts are usually grossly detectable, only ACF comprising 10–110 crypts were selected for histological purposes. Each focus was microdissected as described previously using a surgical microscope (magnification  $\times 25$ ; Ref. 31). Four- $\mu$ m paraffin sections were obtained serially perpendicular to the luminal surface and stained with H&E.

ACF were subdivided into the following categories according to criteria described previously (31): (a) surface hyperplastic type; (b) surface and glandular hyperplastic type; (c) mixed hyperplastic and adenomatous type; and (d) adenomatous type or microadenoma.

**Statistical Methods.** Frequencies of constipation and laxative use among patients with SC, patients with DD, and controls without intestinal diseases were compared using common methods for the analysis of proportion. A comparison between patients with SC and those with DD was also performed regarding the presence of melanosis coli. ACF frequency and multiplicity were analyzed as continuous variables, and the original data were log-transformed for better normal approximation and for variance stabilization (33). However, the original values are shown in the tables to allow better comprehension. One- and two-way ANOVA was performed for testing differences in mean values of ACF frequencies and multiplicity when comparing patients with SC and those with DD. The ORs for having SC or DD with respect to no SC or DD were computed using polytomous logistic regression (34), including age and sex as possible confounders. Because constipation and laxative use were closely related, only one of them was included in the multivariate model at each step. Lastly, we computed the OR for having SC with respect to DD according ACF frequency categorized at two levels: <0.10 and  $\geq 0.10$  ACF/cm<sup>2</sup>. All of the statistical tests were two-tailed and performed at a *P* of 0.05 using the Biomedical Data Processing/Dynamic computer programs (University of California, Los Angeles, CA).

## Results

Fifty-five patients with SC, 41 with DD, and 96 controls without intestinal disease were included in the study. The mean age of patients with SC (65.6 years, range 39–95) did not differ significantly from the age of patients with DD (63.2 years, range 28–90) and controls (64.3 years, range 33–92). The male:female ratio was 1.1 in patients with SC, 0.6 in those with DD, and 0.8 among controls.

Prevalences of history of constipation and of anthranoid laxative use were similar in patients with SC and those with DD, but they were significantly higher in both these groups than among controls (Table 1). Melanosis coli was almost equally common in patients with SC (38.2%) and in those with DD (39%). Using polytomous logistic regression, both SC and DD were associated with constipation (OR for SC, 1.9; 95% CI, 0.9–4.1; OR for DD, 2.8; 95% CI, 1.2–6.3) and with anthranoid laxative use (OR for SC, 5.3; 95% CI, 2.1–13; OR for DD, 4.0; 95% CI, 1.5–11) when compared with controls. No differences were found between patients with SC and patients with DD. When both variables were included in the model, laxative use showed the strongest association with both SC and DD, whereas constipation was not associated with either disease.

Anthranoid laxative use was reported by most patients who suffered from constipation (76.5% of subjects with SC, 68.8% of those with DD, and 44% of controls) and also by

**Table 1** History of constipation and of anthranoid laxative use, and presence of histologically detected melanosis coli among patients with SC, DD, and subjects without intestinal diseases (controls)

	Patients with SC, No. (%)	Patients with DD, No. (%)	Controls No. (%)	P
Total subjects	55 (100)	41 (100)	96 (100)	NS <sup>a</sup>
History of constipation	17 (30.9)	16 (39)	18 (18.8)	0.03
History of anthranoid laxative use	18 (32.7)	11 (26.8)	8 (8.3)	0.005
Melanosis coli	21 (38.2)	16 (39)		

<sup>a</sup> NS,  $P > 0.05$ .

**Table 2** ACF characteristics among patients with SC or DD

ACF characteristics	Patients with SC (n = 55)	Patients with DD (n = 41)	P
Mean area of mucosa (cm <sup>2</sup> )	46.33	43.76	NS <sup>a</sup>
Total number of ACF	499	152	
Number of ACF per subject: mean (SD) <sup>b</sup>	9.07 (5.87)	3.71 (4.27)	<0.0001
Frequency: mean (SD) (ACF/cm <sup>2</sup> )	0.24 (0.18)	0.10 (0.11)	<0.0001
Multiplicity: mean (SD) (crypts/focus)	20.48 (13.41)	22.69 (18.46)	NS

<sup>a</sup> ACF were found in all but 3 patients with SC (94.5%) and in 32 patients with DD (78%).

<sup>b</sup> NS,  $P > 0.05$ .

10.5% of SC patients without history of constipation according to Rome criteria. None of the subjects with DD or controls without constipation reported chronic use of anthranoids. Melanosis coli was about twice as common among subjects with than those without anthranoid laxative use in both patients with SC (55.6% versus 28.9%, respectively) and those with DD (63.6% versus 30%). Melanosis coli was significantly associated with anthranoid laxative use when considering all of the patients together, with an OR of 3.4 (95% CI, 1.3–8.5).

A total of 651 ACF were found; their number per patient, mean frequency, and multiplicity in patients with SC and those with DD are summarized in Table 2. A similar amount of colon mucosa was examined in the two groups. Mean ACF number per subject and frequency were higher in patients with SC than in those with DD ( $P < 0.001$ ), whereas no difference in multiplicity was found.

When considering ACF frequency as a dichotomous variable, a higher proportion of subjects with  $>0.10$  ACF/cm<sup>2</sup> was found among patients with SC than those with DD (70.9% and 15%, respectively;  $P < 0.0001$ ). As a consequence, the ORs for SC were 15.3 (5.0–47.1) for having  $>0.10$  ACF/cm<sup>2</sup> using logistic regression.

ACF analysis among patients with SC or DD according to history of constipation is shown in Table 3. No differences were found according to history of constipation in patients with either SC or DD. Similarly, no association was found between ACF characteristics and anthranoid laxative use (Table 4) or melanosis coli (Table 5) in patients with SC or DD, apart from a higher multiplicity in patients with SC who did not use anthranoid laxatives with respect to those who did.

Three hundred and fifty-seven ACF  $>10$  crypts (54.84%) were found and collected for histology. In patients with SC, histological subtypes of ACF were subdivided as follows: surface hyperplastic ACF 41.8%, surface/glandular hyperplastic ACF 54.8%, and mixed hyperplastic/dysplastic ACF 3.4%. In patients with DD the histological subtypes were subdivided as follows: surface hyperplastic ACF 54.3% and surface/glandular hyperplastic ACF 45.7%. No mixed hyperplastic/dysplastic

ACF was found in the DD group. No adenomatous ACF was detected in either SC or DD group.

## Discussion

The associations of constipation and anthranoid laxative use with colon cancer have been supported mainly by data collected through interviews. The results of these studies are not consistent and do not allow definite conclusions to be drawn on the matter (35). Discrepancies between epidemiological studies may be explained by recall inaccuracy and confounding factors, such as dietary and other lifestyle habits. Constipation in fact disappeared as a risk factor when adjusting for dietary components in some studies (36). The different criteria adopted for the definition of bowel movements and laxative use in the different studies have also been questioned (1).

This study focused on ACF analysis as a support tool for investigating the cause-effect relationship between constipation and laxative use and colon cancer. To ensure comparability of the information collected, we used a stringent standardized system for defining constipation such as the Rome criteria. Therefore, some patients who claimed to use laxatives were otherwise negative in regard to constipation history according to the definition used. We found a higher proportion of patients with constipation and especially anthranoid laxative use among subjects with SC than among controls without intestinal diseases, in agreement with a recent case-control study (7). There was no difference between patients with SC and those with DD, which is not surprising because patients with these diseases probably share common risk factors, such as insufficient dietary fiber and vegetable intake. Melanosis coli is a common side effect of prolonged use of anthranoid laxatives and is suspected of being associated with colon cancer. We confirmed the well-known association of this condition with anthranoid laxative use in both patients with SC and with DD, thus indirectly validating the information on history of laxative use collected through interview. However, melanosis coli was also found in 28.9% of SD patients and 30% of DD patients who did not use laxatives. This is in agreement with a recent observation that melanosis coli is a nonspecific marker of colonic epithelial apoptosis with many possible causes (13). The prevalence of melanosis coli did not differ between patients with SC and those with DD, which does not support the hypothesis of an association between this condition and colon cancer.

ACF are putative preneoplastic lesions of the colon and rectum, and numerous studies support their role as biomarkers of CRC risk (29, 30). However, ACF have been used as an epidemiological tool for evaluating cancer risk in only a few human studies. In an Italian study, ACF frequency in CRC patients was found to be higher among subjects living in a high rather than those living in a low incidence area for the disease (37). Takayama *et al.* (28) found that the administration of sulindac, a nonsteroidal anti-inflammatory drug, significantly reduced the number of ACF in both normal subjects and pa-

Table 3 ACF characteristics in patients with SC or DD according to history of constipation

ACF characteristics	Patients with SC			Patients with DD		
	Constipation (n = 17)	No constipation (n = 38)	P	Constipation (n = 16)	No constipation (n = 25)	P
Total number of ACF	133	366		77	75	
Number of ACF per subject: mean (SD)	7.82 (4.04)	9.62 (6.49)	NS <sup>a</sup>	4.81 (4.56)	3.00 (4.00)	NS
Frequency: mean (SD) (ACF/cm <sup>2</sup> )	0.21 (0.15)	0.256 (0.19)	NS	0.14 (0.13)	0.08 (0.10)	NS
Multiplicity: mean (SD) (crypts/focus)	17.97 (14.41)	21.60 (12.99)	NS	17.84 (15.67)	26.01 (19.87)	NS

<sup>a</sup> NS,  $P > 0.05$ .

Table 4 ACF characteristics in patients with SC or DD according to history of anthranoid laxative use

ACF characteristics	Patients with SC			Patients with DD		
	Laxative use (n = 18)	No laxative use (n = 37)	P	Laxative use (n = 11)	No laxative use (n = 30)	P
Total number of ACF	143	356		52	100	
Number of ACF per subject: mean (SD)	7.94 (4.41)	9.61 (6.44)	NS <sup>a</sup>	4.73 (5.48)	3.33 (3.77)	NS
Frequency: mean (SD) (ACF/cm <sup>2</sup> )	0.21 (0.16)	0.26 (0.18)	NS	0.12 (0.14)	0.10 (0.10)	NS
Multiplicity: mean (SD) (crypts/focus)	13.36 (8.14)	23.94 (14.17)	0.002	18.61 (18.52)	24.05 (18.64)	NS

<sup>a</sup> NS,  $P > 0.05$ .

Table 5 ACF characteristics in patients with SC or DD according to the presence of melanosis coli by histology

ACF characteristics	Patients with SC			Patients with DD		
	Melanosis coli (n = 21)	No melanosis coli (n = 34)	P	Melanosis coli (n = 16)	No melanosis coli (n = 25)	P
Total number of ACF	200	299		66	86	
Number of ACF per subject: mean (SD)	9.00 (5.33)	9.12 (6.25)	NS <sup>a</sup>	4.13 (4.70)	3.44 (4.04)	NS
Frequency: mean (SD) (ACF/cm <sup>2</sup> )	0.23 (0.17)	0.25 (0.19)	NS	0.11 (0.11)	0.10 (0.12)	NS
Multiplicity: mean (SD) (crypts/focus)	25.88 (16.83)	17.38 (9.96)	NS	17.64 (17.50)	26.15 (18.76)	NS

<sup>a</sup> NS,  $P > 0.05$ .

tients with adenoma or cancer. However, to our knowledge, ACF have never been used to assess the risk of CRC because of environmental factors or clinical conditions in humans, apart from one case report of a single patient with melanosis coli, ACF, and colon cancer who had used a variety of laxatives (38).

We investigated ACF frequency and multiplicity through surgical examination, because *in vivo* ACF analysis by magnifying endoscopy, although performed in some studies (28), still lacks definite validation. A weakness in our choice is the lack of biological samples from healthy people, because the ACF system in our study can only be analyzed in patients undergoing colon surgery. However, we enrolled for the comparison patients with DD, a disease that is not associated with colon cancer. We selected only sigmoid colon samples, firstly because sigmoid colon is the most frequent site for both colon cancer and DD, and secondly because of the high variability of ACF frequency among different segments of the large bowel (22, 23, 28, 31, 39, 40). This study confirms previous findings of a higher frequency of ACF in patients with, than those without, colon cancer (23, 28, 31, 39). Patients with  $>0.10$  ACF/cm<sup>2</sup> had a substantially higher risk of having SC with respect to DD, with an OR of  $\sim 15$ . On the contrary, ACF multiplicity and histology did not differ between patients with SC and those with DD. Dysplasia in ACF, which is considered a precursor of colon cancer (28, 40), was found in only a few SC cases (3.4% of total ACF) and in no patients with DD.

No association of ACF characteristics was found with history of constipation or anthranoid laxative use or with pres-

ence of melanosis coli in patients with either SC or DD, suggesting that ACF are indicators of colonic exposure to factors that promote carcinogenesis and are scarcely influenced by constipation itself or laxative use.

In conclusion, this study confirms an epidemiological role of ACF frequency as a biomarker indicative of a high-risk condition for colon cancer development, and suggests that a cause-effect relationship among constipation, anthranoid laxative use, or melanosis coli and colon cancer is unlikely when using ACF as a complementary tool for epidemiological investigation.

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