Risk Factors for Colorectal Cancer in Relation to Number and Size of Aberrant Crypt Foci in Humans

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Abstract

Several characteristics of aberrant crypt foci (ACF) suggest that they are precursors of colorectal cancer, but the factors that promote or inhibit their growth are largely unknown. We conducted a pilot study to explore whether factors associated with risk of colorectal cancer are also associated with number or size of rectal ACF. Thirty-two U.S. veterans, ages 50 to 80 years, were recruited to undergo magnifying chromoendoscopy for imaging of rectal ACF and colonoscopy for identification of polyps or cancer. Participants completed a questionnaire on cigarette smoking, use of nonsteroidal anti-inflammatory drugs (NSAIDs), and family history of colorectal cancer. Fisher's exact test was used to assess the statistical significance of associations between colorectal cancer risk factors and characteristics of ACF. Cochran-Mantel-Haenszel statistics and polytomous regression were used to test the significance of associations adjusted for age. Participants with a history of adenoma had more ACF than those without (age-adjusted $P = 0.02$), but the numbers in the two groups overlapped markedly. Older participants had more ($P = 0.06$) and larger ($P = 0.009$) ACF than younger participants. No associations were identified between either ACF number or size and cigarette smoking, use of NSAIDs, or family history of colorectal cancer. These findings suggest that persons with adenomas have somewhat more rectal ACF than persons without, and that older age is a risk factor for ACF growth. Future research should be directed toward developing techniques to identify ACF that are likely to progress to cancer and the modifiable factors that promote or inhibit such progression. (Cancer Epidemiol Biomarkers Prev 2005;14(3):605–8)

Introduction

Aberrant crypt foci (ACF), clusters of colorectal crypts with abnormal morphology, were first discovered in mice treated with azoxymethane (1). Further investigations established ACF as a biomarker of cancer risk in azoxymethane-treated mice and rats (2-4). In these animals, ACF begin as a single enlarged crypt with a thicker epithelial lining than normal crypts and subsequently expand into larger clusters (1). Nonsteroidal anti-inflammatory drugs (NSAIDs) and certain micronutrients inhibit the initial development or expansion of ACF in azoxymethane-treated rodents (5).

In humans, the association between ACF and malignancy is less clear and factors that promote or inhibit ACF growth are largely unknown. For example, in some studies, persons with colorectal cancer had more (6-8) or larger (9) ACF than those without. However, there are null and inverse associations also (9, 10).

Of the risk factors associated with colorectal cancer, only history of adenoma, age, and NSAIDs have been examined in relation to ACF in humans. In one study, ACFs were related to adenomas (11); in another study, only a small, nonsignificant association was observed (8). Older age has been associated with more numerous ACF (7, 11-13), but this was statistically significant in only one study (11). A clinical trial suggested that NSAIDs can eliminate ACF (11).

As a preliminary step, we examined the relationship between number and size of ACF and selected risk factors for colorectal cancer among 32 adults using a magnifying colonoscope to view and sample ACF.

Materials and Methods

Study Sample. U.S. veterans, ages 50 to 80 years, were eligible to participate. Exclusion criteria were contraindication to colonoscopy; use of anticoagulant medication; history of familial adenomatous polyposis, inflammatory bowel disease, or radiation proctopathy; and allergy to methylene blue or *N*-acetylcysteine.

All participants were recruited from Veterans Affairs Puget Sound Health Care System, Seattle Division. Twenty-four participants were recruited from those scheduled for elective colonoscopy. Clinical indications were history of polyps ($n = 11$), visible gastrointestinal bleeding ($n = 4$), occult blood in stool ($n = 5$), family history of colorectal cancer ($n = 1$), abnormal barium enema ($n = 1$), previous colorectal cancer ($n = 1$), and diarrhea ($n = 1$). To increase enrollment of participants without colorectal neoplasia, eight additional participants were recruited in Veterans Affairs Puget Sound Health Care System as volunteers.

Fred Hutchinson Cancer Research Center and University of Washington Institutional Review Offices approved the study, as did the Research Committee of Veterans Affairs Puget Sound Health Care System. Each participant gave written informed consent before any research activity.

Demographic, Lifestyle, and Clinical Data. Immediately before endoscopy, participants completed a self-administered questionnaire on use of tobacco and NSAIDs and family history of colorectal cancer. The endoscopist recorded information on participant age, sex, history of neoplasia, and clinical indication.

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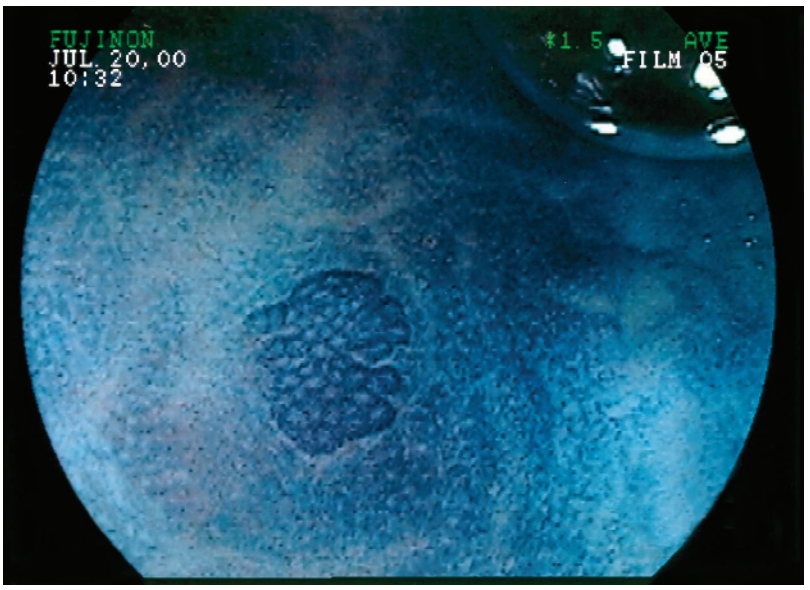

Figure 1. Typical endoscopic appearance of an ACF. This photograph was obtained with a Fujinon EC-410 CM magnifying colonoscope after the rectal mucosa had been stained with 0.2% methylene blue.

Endoscopy. Participants prepared for endoscopy with p.o. polyethylene glycol and bisacodyl. Before imaging rectal ACF, colonoscopy was done to identify colorectal pathology. Data on histology of polyps removed at this colonoscopy or previously were obtained from pathology records.

A Fujinon EC-410CM or CL colonoscope was used to perform magnifying chromoendoscopy (Fujinon Corporation, Saitama City, Japan). This colonoscope has a fixed optical magnification of 35× when endoscopic images are displayed on a 14-in. monitor; the degree of magnification can be increased digitally to a total of 52×. Because assessment of ACF number and size in the colon is technically difficult, we imaged rectal ACF only. To prepare for staining, 10% *N*-acetylcysteine, a mucolytic, was applied to the epithelium with a spray catheter (Olympus washing pipe, CF-PW5V-1; Olympus Corporation, Tokyo, Japan). Subsequently, 0.2% methylene blue was applied.

After staining, two biopsies of normal-appearing epithelium were obtained and ACF distal to the second valve of Houston (~14 cm from the anal verge) were sought. Aberrant crypts were distinguished from normal crypts by deeper color, raised appearance, and larger diameter (11, 14). Before biopsy, ACF were photographed to allow subsequent measurement of size. Standard biopsy forceps were used to obtain up to eight ACF from each participant. Biopsies were fixed in formalin or snap frozen.

Histologic Examination of Biopsies. Formalin-fixed biopsies from the first 15 participants were prepared for histologic review. Biopsies were embedded in paraffin, step-sectioned perpendicular to the lumen in 5-μm sections, mounted onto slides, and stained with H&E.

The pathologist, blinded to endoscopic diagnosis, classified the biopsies as hyperplastic, dysplastic, or without hyperplasia or dysplasia. Hyperplastic tissue had serrated crypt profiles and/or cellular tufting and lacked features of dysplasia (15). Dysplastic tissue displayed one or more abnormal cytologic features: nuclear enlargement, stratification, pleomorphism, hyperchromasia, or increased nuclear-to-cytoplasmic ratios extending to the mucosal surface, with or without architectural distortion (15, 16).

Statistical Analysis. We examined associations between colorectal cancer risk factors and number and median size of rectal ACF. In the first 15 participants enrolled, every ACF observed, up to a maximum of eight, was counted and sampled for histologic review. Because the focus of our earliest work was to test chromoendoscopy technique and to collect samples of ACF, we did not count beyond these eight. Thus, in the first 15 participants, we had exact counts for participants with seven or fewer; the remainder had eight or more. In the later stages of the study, all rectal ACF were counted. We combined these data by classifying ACF number as 0 to 4, 5 to 7, and ≥8. The size of each ACF was determined by counting the number of crypts in a photograph. For each participant, median size was determined from a maximum of eight photos; the mean number of photos per participant was 6.8.

Risk factors examined were age, history of adenoma, NSAID use, smoking status and duration in pack-years, and first-degree family history of colorectal cancer. A history of adenoma meant an adenoma found at this or a previous colonoscopy. One participant with a history of colorectal cancer was included in this category.

For analyses with categorical variables, we adjusted for age using Cochran-Mantel-Haenszel statistics; age strata were ≤65 and >65 years. For pack-years of cigarette smoking, we used polytomous regression with age and pack-years modeled as continuous variables. We used Fisher's exact test to determine the significance of associations with age and family history, and χ^2 test with Cochrane's adjustment (17) for associations between ACF size and prevalence of hyperplasia or dysplasia in ACF. Tests of statistical significance were two-sided.

Results

The mean (±SD) age of the participants was 65.9 (±9.0) years; all but one were men. Twenty-eight of the 32 (87.5%) were Caucasian; four were African-American. Among participants with a history of cigarette smoking (n = 24), 37.5% were current smokers. Current smokers had a mean (±SD) of 33.8 (±40.2) pack-years and former smokers had 44.6 (±35.0). Former smokers had abstained a mean of 23.1 (±17.8) years.

No participant experienced complications from colonoscopy or chromoendoscopy; each had at least two rectal ACF, with a median size of 51.0 crypts (range = 3-455 crypts). Among those with complete ACF counts, the median number was 7.0 (range = 3-29). The typical endoscopic appearance of an ACF is shown in Fig. 1.

Of the 104 biopsies that met the endoscopic criteria for ACF, one had low-grade dysplasia and 44.2% had

hyperplasia. Of the 40 biopsies obtained from normal-appearing epithelium, 39 (97.5%) showed neither hyperplasia nor dysplasia and 1 (2.5%) had hyperplasia. Of ACF with more than the mean number (72 crypts) per focus, 66.7% had hyperplasia or dysplasia. In contrast, only 25.5% of ACF with fewer crypts than the mean had hyperplasia or dysplasia; this difference was statistically significant ($P < 0.001$).

Participants with a history of adenoma had more ACF than those without ($P = 0.02$; Table 1). Participants ages 70 years or older tended to have more ACF than younger participants ($P = 0.06$). Neither use of NSAIDs during the previous year nor family history of colorectal cancer was associated with ACF number.

There was no association between current cigarette smoking and number of ACF; adding recent former smokers (≤2 years of abstinence) to the ''current smoker'' category did not materially alter these results. Furthermore, there was no association between pack-years of cigarette smoking and number of ACF; the age-adjusted odds ratio for a 10 pack-year difference was 1.0 (95% confidence interval, 0.9-1.3).

Participants ages ≥70 years had larger ACF than younger participants ($P = 0.009$), but history of adenoma, NSAID use during the previous year, and family history were not associated with size (Table 2). There was no association between current cigarette smoking and ACF size; adding recent former smokers (see above) did not materially alter these results.

Table 1. Selected risk factors for colorectal cancer in relation to number of ACF

	n	No. of ACF (%)*			*P*
		0-4	5-7	≥8	
Age (y)					
50-59	9	33.3	22.2	44.4	0.06†
60-69	9	33.3	44.4	22.2	
70-80	14	0.0	28.6	71.4	
First-degree relative with a history of colorectal cancer					
No	29	17.2	31.0	51.7	0.76†
Yes	3	33.3	33.3	33.3	
History of adenoma‡					
≤65 y old					
No	5	80.0	20.0	0.0	0.02§
Yes	10	20.0	30.0	50.0	
>65 y old					
No	4	0.0	50.0	50.0	
Yes	13	0.0	30.8	69.2	
Cigarette smoking					
≤65 y old					
Not current	9	22.2	33.3	44.4	0.12§
Current	6	66.7	16.7	16.7	
>65 y old					
Not current	14	0.0	35.7	64.3	
Current	3	0.0	33.3	66.7	
NSAID‖ use at least weekly during the past year					
≤65 y old					
No	6	50.0	16.7	33.3	0.24§
Yes	9	33.3	33.3	33.3	
>65 y old					
No	3	0.0	100.0	0.0	
Yes	14	0.0	21.4	78.6	
Frequency of NSAID‖ use during the past year					
≤65 y old					
0-6 times/wk	8	37.5	25.0	37.5	0.96§
≥7 times/wk	7	42.9	28.6	28.6	
>65 years old					
0-6 times/wk	6	0.0	50.0	50.0	
≥7 times/wk	11	0.0	27.3	72.7	

*Row percentages are presented in the columns below this header.
†Unadjusted *P* from Fisher's exact test.
‡History of adenoma: one or more adenomas identified during the study colonoscopy or at a previous colonoscopy.
§Age-adjusted *P* from Cochran-Mantel-Haenszel test, with age categories of ≤65 and >65 years.
‖Includes aspirin.

Table 2. Selected risk factors for colorectal cancer in relation to median size of ACF

	n	Median number of crypts per ACF within person (%)*			*P*
		≤40	41-80	>80	
Age (y)					
50-59	9	66.7	22.2	11.1	0.009†
60-69	9	66.7	22.2	11.1	
70-80	14	7.1	35.7	57.1	
First-degree relative with a history of colorectal cancer					
No	29	41.4	27.6	31.0	1.0†
Yes	3	33.3	33.3	33.3	
History of adenoma‡					
≤65 y old					
No	5	60.0	20.0	20.0	0.44§
Yes	10	70.0	20.0	10.0	
>65 y old					
No	4	50.0	25.0	25.0	
Yes	13	7.7	38.5	53.8	
Cigarette smoking					
≤65 y old					
Not current	9	66.7	22.2	11.1	0.47§
Current	6	66.7	16.7	16.7	
>65					
Not current	14	21.4	35.7	42.9	
Current	3	0.0	33.3	66.7	
NSAID‖ use at least weekly during the past year					
≤65 y old					
No	6	66.7	0.0	33.3	0.38§
Yes	9	66.7	33.3	0.0	
>65 y old					
No	3	66.7	33.3	0.0	
Yes	14	7.1	35.7	57.1	
Frequency of NSAID‖ use during the past year					
≤65 y old					
0-6 times/wk	8	75.0	0.0	25.0	0.50§
≥7 times/wk	7	57.1	42.9	0.0	
>65 y old					
0-6 times/wk	6	33.3	33.3	33.3	
≥7 times/wk	11	9.1	36.4	54.6	

*Row percentages are presented in the columns below this header.
†Unadjusted *P* from Fisher's exact test.
‡History of adenoma: one or more adenomas identified during the study colonoscopy or at a previous colonoscopy.
§Age-adjusted *P* from Cochran-Mantel-Haenszel test, with age categories of ≤65 and >65 years.
‖Includes aspirin.

Discussion

In this study, persons with a history of adenoma had more rectal ACF than those without ($P = 0.02$). Takayama et al. (11) observed more rectal ACF among Japanese with adenomas than among those without neoplasia ($P < 0.001$). In another chromoendoscopy study, persons with adenomas had no more rectal ACF than those without polyps (odds ratio, 1.05; 95% confidence interval, 0.96-1.15; ref. 8). In all three studies, the frequency distribution of ACF number among persons with adenomas overlapped that among persons without. These variations in strength of association between ACF number and adenomas may be explained by differences in participant characteristics across the studies (e.g., age, diet, ethnicity) or in methods used to stain the rectal mucosa.

Persons with a history of adenoma did not have significantly larger ACF than persons without. The results of earlier studies of ACF size in relation to adenomas and colorectal cancer have been mixed (9-11).

Very little is known about the factors that initiate or promote growth of ACF in humans. If ACF develop into colorectal polyps and subsequently cancer, then some factors associated with risk of colorectal cancer may also affect ACF growth. Here, older persons tended to have more and larger ACF, a finding consistent with previous studies (7, 11-13).

There was no association between NSAID use, cigarette smoking, or family history of colorectal cancer and either ACF number or size. In contrast, use of sulindac has been associated with regression of ACF in one small clinical trial (11). That trial was neither randomized nor blinded, but perhaps certain NSAIDs may be active at the earliest stages of colorectal carcinogenesis.

Here, 54.8% of 104 biopsies of ACF displayed neither hyperplasia nor dysplasia, 44.2% had hyperplasia and one had dysplasia, data consistent with those of Adler et al. (8). Although Takayama et al. (11) identified a similar proportion of ACF without hyperplasia or dysplasia (57%), dysplasia was present in 21%. In these three chromoendoscopy studies, the relatively high proportion of ACF without hyperplasia or dysplasia cannot be entirely attributed to difficulties with sampling small lesions because some ACF extracted from colon resection specimens *ex vivo* also lack hyperplasia or dysplasia (9, 13, 14). The higher prevalence of dysplastic ACF in the Japanese study compared with the U.S. studies may result from differences in the ethnicity, diet, or environmental exposures of the study populations. Different approaches to imaging ACF and to diagnosing dysplasia may also be relevant.

There are some limitations in the design of the current study. The primary purpose was to explore the relationships between colorectal cancer risk factors and prevalence of rectal ACF as a preliminary step toward larger-scale epidemiologic studies. Given the small sample, we could adjust for confounding only to a limited degree and may not have identified some associations because of insufficient power. Our participants were recruited from a veterans' hospital and, therefore, are not representative of the general population on sociodemographic characteristics, medication use, and comorbid conditions. Finally, because assessment of ACF number and size in the colon is technically difficult, we studied rectal ACF only.

Several lines of evidence indicate that ACF are the earliest identifiable precursors of colorectal cancer. In azoxymethane-treated rats, tumors were 17 times more likely to develop in areas that previously contained an ACF than elsewhere (2). K-*ras* mutations are present in ~40% to 90% of ACF from people without familial adenomatous polyposis (11, 12, 18). Microsatellite instability (19, 20), APC mutations (18, 21), and dysplasia (6, 9, 11, 13, 14) have also been identified in human ACF. The absence of dysplasia or genetic abnormalities in some ACF does not exclude the possibility that they are precursors of cancer. Indeed, other preneoplastic conditions, such as Barrett esophagus, adenomas, and ulcerative colitis, lack some genetic and histologic features of their corresponding tumors (22-24).

In conclusion, this study suggests that persons with adenomas have somewhat more rectal ACF than persons without, and that age is a risk factor for ACF growth. Although ACF are likely to represent an early step in progression toward human colorectal cancer, neither ACF size nor number has been shown to be predictive of progression. Therefore, neither of these ACF characteristics can be considered valid intermediate end points for cancer prevention studies at this time. Future research should be directed toward developing techniques to identify ACF that are likely to progress and the modifiable factors that promote or inhibit such progression.

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