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One Year Recurrence of Aberrant Crypt Foci

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Abstract

Introduction—Aberrant crypt foci (ACF) are putative precursors of colorectal adenomas and have been postulated as a potential biomarker for colorectal cancer. Few studies have followed subjects after ACF removal to monitor recurrence.

Methods—Subjects enrolled in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial were recruited for a study of ACF. A standardized protocol using magnified endoscopy and mucosal staining with methylene blue was implemented to detect rectal ACF. After removal of all baseline ACF, subjects returned one year later and recurrent ACF were observed and biopsied.

Results—A total of 434 of 505 (86%) subjects observed at baseline returned for the year 1 exam. The mean number of ACF at year 1 was strongly correlated with the number at baseline; subjects with 0, 1, 2–3, 4–6, and 7+ ACF at baseline had a mean of 1.2, 1.4, 1.7, 3.0 and 5.5 ACF, respectively, at year 1. ACF prevalence and mean count at year 1, 61% and 1.93, respectively, were only slightly lower than the corresponding values at year 0, 69% and 2.25. The locations of ACF at year 1 and baseline were significantly correlated. Of 96 ACF assessed for histology, 70 (73%) were hyperplastic and none dysplastic.

Conclusion—After removal of ACF at baseline, ACF counts one year later are only slightly reduced and are significantly correlated with the baseline ACF count. The results of this study do not support a role for ACF in clinical practice.

Introduction

Aberrant crypt foci (ACF) are alterations in the colonic mucosa that have been proposed as one of the earliest stages of the carcinogenic pathway in the colorectum, and a putative precursor to adenomatous polyps [1,2]. Understanding the dynamics of ACF over time including their persistence, regression, and recurrence, may yield important clues to the dynamics of adenomatous polyps, lesions further down the carcinogenic pathway. While a number of studies have examined ACF at a single time point [2–5], few have examined ACF longitudinally over time. The longitudinal studies that have been performed have been small, examining less than fifty subjects [1,6].

The Prostate, Lung, Colorectal, and Ovarian (PLCO) Screening Trial is a randomized, controlled study of cancer screening, including study of flexible sigmoidoscopy for early

detection of colorectal cancer [7]. In 2003 an ancillary study of ACF was initiated at four screening centers. Standardized protocols and definitions for ACF detection were implemented. The findings of the initial ACF exam showed good comparability across centers and an overall prevalence of rectal ACF of 68% 8. A report on the natural history of ACF from the pilot phase of the protocol, where ACF were identified but not biopsied or removed and a repeat examination was performed one year later, showed a considerable dynamic to ACF detection; roughly half of the ACF at baseline appeared to have regressed at year one but these were replaced with an approximately equal number of apparently newly formed lesions 9. In this investigation, we report on rectal ACF recurrence one year after clearing the rectum of all prevalent ACF, to determine new ACF formation.

Methods

The methods for the ACF study have been previously described [8]. Briefly, subjects enrolled in the Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial were eligible for the ACF study if they were screened at one of four screening centers (Georgetown University, Marshfield Clinic, University of Pittsburgh, and Washington University) participating in the ACF study if they received an adequate baseline PLCO flexible sigmoidoscopy (FSG) screening exam with no finding of cancer. In addition, subjects with a positive screen had to have undergone follow-up colonoscopy (or sigmoidoscopy) to determine distal adenoma status since the PLCO FSG exam did not biopsy or remove lesions. Eligible subjects were recruited at the four centers on a stratified basis so as to enroll approximately equal numbers of subjects with no distal adenomas, non-advanced distal adenomas, and advanced distal adenomas at baseline PLCO screen. The definition of an advanced adenoma was size ≥ 1cm, or villous histology, or high grade dysplasia. Information on smoking history, BMI, and family history of colorectal cancer was obtained from a demographics and medical history questionnaire administered to all PLCO subjects at baseline. Medication use (aspirin, NSAIDs, and statins) was assessed at the time of each ACF exam.

Study subjects were scheduled for two ACF exams one year apart. At each exam, subjects underwent a modified bowel prep of clear liquids and phosphasoda and tap water enemas to clear the rectum. The rectum was sprayed with 60 ml of Mucomyst to remove adherent mucus before staining the mucosa of the rectum for 2 minutes with 60 ml of methylene blue dye. After washing out the excess dye, the distal rectum up to the middle rectal valve was examined using the Fujinon ES-4105CE5 magnifying sigmoidoscope, which allowed for 4× magnification).

ACF were defined as colonic crypts with a larger diameter than normal mucosa, having a thicker epithelium, and more darkly staining than normal crypts. Lesions elevated greater than 2 mm were considered to be polyps and not counted as ACF. When an ACF was identified, it was photo documented, the location (centimeters from the anal verge) was recorded, and the lesion was biopsied with a cold biopsy forceps. Also, three biopsies of normal rectal mucosa were obtained as control specimens. Biopsies were either fixed in formalin or frozen in liquid nitrogen in a standardized manner and sent to the UCLA Medical Center Department of Pathology for histologic analysis. For budgetary reasons, only a (random) sample of the fixed biopsies were evaluated for histology. ACF biopsy specimens were categorized as hyperplastic, mixed hyperplastic/dysplastic, dysplastic, or normal. Detailed shipping and processing techniques for the specimens, as well as the specific criteria used for histologic classification, have been previously reported [8].

Statistical Analysis

The statistical significance of the difference in ACF prevalence at year 0 and year 1 was assessed by McNemar's test for matched pairs. The significance of differences in ACF count between the two exams was assessed by utilizing the signed rank test on the paired differences.

A multiple logistic regression model was used to determine the association of various risk factors on ACF recurrence at year 1. Factors evaluated were sex, age, baseline adenoma status (no adenoma, non-advanced adenoma, advanced adenoma) smoking history (current, former, never), BMI (< 25, 25–30, >30), family history of colorectal cancer, and mediation use (aspirin, NSAIDS, statins). The model was also used to test whether screening center was significantly associated with ACF prevalence.

To examine the relationship between ACF location at baseline and year 1 we examined the group of subjects with at least one ACF at both exams. For all ACF observed at year 1, we evaluated how many were within 1 cm (and 2 cm) of a baseline ACF in the same subject. To determine the expected percentage that were within this distance, we randomly permuted the baseline and year 1 location data so that each subject's baseline ACF locations were combined with another random subject's year 1 locations. In order to control for any effect of ACF number at each visit, permutations were all done within the same ACF count total at baseline and year 1 (i.e., subjects with 1 ACF at baseline and 2 ACF at year 1 were permuted only with other such subjects).

Results

Of 505 subjects enrolled in the main phase of the ACF study, 434 (86%) returned for the 2nd (year 1) ACF exam. The characteristics of the returning and non-returning cohorts are displayed in table 1. Returning subjects were similar to non-returning subjects except returning subjects were significantly younger, less likely to be a current or former smoker, and were less removed in time from the baseline PLCO FSG exam. A total of 66% of returning subjects were male and 53% were aged 70 or over. About half were daily aspirin users and 55% were former or current smokers. Almost 60% had a history of adenoma at the initial FSG exam for the PLCO Trial, which occurred on average 8 years before the baseline (year 0) ACF exam. The mean time from the baseline to the year 1 ACF exam was 358 ± 51 (SD) days.

Table 2 shows ACF prevalence and counts at year 0 (baseline) and year 1 for returning subjects. Prevalence was slightly, though statistically significantly, higher at baseline (69%) than year 1 (61%), as was the mean number of ACF (2.25 versus 1.93). The proportion of subjects having 4 or more ACF was similar at the two exams, 24% at baseline versus 21% at year 1 (p=0.4).

The number of ACF at year 1 was strongly associated with the number at baseline (Table 3). Only 44% of subjects with no ACF at baseline had any ACF at year 1, as compared to 64–73% of subjects with 1–6 ACF at baseline and 90% of subjects with 7 or more ACF at baseline. The mean number of ACF at year 1 increased steadily with the number of ACF at baseline, rising from 1.2 in the no ACF at baseline to 5.5 in the 7+ ACF at baseline group (Table 3). The correlation of the number of ACF at baseline and year 1 was r=0.44 (p < 0.0001).

Table 4 shows ACF prevalence and mean count by center. Each quantity differed significantly across screening centers (p < 0.0001) at both year 1 and baseline; further, the relative ranking of the centers in terms of prevalence and mean count was the same at baseline as at year 1. Since some centers consistently identified greater numbers of ACF, and others consistently lower numbers, across exam years, and because subjects were seen at the same center for both exams, some of the overall correlation in ACF count across exams could be due to the confounding effect of center. However, within centers, the correlation in ACF count at baseline and year 1 was statistically significant at all but one center, with a correlation coefficient ranging from 0.26 to 0.62 (Table 4).

In a multiple logistic regression model examining age, gender, baseline adenoma status, smoking, BMI, family history of CRC, and medication use (aspirin, NSAIDS, statins), the only significant factor associated with ACF prevalence was former smoking (OR=1.8; 95% CI 1.1–

2.8); current smoking had an OR of 1.2 (95% CI 0.6–2.6). Obesity (BMI > 30) was found to have a non-significant OR of 1.4 (95% CI 0.8–2.1).

The analysis of ACF location at baseline and year 1 showed that among the 206 subjects with at least one ACF at both visits, 60.0% of year 1 ACF were within 1 cm of a baseline ACF, compared to an expected percentage of 45.7% ($p < 0.0001$). The comparable percentages for identifying ACFs within 2 cm of a baseline ACF were 75.4% observed versus 61.1% expected ($p < 0.0001$).

A total of 96 observed ACF at year 1 were evaluated for histology. Of these, 70 (73%; 95% CI 64–82%) were found to be histologic ACF, all hyperplastic. There was no significant difference in histologic confirmation rates across screening centers.

Discussion

In this study, rectal ACF were removed and the rectum was re-assessed one year later. Despite the fact that all observed ACFs were removed at the baseline exam, the prevalence and mean number of ACF observed at year 1, 61% and 1.93, respectively, was only slightly, albeit statistically significantly ($p=0.02$), lower than that observed at baseline, 69% and 2.25. There was also a strong, statistically significant correlation between the prevalence and number of ACF at baseline and what was found one year later ($p < 0.0001$).

A number of explanations may explain these observations. The correlation of baseline and year one counts could be explained by an underlying tendency in some subjects to develop ACF; this tendency would then manifest itself in elevated counts at both exams. Alternatively, or additionally, some ACF may have not been removed at baseline, and these may have persisted at year one, so missed ACF may help explain the similarity in ACF count one year apart. Evidence suggests that the endoscopic detection of ACF is flawed, with considerable inter-observer variability [10].

The year 1 ACF count is similar to the baseline ACF count, whether the ACFs observed at baseline are removed, as in the current study, or just observed, as occurred in our pilot, natural history study, where ACF at baseline were observed but not removed [9]. The mean number of ACF observed at year 1 when baseline ACF were left in situ was only slightly higher, 2.3, than the year 1 mean number observed in the current study of 1.93 (average baseline counts were similar in the two studies). That baseline removal had only a small effect on the year 1 count can be explained, at least in part, by the phenomenon of ACF regression. In the natural history study, 57% of baseline ACF were estimated to have regressed and 43% persisted, one year later [9]. If many ACF regress within a year, then removing ACF at baseline will not have a great affect on the ACF count one year later.

Few studies have examined ACF over time to evaluate ACF recurrence after removal. In the Adenoma Prevention with Celecoxib Trial, 45 subjects were examined at baseline and re-evaluated one year later [6]. The mean number of ACF at baseline and year 1 were similar, 8.3 and 6.2, respectively. However, the numbers of ACF were substantially higher than that seen in the current study, an observation which points to variability in ACF reproducibility which hinders comparisons across studies [10].

Parallels between ACF and adenomatous polyps are notable. In this study the number of ACF at baseline and year 1 were strongly correlated, with a correlation coefficient of 0.44. Studies of adenomas have repeatedly reported a similar finding; namely, that after polypectomy, the number of adenomas at baseline is predictive of the number seen at repeat colonoscopy at 1–4 years [11–13]. We also observed a correlation in the location of ACF at baseline and at year 1. A similar finding has also been observed with adenomas; adenoma location at baseline and

first surveillance are significantly correlated [12]. Regression, which was observed with ACF in our natural history study, has also been hypothesized to occur with adenomas [14].

In an analysis of the baseline ACF exam in this cohort, we reported that cigarette smoking was associated with higher ACF prevalence and increased BMI was associated with lower ACF prevalence [8]. In this study of recurrent ACF, we also found a significant association with cigarette smoking; however, somewhat curiously, a significant OR was only seen for former smoking (OR=1.8), and not current smoking (OR=1.2). At baseline, ACF prevalence was associated more strongly with current (OR=2.6) as opposed to former (OR=1.6) smoking. Other studies have shown smoking to be a risk factor for prevalent ACF. For example, Moxon et al. showed that smokers had a significantly greater number of ACF than non-smokers and also demonstrated a significant dose-response effect (on ACF count) with number of pack years [3].

Our previous finding that increased BMI was inversely associated with ACF was unexpected, since elevated BMI has been considered to be a risk factor for adenomas and colorectal cancer. Additionally, Swede et al. showed increased BMI to be associated with increased number of prevalent ACF [4]. Our result here, of a modest, but not statistically significantly elevated risk for recurrent ACF in obese subjects (OR=1.4) seems more in line with the literature than our earlier finding from the baseline exam.

This study sheds additional light on the role for ACF in research and clinical practice. In our multi-center study, we found serious limitations in ACF reproducibility, and a considerable dynamic to ACF progression, with evidence for regression and initiation within a relatively short time frame of 1 year [9,10]. Because of problems in the reliability of detection, we are skeptical of the use of ACF to predict clinical outcomes, and don't believe there is a role for ACF detection in clinical practice [2,15]. Additionally, in contrast to animal models, where dysplastic ACF are commonly produced, dysplastic ACF are rarely detected in humans [2]. Thus, the conclusion from animal studies, that there likely is a link between preventing ACF and preventing colorectal cancer, may not be applicable in humans.

In conclusion, removal of ACF at baseline did not result in a markedly reduced number of ACF observed one year later, and the number of ACF observed at year 1 was generally comparable to the baseline level. The locations of ACF removed at baseline and those observed at year one were significantly correlated. Parallels between ACF and adenomas are notable, including a tendency for some individuals to develop recurrent lesions in similar locations, as well as the possibility of spontaneous regression. At this time however, there is no defined role for ACF detection in clinical practice.

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Table 1

Characteristics of returning and non-returning subjects in the ACF study.

	Returned for Year 1 Exam (n=434)	Did not Return (n=71)	P-value [1]
Age ≥ 70 [2]	53%	68%	0.02
Male	66%	63%	0.62
Current or Former Smoker	55%	73%	0.005
Family History of CRC	13%	7%	0.18
Daily Aspirin Use at Year 0	46%	49%	0.61
Daily Aspirin Use at Year 1	47%	-	
Adenoma Status at PLCO Baseline Exam			0.48
None	43%	41%	
Non-advanced	31%	38%	
Advanced	26%	21%	
Mean time lag from PLCO Baseline Exam to Year 0 ACF Exam (yrs)	8.0	8.8	0.001

[1] Comparison between those who did and those who did not return.

[2] At baseline exam (year 0)

Table 2

ACF Prevalence and Count at Year 0 and Year 1 among returning subjects.

	Year 0	Year 1	P Value
N=434			
Subjects with ACF: N (%)	298 (69)	266 (61)	0.02
Mean (SD) ACF Count	2.25 (2.6)	1.93 (2.4)	0.02
Mean (SD) ACF Count (among those with ≥ 1 ACF)	3.28 (2.6)	3.15 (2.3)	0.5
ACF Count Distribution: N (%)			0.005
0	136 (31)	168 (39)	
1	67 (15)	85 (20)	
2–3	127 (29)	89 (21)	
4–6	83 (19)	65 (15)	
7+	21 (5)	27 (6)	

Table 3

ACF Count at Year 1 by ACF Count at Year 0

		# of ACF at Year 1						
		0	**1**	**2–3**	**4–6**	**7+**	**Any ACF**	**Mean #**
# of ACF at Year 0	N	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	
0	136	76 (56)	22 (16)	25 (18)	10 (7)	3 (2)	60 (44)	1.2
1	67	22 (33)	27 (40)	12 (18)	3 (5)	3 (4)	45 (67)	1.4
2–3	127	46 (36)	28 (22)	29 (23)	20 (16)	4 (3)	81 (64)	1.7
4–6	83	22 (27)	8 (10)	22 (27)	21 (25)	10 (12)	61 (73)	3.0
7+	21	2 (10)	0	1 (5)	11 (52)	7 (33)	19 (90)	5.5

Note: Correlation in ACF count at year 0 and year 1 within subjects was 0.44 ($p < 0.0001$)

Table 4

ACF prevalence and mean count across centers.

	Center			
	1	**2**	**3**	**4**
N	85	121	133	95
Year 0				
Prevalence	79%	74%	59%	67%
Mean Count	3.6	2.4	1.3	2.2
Year 1				
Prevalence	78%	70%	41%	63%
Mean Count	3.6	2.0	0.8	1.9
Correlation of Year 0 and Year 1 count in subjects (p-value)	0.62 (<0.0001)	0.26 (0.004)	0.27 (0.001)	0.12 (0.3)

Note: $p < 0.0001$ for comparison across centers for both prevalence and mean count at both baseline and year 1. Prevalence and mean count for year 0 are calculated only for returning subjects.