One Year Recurrence of Aberrant Crypt Foci

Paul F. Pinsky1, James Fleshman2, Matt Mutch2, Christopher Rall3, Aline Charabaty4, David Seligson5, Sarah Dry6, Asad Umar1, and Robert E. Schoen6

Abstract
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. Subjects enrolled in the Prostate, Lung, Colorectal, and Ovarian 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 1 year later and recurrent ACF were observed and biopsied. 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 to 3, 4 to 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, respectively). The locations of ACF at year 1 and baseline were significantly correlated. Of 96 ACF assessed for histology, 70 (73%) were hyperplastic and none were dysplastic. After removal of ACF at baseline, ACF counts 1 year later were only slightly reduced and were 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, might yield important clues to the dynamics of adenomatous polyps, lesions further down the carcinogenic pathway. Although 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 done have been small, examining fewer than 50 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 (FSG) 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, in which ACF were identified but not biopsied or removed and a repeat examination was done 1 year later, showed a considerable dynamic to ACF detection; roughly half of the ACF at baseline seemed to have regressed at year 1 but these were replaced with an approximately equal number of apparently newly formed lesions (9). In this investigation, we report on rectal ACF recurrence 1 year after clearing the rectum of all prevalent ACF to determine new ACF formation.

Materials and Methods
The methods for the ACF study have been previously described (8). Briefly, subjects enrolled in the 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 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 because 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...
Table 1. Characteristics of returning and non-returning subjects in the ACF study

<table>
<thead>
<tr>
<th>Returned for year 1 exam (n = 434)</th>
<th>Did not return (n = 71)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥ 70†</td>
<td>53%</td>
<td>68%</td>
</tr>
<tr>
<td>Male</td>
<td>66%</td>
<td>63%</td>
</tr>
<tr>
<td>Current or former smoker</td>
<td>55%</td>
<td>73%</td>
</tr>
<tr>
<td>Family history of colorectal cancer</td>
<td>13%</td>
<td>7%</td>
</tr>
<tr>
<td>Daily aspirin use at year 0</td>
<td>46%</td>
<td>49%</td>
</tr>
<tr>
<td>Daily aspirin use at year 1</td>
<td>47%</td>
<td>—</td>
</tr>
<tr>
<td>Adenoma status at PLCO baseline exam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>43%</td>
<td>41%</td>
</tr>
<tr>
<td>Nonadvanced</td>
<td>31%</td>
<td>38%</td>
</tr>
<tr>
<td>Advanced</td>
<td>26%</td>
<td>21%</td>
</tr>
<tr>
<td>Mean time lag from PLCO baseline exam to year 0 ACF exam (y)</td>
<td>8.0</td>
<td>8.8</td>
</tr>
</tbody>
</table>

*Comparison between those who did and those who did not return.
†At baseline exam (year 0).

no distal adenomas, non–advanced distal adenomas, and advanced distal adenomas at baseline PLCO screen. The definition of an advanced adenoma was size (≥1 cm), villous histology, or high-grade dysplasia. Information on smoking history, body mass index (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, nonsteroidal anti-inflammatory drugs, and statins) was assessed at the time of each ACF exam.

Study subjects were scheduled for two ACF exams 1 year apart. At each exam, subjects underwent a modified bowel preparation 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 40× 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 >2 mm were considered to be polyps and not counted as ACF. When an ACF was identified, it was photographed, 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 University of California at Los Angeles 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 using the signed rank test on 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 medication use (aspirin, nonsteroidal anti-inflammatory drug, 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. 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 one ACF at baseline and two 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 second (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 that 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 days (SD).

Table 2 shows ACF prevalence and count at year 0 (baseline) and year 1 for returning subjects. Prevalence was slightly, although 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 four 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, compared with 64% to 73% of subjects with one to six ACF at baseline and 90% of subjects with seven 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; furthermore, the relative ranking of the centers in terms of prevalence and mean count was the same at baseline as at year 1. Because 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 colorectal cancer, and medication use (aspirin, nonsteroidal anti-inflammatory drug, statins), the only significant factor associated with ACF prevalence was former smoking [odds ratio (OR), 1.8; 95% confidence interval, 1.1-2.8]; current smoking had an OR of 1.2 (95% confidence interval, 0.6-2.6). Obesity (BMI > 30)

Table 2. ACF prevalence and count at year 0 and year 1 among returning subjects

<table>
<thead>
<tr>
<th></th>
<th>Year 0</th>
<th>Year 1</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 434</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects with ACF, n (%)</td>
<td>298 (69)</td>
<td>266 (61)</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean (SD) ACF count</td>
<td>2.25 (2.6)</td>
<td>1.93 (2.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean (SD) ACF count (among those with ≥1 ACF)</td>
<td>3.28 (2.6)</td>
<td>3.15 (2.3)</td>
<td>0.5</td>
</tr>
<tr>
<td>ACF count distribution, n (%)</td>
<td>136 (31)</td>
<td>168 (39)</td>
<td>0.005</td>
</tr>
<tr>
<td>0</td>
<td>67 (15)</td>
<td>85 (20)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>127 (29)</td>
<td>89 (21)</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>21 (5)</td>
<td>27 (6)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. ACF count at year 1 by ACF count at year 0

<table>
<thead>
<tr>
<th>No. of ACF at year 1</th>
<th>0</th>
<th>1</th>
<th>2-3</th>
<th>4-6</th>
<th>7+</th>
<th>Any ACF</th>
<th>Mean no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ACF at year 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>136</td>
<td>76 (56)</td>
<td>22 (16)</td>
<td>25 (18)</td>
<td>10 (7)</td>
<td>3 (2)</td>
<td>60 (44)</td>
</tr>
<tr>
<td>1</td>
<td>67</td>
<td>22 (33)</td>
<td>27 (40)</td>
<td>12 (16)</td>
<td>3 (5)</td>
<td>3 (4)</td>
<td>45 (67)</td>
</tr>
<tr>
<td>2-3</td>
<td>127</td>
<td>46 (36)</td>
<td>28 (22)</td>
<td>29 (23)</td>
<td>20 (16)</td>
<td>4 (3)</td>
<td>81 (64)</td>
</tr>
<tr>
<td>4-6</td>
<td>83</td>
<td>22 (27)</td>
<td>8 (10)</td>
<td>22 (27)</td>
<td>21 (25)</td>
<td>10 (12)</td>
<td>61 (73)</td>
</tr>
<tr>
<td>7+</td>
<td>21</td>
<td>2 (10)</td>
<td>0</td>
<td>1 (5)</td>
<td>11 (52)</td>
<td>7 (33)</td>
<td>19 (90)</td>
</tr>
</tbody>
</table>

NOTE: Correlation in ACF count at year 0 and year 1 within subjects was 0.44 (P < 0.0001).
was found to have a nonsignificant OR of 1.4 (95% confidence interval, 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 with 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% confidence interval, 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 1 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, respectively). There was also a strong, statistically significant correlation between the prevalence and number of ACF at baseline and what was found 1 year later ($P < 0.0001$).

A number of explanations might explain these observations. The correlation of baseline and year 1 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 1, therefore missed ACF may help explain the similarity in ACF counts 1 year apart. Evidence suggests that the endoscopic detection of ACF is flawed, with considerable interobserver variability (10).

The year 1 ACF count was 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, in which 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 (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 1 year later (9). If many ACF regress within a year, then removing ACF at baseline will not have a great effect on the ACF count 1 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 1 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; i.e., that after polypectomy, the number of adenomas at baseline is predictive of the number seen at repeat colonoscopy at 1 to 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 nonsmokers and also showed 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 because 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 multicenter 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 with animal models, in which 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, might not be applicable in humans.

In conclusion, removal of ACF at baseline did not result in a markedly reduced number of ACF observed 1 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 1 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 12/04/2009; revised 03/31/2010; accepted 04/01/2010; published OnlineFirst 06/22/2010.

References

One Year Recurrence of Aberrant Crypt Foci
Paul F. Pinsky, James Fleshman, Matt Mutch, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-09-0257

Cited articles
This article cites 15 articles, 2 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/3/7/839.full#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://cancerpreventionresearch.aacrjournals.org/content/3/7/839.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerpreventionresearch.aacrjournals.org/content/3/7/839.
Click on “Request Permissions” which will take you to the Copyright Clearance Center's (CCC) Rightslink site.