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# Epidemiology of Colonic Aberrant Crypt Foci: Review and Analysis of Existing Studies

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## Abstract

Since first described in a rodent model in 1987, aberrant crypt foci (ACF) in the colon have been shown to exhibit many of the molecular features of the more advanced colonic neoplasms including cancer. Therefore, they may be early lesions with potential for progression, and be valuable biomarkers for reduction of risk of colorectal cancer (CRC). For this review, we searched PubMed, and reference lists of recent publications, for studies which reported on associations of features of ACF in humans, such as number or size, with subject characteristics, such as age or family history of CRC. Over 150 papers have reported on ACF in humans. However, the vast majority of these publications are concerned with molecular and morphological features of biopsied lesions, and not their epidemiology. None of the epidemiological studies were of optimum design, primarily due to their absence of a well-defined subject sampling frame or method. Given their 'first-generation' nature, consistent findings were of increased ACF number with age and with synchronous advanced colonic neoplasia. One study reported a higher mean number of ACF in subjects with a family history of CRC than in those without. The strongest evidence on the ability of ACF to predict a diagnosis of CRC will be from prospective studies with baseline ACF assessment in a large sample of disease-free persons (many thousands) who are followed carefully for many years. In the interim, because ACF are asymptomatic, well-designed cross-sectional studies are feasible and will yield valuable information on the relation of ACF to the known risk factors for CRC. This information can then be used to improve the design of prospective studies, and of clinical intervention trials that use ACF as an intermediate endpoint.

## Introduction

Colorectal cancer (CRC) is the second most often diagnosed cancer in westernized nations and shows a wide international variation in risk [1]. It is the second most common cause of cancer death in America [2]. Reasons for the high risk in westernized countries are only partially understood. Diet is believed to play a key role, particularly red and processed meat consumption. In addition, risk of colon cancer has been associated with family history and inversely associated with aspirin use and exercise.

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CRC is thought to progress through several morphological stages, including polyp formation and malignant conversion [3]. A number of genetic alterations, including mutations in the K-ras, p53 and APC genes, have been shown to accompany disease progression [4]. The earliest identifiable lesion on this pathway may be the aberrant crypt foci (ACF).

## Aberrant Crypt Foci

Since the first report of ACF in a rodent model by Bird in 1987 [5], there has been increasing interest in their biology. ACF are pre-polyp abnormalities identified in single crypts by high magnification chromendoscopy using either methlyene blue or indigo carmine. Colonic ACF may represent early lesions capable of progression to CRC and/or be predictive markers of future risk. Kinzler and Vogelstein [3] place ACF as the earliest identifiable morphological change on the pathway to CRC. However, it has been shown that ACF are a heterogeneous group of lesions, and some may be important in CRC development and others not. Elucidation of the distribution of ACF in the general population, and the association of ACF with personal characteristics will advance our understanding of their biological meaning.

The epidemiology of ACF should be similar to that of CRC if they are precursor lesions and/or if their presence is a good predictor of future CRC development. Risk factors for CRC should also predict number of ACF, depending on how early in the pathogenic process they operate. If they operate at the earliest stages, then they should also predict formation of ACF.

The potential benefit of understanding ACF is significant. If the number of ACF is a good indicator of future CRC risk, then it can be used clinically for assessment of risk, screening guidelines, and intervention strategies. Also, if ACF, or an identifiable subset of these lesions, are strongly associated with progression to CRC, then their removal may reduce future risk.

ACF are beginning to be used as markers in intervention trials for risk-reduction of CRC. There are currently two NCI-sponsored trials listed at ‘clinicaltrials.gov’ that use number of ACF in the distal colon/rectum as the primary endpoint. One led by the Mayo Clinic compares sulindac, atorvastatin, inulin, and placebo over a 6-month intervention (NCT00335504), and the other led by MD Anderson Cancer Center compares sulindac, aspirin, ursodiol, and placebo over a 12-month intervention (NCT00062023). A better understanding of the relation of ACF to risk factors for CRC will provide more guidance for design of future intervention trials.

## Rationale for this Review

For the experimental biologist, ACF may offer the opportunity to observe the very earliest molecular alterations on the multi-step pathway to CRC. To date, hundreds of publications on this basic biology in animal models have appeared, and the number is growing rapidly. The first papers on ACF in humans began to appear in the early 1990s [6-8]. Since then, many more have been published. Some of these are descriptive studies of clinical features of ACF in various groups of patients. There is also a limited number of studies which could be described as ‘epidemiological’ in that they provide estimates of the associations of ACF features (e.g., number, histological grade, size) with demographic characteristics of the patients in whom they have been observed (e.g., age, family history of CRC, aspirin use). None of these studies have been of optimal epidemiological design, and so should be viewed as ‘first-generation’ investigations.

It is the purpose of this review to describe the first generation investigations of the epidemiology of ACF, and then from that perspective to provide suggestions for potential future directions for the next generation of studies. For in-depth review of the experimental and human observational evidence on the phenotypes and genetic and epigenetic spectra of ACF, see

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Pretlow and Pretlow [9] and Alrawi et al. [10]. For detailed description of histopathological features of ACF see Hurlstone and Cross [11] and Di Gregorio et al. [12].

We will focus on the evidence for an association of ACF with synchronous findings of advanced colonic neoplasia and with specific patient characteristics and risk factors for subsequent CRC development. There are two broad objectives to this effort. First is to contribute to the rapidly expanding information in support of ACF as an early preneoplastic lesion on the pathway to CRC. Second is to consider whether a relatively easily measured aspect of ACF in the distal portion of the colon/rectum, such as number or size, could be used as an accurate estimate of the risk level of an individual. For the first objective, considerable experimental, clinical, and observational data has accumulated: many of the genetic and phenotypic alterations seen in CRC are also seen in ACF [9]. Even if these associations can be established beyond doubt, however, the use of ACF as a marker of future risk for an individual may still not be completely effective. Clearly, only a small subset of ACF could possibly progress, a logic based on the fact that almost all persons over age 50 have at least one ACF in the distal 20 cm of colon/rectum, yet cumulative lifetime risk of CRC is only about 5% [13]. Unless and until a molecular signature is uncovered that distinguishes those ACF with malignant potential from those without inherent risk, the mere presence of ACF might offer little information for the patient. However, the converse is also true: even if a very small fraction of ACF, or none at all, ever progress to CRC, their number (or other feature) may still be a useful marker of future risk of CRC. This would hold true if the presence of ACF is an indicator of processes in the colon that are also generating altered cells and other lesions which themselves are on the path to CRC.

## Risk Factors for CRC

The known heritable forms of colon cancer, including inherited high-risk syndromes familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC), confer a greatly increased risk of colorectal cancer and account for up to 6% of cases [14,15]. Another 10% to 30% of cases of CRC are associated with a family history of CRC in a first degree relative [16]. This family history effect may have a heritable genetic basis [17] in addition to shared environmental conditions among families [18,19]. Lichtenstein et al. [20] estimate that 35% of CRC is due to heritable factors based on analysis of twin cohorts in the Nordic countries.

Johns and Houlston [21] provide estimates for the relative risks of CRC according to the ageat-diagnosis and number of first degree relatives who have been diagnosed with CRC. Overall, at least one first degree relative with CRC conferred a relative risk of 2.25. The relative risk associated with more than one relative was 4.25; one relative with CRC under age 45 was 3.87; an afflicted parent was 2.26; and an afflicted sibling was 2.57.

It has been estimated that 70%-90% of CRC is influenced by diet [22]. Although the mechanism by which diet affects colon carcinogenesis is not entirely clear, epidemiological data point to the consumption of red meat, and perhaps low intake of fiber [23] and of fruits and vegetables [24,25]. Lack of physical activity and obesity have been associated with increased risk [26]. It is difficult, however, to disentangle effects of diet, obesity, and physical activity, in epidemiological studies.

A consistent finding for colon cancer is an association with red and processed meat intake [27,28]. Red meat components hypothesized to be contributory factors to colon cancer etiology include DNA-reactive heterocyclic amines formed during cooking [23,29]; animal fat, which may act by increasing intracolonic concentrations of membrane-damaging bile acids and fatty acids [30-33]; and, iron content of red meat [34-38]. Most dietary iron is not absorbed but is concentrated in the feces at levels that may be up to 10-fold higher than that found in tissues [39]. Moreover, feces contain high levels of bile pigments (e.g. bilirubin and biliverdin) that can complex with iron in a form that is capable of supporting the Fenton reaction [39,40].

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However, there is no consensus to date as to what aspect of red meat is most important as a potential colonic carcinogen.

Regular aspirin use has been associated with a reduced risk of colorectal cancer [41]. Aspirin and non-aspirin NSAIDS have also been reported to reduce occurrence of adenomas, and even contribute to their regression [42]. In animal models, aspirin has been shown to reduce ACF formation in rat colon [43].

Two postulated mechanisms for CRC pathogenesis that may have both dietary and familial components are insulin resistance and chronic inflammation [41,44,45]. Taken together, these two mechanistic models for etiology of CRC provide a framework within which much of the epidemiology of CRC may be interpreted. For example, risk reduction with aspirin use and risk elevation with body mass index, may be explained by reduction in inflammatory processes and increased tissue exposure to insulin, respectively. However, neither mechanism has yet been unequivocally demonstrated.

An interesting and perhaps clinically important question is what CRC risk factors also predict occurrence and pathobiology of ACF. Once the answer to this question is established, it could lead to the use of ACF occurrence as a useful marker of future risk of CRC.

## Studies of Human ACF

For this review, we searched PubMed, and reference lists of recent publications, for studies which reported on associations of features of ACF in humans, such as number or size, with subject characteristics, such as age, presence of synchronous advanced neoplasia, NSAID use, or family history of CRC. Over 150 papers have reported on ACF in humans, but only 13 publications could be defined as ‘epidemiological’ in that some estimate of association between presence of ACFs and subject characteristics are given. The vast majority of publications on human ACF are concerned with molecular biological features of biopsied lesions.

The published epidemiological studies are summarized in Table 1. Each of these studies reports an estimate of the degree of association of subject characteristics (e.g., age, family history of CRC, synchronous advanced neoplasia, etc.) with features of ACF (e.g., number, size, histological grade, etc.). The question that emerges is: how valid are these estimates? There are two crucial aspects of the epidemiological studies that must be considered when assessing validity: 1) for internal validity; method of sampling and assessment of ACF (potential measurement bias), and assessment of cofactors (potential confounding bias), and 2) for external validity (i.e., generalizability); subject sampling frame and method for subject selection. For the purposes of determining whether ACF can be used as a screening tool for degree of risk of CRC, both issues are paramount.

In contrast, there are many published human studies for which these epidemiological criteria are less important. For example, Luo et al. [46] sampled a total of 32 ACF and adjacent normal tissue from resected colons of 28 patients. They assessed loss of heterozygosity (LOH) at several chromosomal locations, microsatellite instability (MSI) at several locations, and the expression of APC and beta-catenin proteins. Although relevant clinical details for each patient and their diagnosis are provided, there is no indication of how the patients were sampled and from what underlying population group they derive. Nor is there any indication of how the particular ACF were chosen for analysis from all the ACF found in each patient. These issues, however, have little bearing on this study because of the nature for their inquiry. They found LOH and/or MSI in 8 of the 32 ACF examined, and further note that 6 of these ACF showed no features of dysplasia. In each of these 8 ACF, expression of APC and beta-catenin was normal. They also reported that LOH was found most frequently at a location including *PTPRJ*, the protein tyrosine phosphatase receptor type J gene, suggesting this may be a new

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colonic tumor suppressor. This study demonstrates an important 'proof of principle' that LOH and MSI can occur in ACF before altered expression of APC or beta-catenin, and suggest that these alterations can be very early markers of progression to CRC. This is a potentially important observation bearing on the sequence of events in the carcinogenic process. Our description of Luo et al. [46] is provided as a guide to the kind of human study not included in our review of the epidemiology of ACF. Another example, from our laboratory, is the investigation of Greenspan et al. [47] in which methylation status of the putative tumor suppressor *RASSF1A* in human ACF was examined. Greenspan et al. [47] used methylation-specific PCR and found that up to 30% of aberrant crypts sustained hypermethylation of *RASSF1A* whereas adjacent normal mucosa was unmethylated at this site. In addition, *RASSF1A* hypermethylation and *K-ras* mutations were not mutually exclusive. This is also a potentially important mechanistic observation but not included in our review of the epidemiology.

## Review of Epidemiological Studies

Of the 13 studies outlined in Table 1, six used surgical specimens for analysis, and seven used biopsy specimens obtained upon colonoscopy. These studies have estimated the prevalence and/or histological grade of ACF in 'normal' subjects, and subjects with colonic polyps or colon cancer [8,48-59], although the definition of 'normal' varies widely. None of these studies has been population-based, and very few have included patients with no apparent risk factors for CRC.

Roncucci et al. [8] selected 5 patients with familial adenomatous polyposis (FAP), 12 patients with colon cancer, and 10 patients with benign conditions of the large bowel (BD) from Toronto hospitals for examination of the resected colons. The method for subject selection was not given. They reported that 6 of 10 subjects with BD had at least one detectable ACF. In contrast, all 12 patients with colorectal cancer had at least one ACF, and a mean of $0.37/cm^2$ on the colon surface area they examined. All 5 subjects with colorectal cancer and FAP had at least one ACF, with a mean of $20/cm^2$.

A later study by Roncucci et al. [48] was designed to determine whether ACF were found in greater number in resected colons from CRC patients from two different regions in Italy: one with twofold higher incidence of CRC (26 from northern Italy) than the other (32 from southern Italy). They reported a significantly higher ACF count in the patients from the higher CRC risk region. Crypt size and histological grade, however, were not different. How the patients were chosen from those available was not reported.

Takayama et al. [49] conducted a study of ACF number, size, and dysplastic features in a group of 171 'normal' subjects, 131 patients with adenoma, and 48 patients with colon cancer in Sapporo, Japan. 'Normal' was defined as "having no apparent lesions of the colon on endoscopy". It is not clear from this description whether 'normal' subjects did or did not have symptoms or indications which lead to their exam; the reasons for the colonoscopies were not given. Prevalence of ACF in the distal rectal region increased with age in all groups with 54% of normal subjects aged 40-49 having at least one ACF, and 3.6% having at least one dysplastic ACF. By age 70 and older, 69% had at least one ACF and 10.3% had at least one dysplastic ACF. Among the patients with adenomas, ACF prevalence was higher, and among those with colon cancer, was higher still, with 100% of colon cancer patients of all ages having at least one ACF, and over 50% having at least one dysplastic ACF. Neither family history nor prior use of NSAIDs was reported upon, although a small intervention trial with sulindac was described.

Shpitz et al. [50] examined ACF in 76 colon cancer patients and in 17 patients with benign colon disease selected over a two period from clinics in Tel Aviv, Israel. It is not stated how

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many patients were eligible from the clinics over this time span nor how those examined were selected from the total available. They reported a high prevalence of ACF in both groups, with more in the colon cancer subjects. In addition, more ACF were found in the distal than proximal colon.

Bouzourene at al. [51] obtained resected colons from 26 CRC patients, 4 patients with adenoma, and 7 patients with non-neoplastic colon disease (BD) in Switzerland. They evaluated histology of 378 of the 508 ACF counted in this patient group, and reported that ACF density was less in the CRC patients than in the 7 BD patients although number of dysplastic ACF was higher in the CRC patients. Median size of ACF was progressively larger from normal histology to hyperplastic to dysplastic.

Nascimbeni et al. [52,53] conducted two studies with some overlap in patient groups. In both studies, resected colon was obtained from CRC patients and from patients with diverticular disease, and ACF were counted and compared. In the first study, a significantly higher concentration of ACF was seen in the cancer patients. In the second study, a questionnaire was administered which included family history, but the results for family history were not reported. In addition, the second study reported on a lack of association of laxative use and constipation with risk of CRC by comparing these factors between the CRC patients and a group of normal controls who were also asked these questions. ACF were not counted in the control group.

Adler et al. [54] selected 90 subjects from the daily colonoscopy list at the Mayo Clinic in Rochester, MN. Thirty were subjects with normal colons by endoscopy, 30 had adenomatous polyps, and 30 had colon cancer (the ‘normal’ group was not from routine screening colonoscopy, but rather persons who came to clinic with a complaint such as ‘altered bowel habit’). The distal 10 cm of the rectum was evaluated in each patient. Among 30 normal subjects, 23 had at least one ACF, and the median among the group was 3.5; among 30 subjects with polyps, 25 had at least one ACF, and the median was 4.0; among 30 patients with colon cancer, 28 had at least one ACF, and the median was 7.5. The differences remained after logistic regression adjustment for age and gender, but information on other factors that might affect ACF development was not included such as family history or tobacco. The Adler et al. [53] study did not report on histological grade of the ACF that were found.

Hurlstone et al. [55] conducted colonoscopy on a large series of patients in England. From 2,559 patients receiving colonoscopy for a variety of symptoms and indications by the first author from 2000 to end of 2004, 1000 were chosen after various exclusions including the presence of ‘protruding’ adenomas or cancer (JRSC class Ip, Is, Ips). Among this group, 574 were endoscopically normal, 281 had a ‘flat’ adenoma, and 14 had ‘flat’ cancer. After total colonoscopy, ACF were counted by chromendoscopy in the rectum. Mean numbers of ACF were 1, 9, and 38 respectively, and the proportion dysplastic was much higher in the cancer group. There was not adjustment for potential risk factors for CRC.

Rudolph et al. [56] recruited 32 patients from the VA Health System in the Seattle area who were scheduled for colonoscopy for a variety of indications such as history of polyps or visible GI bleeding. They found a significant association of ACF number and size with age (50 to 80 years of age). Rudolph et al. [56] also found a significant elevation of ACF number in patients with a personal history of adenoma. They reported no association of ACF number and a family history of CRC but this was based on only 3 subjects.

Moxon et al. [57] conducted a study designed to test a specific hypothesis that folate would be associated with reduced numbers of ACF. They recruited 83 asymptomatic patients referred for screening colonoscopy in Chicago of whom 61 were African-American. These patients completed a detailed demographic and lifestyle questionnaire, as well as a food frequency questionnaire. It is not stated how the patients were selected from all of those eligible during

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the study period. At exam, 1081 rectal ACF (distal 15 cm), 18 adenomas, 7 hyperplastic polyps, and 1 cancer were observed. No association with folate was found, but they did report significant increases with tobacco use and age, but not with NSAID use nor alcohol intake.

Seike et al. [58] examined 386 colonoscopy patients in Chiba University Hospital, Japan with the objective to determine if the number of counted ACF in the distal 15 cm of rectum was a good predictor of the synchronous presence of advanced neoplasms (certain adenomas and cancer) throughout the colon/rectum. There were patient exclusion criteria, and although not explicitly stated, it appears that most (or all) of those eligible over the study period from the study hospital were included. These were not asymptomatic patients coming for a screening exam; they came for a variety of indications that are listed in the paper. The study was conducted in a blinded manner in which the total colonoscopic examination was conducted by one endoscopist, and only after its completion did a second endoscopist enter the procedure suite and conduct chromendoscopy for ACF count in the rectum. They reported that ACF count was a strong predictor of advanced neoplasms in both the colon and also in the rectum. The authors did not report on any other factors related to number of ACF in the rectum.

Stevens et al. [59] tested the hypothesis that the number of ACF would be higher in patients with a family history of CRC than in those without. The results were consistent with prediction. Subjects were convenience sampled from patients coming for either a screening colonoscopy, or a surveillance examination based on a previous indication such as a past finding of adenoma. The family history information included only whether the patient had at least one first-degree relative who had previously been diagnosed with CRC; information on the age at which this diagnosis occurred was not available. None of the 103 subjects were found to have CRC nor advanced adenoma at the time of the examination.

As evident in this review, the published epidemiological studies of ACF conducted to date have been relatively small in size with the exception of Hurlstone et al. [55], Seike et al., [58], and Takayama et al. [49]. None has sampled disease-free persons from a well-defined underlying population at risk, although some have been closer to this ideal than others. In addition, none have been exhaustive in identification of potential confounders of the associations reported. Therefore, the findings of all these studies may be subject to confounding bias and lack of generalizability.

## Findings from the First-Generation Studies

In Table 1, the last column describes the sampling method for subject selection. Each of the studies does explain from what institution the subjects came, but most give no further detail. For example, there is no discussion of the total number of subjects who were available, how the particular subjects were selected, nor what underlying population the institution serves.

Table 2 presents a summary of the key findings of each of the studies reviewed. Table 3 provides a summary of the main findings from the epidemiological studies so far reported. The total number of ACF in patients over age 50 was found to be higher in older patients than middle-aged patients in the studies that presented data on age [48,49,50,56,57,59]. Of the three studies examining family history of CRC, two reported no association [56,57; based on only 3 and 4 subjects] whereas one study [59; based on 43 subjects] reported a significantly higher ACF number in patients with a family history compared to those without. Use of NSAIDs was examined in three studies, two of which reported no association [56,57] and one that reported lower ACF count in users [49]. Tobacco use (cigarette smoking) was examined in two studies, one reporting no association [56] and one reporting a strong association [57]. All but one study reported higher ACF count in patients with synchronous advanced neoplasia, and the one exception did report more dysplastic ACF in patients with CRC [51]. Only one study [57]

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reported on alcohol use and an aspect of diet (folate), and reported neither to be associated with number of ACF.

Several of these first-generation studies have provided encouraging evidence that the count of ACF in the distal 10 to 20 cm of colon/rectum is associated with the synchronous presence of advanced neoplasms, both adenomas and cancers. A number of the studies have also reported evidence in support of associations of ACF frequency with a limited set of risk factors for CRC, although there are conflicts in some of the findings. However, given the small size of most of the studies, these conflicts may be due to chance.

Evidence on whether the number of ACF (or other molecular or morphological features) may be a good predictor of subsequent risk of CRC has generally been assessed in two ways: 1) is the number of ACF related to known risk factors for CRC? and 2) is the number of ACF associated with the presence of synchronous CRC or advanced adenoma? For the second, a limitation of the studies which have reported associations of ACF with synchronous advanced neoplasia is that they have little data on other personal characteristics such as tobacco use, exercise, family history and NSAID use. Therefore the associations may have been confounded, and may not apply to persons at average risk (i.e., no obvious risk factors, which is the majority of cases of CRC).

## Next Generation Studies

The strongest evidence on whether features of ACF in the distal colon/rectum predict later risk of CRC will come from prospective studies with colonoscopic ACF assessment on large samples of disease-free persons (many thousands) who are followed carefully for many years. Such studies might benefit from stratified sampling on known risk factors for CRC (e.g., family history).

However, in the interim, useful indirect information has been gathered in the ‘first-generation’ epidemiological studies reviewed here. Next generation studies can also be cross-sectional in design but should include a larger number of subjects using better defined sampling schemes as well as a more thorough characterization of subject demographics, personal habits (e.g., smoking and exercise), family history, and diet. Cross-sectional designs are feasible and valuable approaches because ACF are asymptomatic, and their presence should have no impact on a study subject's accurate recall of personal information. In addition, it is extremely unlikely that the presence of ACF, per se, would alter a person's likelihood of obtaining a colonoscopy, again due to their complete lack of symptomology. For optimum design, large prospective studies will benefit greatly from these second-generation cross-sectional analyses. Likewise, the conception and design of future intervention trials should be based on sound evidence linking particular CRC risk factors with ACF occurrence.

## Prediction and Prevention

Reliable prediction of future risk of CRC from observation of ACF in the distal colon/rectum may become a reality, as discussed in this paper. Advances toward this goal require much more epidemiological and clinical study of the association of ACF with risk factors for CRC, and ultimately from prospective studies of cancer incidence.

In addition, if specific molecular signatures in ACF of malignant potential are discovered (for example, by gene expression profiling; [60]), then for at least those lesions in the distal colon/rectum that are examined, their removal could decrease future risk and result in cancer prevention.

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**Table 1**

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Epidemiological studies of colonic aberrant crypt foci.

| Study | n | Patients | Tissue Source | ACF Density or number | Subject sampling method |
|---|---|---|---|---|---|
| Roncucci et al., (8)<br>Toronto, Canada | 27 | 5 FAP<br>12 CRC<br>10 BD | Surgery | Mean no. ACF per $cm^2$<br>FAP = 19.9*<br>CRC = 0.37<br>BD = 0.18<br>** P <0.01 FAP versus each group* | Not described |
| Roncucci et al., (48)<br>Italy | 58 | All CRC:<br>32 high risk area<br>26 low risk area | Surgery | All patients = 0.103 per $cm^2$<br>Higher ACF density in high risk compared to low risk region (*P = 0.001*) | Not described |
| Takayama et al., (49)<br>Japan | 350 | 171 Normal<br>131 Adenoma<br>48 CRC<br>Plus, 20 on prospective Suldinac Study | Magnifying colonoscopy lower rectum<br>Full exam (n=3) | Median no. ACF<br>Normal = 1*<br>Adenoma = 5**<br>CRC = 26<br>** P < 0.001 versus adenoma*<br>***P < 0.001 versus CRC* | Patients were persons referred for colonoscopy at a hospital in Japan. The reasons for the referrals are not given. At exam, 49 had CRC, 142 had adenoma, and 179 had neither (called 'normal') |
| Shpitz et al., (50)<br>Tel Aviv, Israel | 93 | 76 CRC<br>17 BD | Surgery | Mean no. ACF per $cm^2$ Age group:*<br>< 50 + 0.076<br>51-60= 0.074<br>61-69 = 0.099<br>70+ = 0.120<br>** P = NS*<br>Significantly more ACFs in CRC than BD<br>CRC: More ACFs in distal versus proximal colon (*P=0.01*) | Not described |
| Bouzourene et al., (51)<br>Switzerland | 37 | 26 CRC<br>4 Adenoma<br>7 BD | Surgery | Median no. ACF per $cm^2$<br>CRC = 0.032<br>Adenoma = 0.011<br>BD = 0.130 | Not described |
| Nascimbeni et al., (52)<br>Italy | 103 | 76 CRC<br>27 diverticular disease (DD) | Surgery | Mean no. ACF per $cm^2$<br>CRC = 0.200*<br>DD = 0.070<br>** P <0.05* | Not described |
| Nascimbeni et al. (53)<br>Italy | 96 | 55 sigmoid cancer<br>41 diverticular disease | Surgery | Mean number ACF<br>CRC = 9.1<br>DD = 3.7<br>Mean crypt count not different | Not described |
| Adler et al., (54)<br>Minnesota, USA | 90 | 30 Normal<br>30 Adenoma<br>30 CRC<br>All with indication for colonoscopy | Magnifying colonoscopy distal 10 cm | Mean no. ACF per patient<br>Normal = 5.0<br>Adenoma = 6.9<br>CRC = 9.9 | Patients selected from the 'daily colonoscopy list with selection based on the indication for the procedure'. 30 were selected because they were known to have CRC and this was to be their preoperative exam; 30 were known to have adenoma; 30 were not known to have either of these, but were referred for exam due to a symptom.<br>Method of selection among these groups is not given. |
| Hurlstone et al., (55)<br>United Kingdom | 869 | 574 Normal<br>281 Adenoma<br>14 CRC<br>All with indication for colonoscopy | Magnifying colonoscopy distal 10 cm<br>Entire colon examined for CRC and adenomas | Median no. ACF per patient<br>Normal = 1<br>Adenoma = 9<br>CRC = 38 | Sampling frame was all 2,559 patients receiving a colonoscopy performed by one endoscopist between January, 2000 and January, 2004 from a single hospital in Sheffield, England. Reasons for exam referral are given. Exclusion criteria are stated, leaving 1,000 eligible. 869 of these were |

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Defined as percent of patients with at least 1 ACF.

Abbreviations:

FAP = Familial adenomatous polyposis

CRC= Colorectal cancer

BD= Benign disease

DD = Diverticular disease

NS = Non-signficant

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**Table 2**

Findings from the 'first-generation' epidemiological studies of aberrant crypt foci. Either number entire colon in those studies using resected surgical tissue, or number in distal rectum in those studies using colonoscopy.

| Study | n | Older age | Familiy history | NSAID | Cancer vs. adenoma, vs. normal | Distal vs. proximal | Tobacco |
|---|---|---|---|---|---|---|---|
| Roncucci et al., (8) Toronto, Canada | 27 | NR | 5 patients with FAP had highest ACF count | NR | Higher count in CRC patients than BD patients Much higher mean count in the 5 FAP patients ACF size smaller in FAP than CRC or BD | NR | NR |
| Roncucci et al., (48) Italy | 58 | Higher count in older patients | NR | NR | Only included CRC patients | Higher number of ACF in distal than proximal colon | NR |
| Takayama et al., (49) Japan | 350 | increased | NR | lower | ACF number associated with number of adenomas Substantially more in patients with CRC | NR | NR |
| Shpitz et al., (50) Tel Aviv, Israel | 93 | increased | NR | NR | Higher number in CRC patients than in BD patients | Higher number of ACF in distal than proximal colon Stronger association of ACF number in distal than proximal colon | NR |
| Bouzourene et al., (51) Switzerland | 37 | NR | NR | NR | Lower in CRC patients overall, but higher for dysplastic ACF | NR | NR |
| Nascimbeni et al., (52) Italy | 103 | NR | NR | NR | Higher in cancer than non-cancer | Higher ACF count in distal colon than proximal | NR |
| Nascimbeni et al. (53) Italy | 96 | NR | NR | NR | Higher in CRC than in DD | NR | NR |
| Adler et al., (54) Minnesota, USA | 90 | NR | NR | NR | Higher ACF count in patients with CRC than those with adenoma which is higher than the count in 'normal' patients | NR | NR |
| Hurlstone et al., (55) United Kingdom | 869 | NR | NR | NR | Mean in synchronous 'normal' = 1 adenoma = 9 cancer = 38 | NR | NR |
| Rudolph et al., (56) Seattle, USA | 32 | increased | NA Only 3 family history positive patients | No association | NR | NR | No association |
| Moxon et al., (57) Chicago, USA | 83 | increased | NA Only 4 with family history | No association | Higher in advanced neoplasia | NR | increased |
| Seike et al., (58) Chiba, Japan | 386 | NR | NR | NR | Increased for synchronous colon advanced neoplasia and for rectal cancer advanced neoplasia | Stronger for distal advanced neoplasia | NR |
| Stevens et al., (59) Connecticut, USA | 103 | Increased among those with no family history of CRC | Increased 43 with family history | NR | NR | NR | NR |

NR = not reported

NA = no association

BD = benign colonic disease

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**Table 3**

Summary of associations of number of ACF with demographic and clinical features of patients from the first generation epidemiological studies of ACF.

| Feature | Number of studies reporting | Number reporting no association | Number reporting association |
|---|---|---|---|
| Age | 6 | 0 | 6 |
| Family History | 3 | 2 (based on 3 and 4 subjects) | 1 (based on 43 subjects) |
| NSAIDS | 3 | 2 | 1 (inverse) |
| Synchronous neoplasia | 10 | 1 | 9 |
| Tobacco | 2 | 1 | 1 |
| Alcohol | 1 | 1 | 0 |