Natural history of human aberrant crypt foci and correlation with risk factors for colorectal cancer

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Abstract. Although aberrant crypt foci (ACF) are estimated to have potential usefulness as a biomarker for colorectal carcinoma (CRC), this remains uncertain because the natural history of ACF has not been well-clarified. To determine the usefulness of ACF as a surrogate marker for CRC, it is necessary to understand the natural history of ACF. A total of 82 subjects who underwent total colonoscopy and whose ACF number was examined at least 2 times at Yokohama City University Hospital were enrolled. We retrospectively evaluated the changes in the ACF number at four different surveillance periods (6 months, 1 year, 2 years, 3 years) and in groups with and without colorectal neoplasms. Furthermore, we classified the subjects into an increased ACF group and a no change/decreased ACF group, and investigated the relationship between the changes in the ACF number and known risk factors for CRC. No significant differences were observed in the ACF number between the first and second observations in any surveillance period groups, and in the groups classified according to the presence or absence of colorectal neoplasms. There were no significant differences between the increased and no change/decreased ACF group in terms of gender, smoking habit, current alcohol consumption, age, BMI, HbA1c or serum triglyceride level (TG), whereas a significant difference between the groups was observed in the serum total cholesterol level (TC) (P=0.012). ACF are a reliable surrogate marker that are not affected by any risk factors for adenomas or CRC, except TC, and may therefore be considered as a useful marker in chemopreventive trials.

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Introduction

Aberrant crypt foci (ACF), which are clusters of colorectal crypts with abnormal morphology, were initially identified as the earliest recognizable lesions on the colonic mucosa of rodents exposed to colorectal carcinogens (1-3). Numerous animal studies have demonstrated ACF as an important predictor of colorectal carcinoma (CRC) development. In humans, ACF were first identified in the colonic mucosa of surgical specimens, shortly after their description in animals. More recently, they were identified *in vivo* by high-magnification chromoendoscopy (HMCC). ACF have emerged over the last decade as putative precursors to colorectal adenomas, and therefore as a potential biomarker for CRC in humans.

Cross-sectional studies have shown a higher rate of ACF occurrence in patients with CRC and adenomas as compared with that in normal patients, which suggests the potential usefulness of ACF as a surrogate marker of CRC (4). However, there are many questions raised by recent studies, such as whether ACF progress spontaneously or not, and whether ACF growth might be correlated with known risk factors for CRC (1,5). There is only one literature report relating to the natural history of ACF, by Schoen *et al* in 2008 (2). This study examined the reproducibility of ACF detection over a 1-year period of observation, however, only 43% of ACF could be specifically re-identified, and more than 50% of the subjects had new ACF. The considerable dynamic to ACF detection in this study may be due to progression or regression of the ACF themselves, lack of adequate experience of the examiners in performing the examination for ACF, and inaccuracy in identifying the size and location of the ACF (2). Schoen *et al* also showed difficulty in re-identifying ACF at the 1-year examination (2). As a result, the natural history of ACF has not yet been well-clarified. Knowledge of the natural history of ACF is needed to understand their potential usefulness as a surrogate marker for CRC.

Furthermore, previous studies have shown the existence of a relationship between the risk factors for CRC and the size

and number of ACF (4,6-9), however, there are no studies showing the relationship between the known risk factors for CRC and the changes in the number of ACF over time. Very little is known about the factors that promote or inhibit ACF growth in humans.

In this study, we retrospectively examined the changes in the number of ACF over time and investigated their correlation with known risk factors for CRC in 82 subjects.

Materials and methods

Study population. Patients who underwent a total colonoscopy and examination of the ACF number at least 2 times at Yokohama City University Hospital from 2004 to 2010 were eligible to participate in this study. The following were the patient exclusion criteria; history of familial adenomatous polyposis, hereditary non-polyposis colorectal cancer, inflammatory bowel disease, invasive cancer, prior large-bowel resection except appendectomy, and regular aspirin or NSAID use. A total of 82 subjects were enrolled in this study. The first and the second examinations were performed by the same examiner after the different four surveillance periods (6 months, 1 year, 2 years, 3 years).

Data sampling. We collected data including the age, gender, comorbidities, past medical history, body mass index (BMI), daily alcohol consumption, and smoking habit from the past records. Subjects were also reviewed for information on the blood data, including HbA1c, serum total cholesterol levels (TC), and serum triglyceride levels (TG), which were calculated just before the first examination.

Endoscopy. All subjects were prepared for endoscopy with a polyethylene glycol-based solution. Total colonoscopy was performed before the examination for ACF. Data on the histology of the polyps removed or biopsied at colonoscopy or previously were obtained from the pathology records. A Fujinon EC-490ZW5/M colonoscope (Fujinon Toshiba ES System Co., Ltd., Tokyo, Japan) was used to conduct magnifying chromoendoscopy. Because of the difficulty in assessment of the number and size of ACF of the entire colon, we examined ACF only from the anal verge to the middle Houston valve. Methylene blue dye (0.2%) was administered to coat the mucosal surface, followed by adequate washing with warm tap water. After 2 min of staining the mucosa, the excess dye was removed carefully by washing with water, and then observation for ACF was commenced. We examined the size and number of the ACF, and investigated the changes in the number of ACF between the first and second observations.

Criteria used to identify and define ACF on endoscopy. ACF are clusters of crypts visualized after staining with methylene blue that appear 'different' from the surrounding mucosa. However, what characterizes them as different varies across the literature. Most commonly, ACF are identified as clusters of crypts that stain darker than the surrounding normal mucosa (4,7,11,18). However, larger crypt size (4,7), raised appearance (7,12,18), thicker epithelial lining (4), dilated or slit-like crypt lumen (12,19), and increased pericryptal area (19) as compared
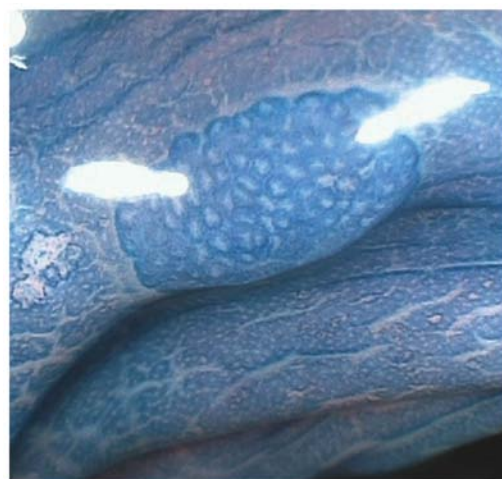

Figure 1. Endoscopic photograph of an ACF with the typical features; clusters of crypts that stain darker than the surrounding normal mucosa, larger crypt size, raised appearance, thicker epithelial lining, dilated or slit-like crypt lumen, and increased pericryptal area.

Table I. Number and mean age (years) of the subjects enrolled in this study.

	Total	Male	Female	P-value[a]
No. of subjects	82	53	29	
Mean age ± SD	63.6±11.1	63.6±10.4	63.5±12.6	0.83

[a]P-value calculated by Student's t-test.

with the surrounding normal mucosa are other frequently used criteria to identify ACF (5). We also used these characteristics as criteria for the diagnosis of ACF. An endoscopic photograph of an ACF with the typical features is shown in Fig. 1.

Statistical analysis. First, we used a paired t-test to evaluate the changes in the number of ACF in each surveillance period. At the same time, we assessed whether or not the present or past colorectal neoplasms were correlated with the change in the number of ACF.

Next, we classified the patients into 2 groups (increased ACF group and no change/decreased ACF group), and examined whether there were significant differences between these two groups in the known risk factors for CRC, such as gender, smoking habit and current alcohol consumption by using the χ^2 test, and the mean age, BMI, HbA1c, TC and TG by using the Student's t-test.

Results

Changes in ACF number in the four different surveillance periods and in the groups with and without colorectal neoplasms. A total of 82 subjects, 53 men and 29 women, aged 35 to 81 years, were enrolled in this study, as shown in Table I. At the first examination, the mean ± SD age of the participants was 63.6±11.1 years (male, 63.6±10.4, female, 63.5±12.6, P=0.83).

Table II. Changes in the numbers of aberrant crypti foci (ACF) in the four different surveillance periods and in the groups with and without colorectal neoplasms (mean ± SD).

| | N | No. of ACF | | P-value[a] |
		First observation	Second observation	
Classification according to the four different surveillance periods				
6 months	31	5.13±5.87	4.81±5.04	0.64
1 year	30	3.87±4.61	3.90±4.47	0.96
2 years	15	4.67±4.12	3.93±4.40	0.15
3 years	6	4.50±4.05	4.00±3.85	0.72
Total	82	4.53±4.97	4.26±4.58	0.46
Classification according to with and without colorectal neoplasms				
Normal group	36	3.68±3.11[b]	3.26±3.54	0.23
Adenoma group	36	3.89±4.03[b]	4.42±4.32	0.39
Carcinoma group	10	7.30±7.48[b]	6.00±7.19	0.35
Total	82	4.53±4.97	4.26±4.58	0.46

[a]P-value calculated by paired t-test comparing the numbers of ACF between the first and second observation. [b]Significant differences were observed between the normal and carcinoma group (P=0.02), and the adenoma and carcinoma group (P=0.03), which were calculated by Student's t-test.

Table III. Observation period, age and gender of the 3 groups classified according to with and without colorectal neoplasms (mean ± SD).

	Total	Normal	Adenoma	Carcinoma
Observation period (years)[a]	1.1±0.6	1.4±0.9	1.0±0.6	0.8±0.2
Age (years)[b]	63.6±11.1	63.8±12.7	65.0±9.7	58.9±8.3
Gender (Male/female)	82 (53/29)	36 (23/13)	36 (24/12)	10 (6/4)

[a]P=0.06 and [b]P=0.29, calculated by Kruskal-Wallis test.

Table II summarizes the changes in the number of ACF in the four different surveillance periods and in the groups with and without colorectal neoplasms. The numbers of ACF at the first and second observations were 5.13±5.87/4.81±5.04 (P=0.64) in the 6 months group, 3.87±4.61/3.90±4.47 (P=0.96) in the 1-year group, 4.67±4.12/3.93±4.40 (P=0.15) in the 2-years group, and 4.50±4.05/4.00±3.85 (P=0.72) in the 3-years group. In total, there was an average of 4.53±4.97/4.26±4.58 (P=0.46) ACF present at the first and second observations. There were no significant differences between the first and second observations in each group or overall, as mentioned above, but this grouping did not take into account the potential variation related to the presence of colonic neoplasms. Therefore, we classified the patients into three groups according to whether or not they had present or past colorectal neoplasms (normal group, adenoma group and carcinoma group), and evaluated the changes in the number of ACF in each group. There was a mean of 3.68±3.11/3.26±3.54 (P=0.23) ACF observed at the first and second observations in the normal group, 3.89±4.03/4.42±4.32 (P=0.39) in the adenoma group, and 7.30±7.48/6.00±7.19 (P=0.35) in the carcinoma group. Thus, no significant changes in the number of ACF between the first

and second observations were observed in any group, even the groups classified according to the presence or absence of colorectal neoplasms. On the other hand, there were significantly increased ACF observed at the first observation in the carcinoma group compared with those in the normal group, and those in the adenoma group (P=0.02, P=0.03 respectively). Although there were no statistically significant differences, more ACF were observed at the second observation in the carcinoma group than in the normal group and adenoma group.

Table III summarizes the mean observation period, age and gender of the 3 groups classified according to with and without colorectal neoplasms. The mean observation periods were 1.4±0.9 years in the normal group, 1.0±0.6 years in the adenoma group, and 0.8±0.2 years in the carcinoma group, in which no statistically significant differences were observed (P=0.06). In addition, there were also no statistically significant differences observed in the mean age of these 3 groups (P=0.29).

Relationship between the changes in the number of ACF and known risk factors for CRC. Finally, we analyzed whether or not each known risk factor for CRC was correlated with

Table IV. Relationship between the changes in the number of ACF number and known risk factors for CRC.

| | No. of ACF | | | |
	Total	Increase	No change/decrease	P-value
No. of subjects	82	28	54	
Gender (Male/female)	53/29	15/13	38/16	0.13[a]
Smoking, n (%)				0.44[a]
Yes	37 (45)	11 (39)	26 (48)	
No	45 (55)	17 (61)	28 (52)	
Alcohol intake, n (%)				0.09[a]
Yes	43 (52)	11 (39)	32 (59)	
No	39 (48)	17 (61)	22 (41)	
Mean age ± SD (years)	63.6±11.1	61.6±9.2	65.6±10.2	0.09[b]
BMI ± SD (kg/m^2)	22.9±3.1	22.9±2.7	22.9±3.2	0.97[b]
HbA1c ± SD (%)	5.66±1.03	5.63±0.95	5.67±1.07	0.89[b]
TC ± SD (mg/dl)	211.6±34.1	225.5±34.4	204.5±32.7	0.012[b, c]
TG ± SD (mg/dl)	146.8±71.1	162.0±90.7	138.9±57.9	0.19[b]

[a]P-value calculated by χ^2 test; [b]P-value calculated by Student's t-test; [c]Significant differences were observed between the increased ACF group and the no change/decreased ACF in TC. BMI, body mass index; HbA1c, hemoglobin A1c; TC, serum total cholesterol; TG, serum triglyceride level.

the changes in the number of ACF (shown in Table IV). We divided the subjects into the increased ACF group and the no change/decreased ACF group so as to clarify what is associated with ACF increase. Subjects in whom the number of ACF had increased at least by one between the first and second observations were classified as the increased ACF group, and those in whom the number of ACF had decreased or not changed between the observations were classified as no change/decreased ACF group. There were 28 subjects in the increased ACF group, and 54 subjects in the no change/decreased ACF group. The proportion of the subjects who smoked habitually was 39% in the former group, and 48% in the latter group. Similarly, the proportion of those who were current alcohol consumers was 39% in the former group and 59% in the latter group. No significant differences were seen between the two groups in terms of gender, smoking habit, or daily alcohol intake. The other factors were as follows: mean age ; 61.6±9.2 years old vs. 65.6±10.2 years old, BMI; 22.9±2.7 kg/m^2 vs. 22.9±3.2 kg/m^2, HbA1c; 5.63±0.95% vs. 5.67±1.07%, TC; 225.5±34.4 mg/dl vs. 204.5±32.7 mg/dl, TG; 162.0±90.7 mg/dl vs. 138.9±57.9 mg/dl. There were also no significant differences between the groups in the mean age, BMI, HbA1c or TG, except TC (P=0.012). In summary, there were no significant differences between the increased ACF group and the no change/decreased ACF in terms of gender, smoking habit, daily alcohol intake, age, BMI, HbA1c or TG, whereas a significant difference in the TC was observed between the two groups.

Discussion

CRC is a major neoplasm worldwide, and its prevalence and mortality have been increasing (15,16). In particular, CRC is one of the most common causes of cancer-related death in developed countries (17). A paradigm shift from surveillance for early detection of cancer or adenomas to new preventive strategies, including chemoprevention, is needed to lower the burden of this disease (14). Several studies using HMCC for rectal ACF detection have reported a stepwise increase in the prevalence and frequency of rectal ACF in patients with a normal colon, adenoma and carcinoma (5-7,10-13). Because of the epidemiologic and genetic association of ACF with colorectal neoplasms, ACF may be a potential biomarker for CRC (2). For example, ACF could be used as endpoints in studies comparing individuals with and without risk factors for CRC and in studies evaluating chemopreventive agents. Because of the high prevalence of the ACF, studies using ACF as an endpoint can have a smaller sample size, and the dose-response relationship could be potentially assessed using the effect on the number of ACF (1). Recently, there have been several chemoprevention trials using ACF as a surrogate marker for CRC. Metformin has been reported to be an antidiabetes drug with a chemopreventive effect against CRC, not only in rodents but also in humans, by using ACF as a surrogate marker for CRC (14,21).

If the natural variation of ACF is substantial, a chemoprevention agent must produce significantly greater regression of ACF than that which could be explained as a natural variation (2). However, there is only one previous report that has shown the natural changes in the number of ACF over time. Further knowledge of the natural history of ACF is necessary to understand their potential usefulness as a surrogate marker for CRC.

Our investigation of the natural variability of ACF in the 82 subjects showed that the number of ACF did not change significantly over time. When the subjects were divided

according to four different surveillance periods from 6 months to 3 years, there were no significant differences between the first and second observation in any group. However, classification only according to the surveillance period is not sufficient to evaluate the natural variations of ACF, because having past or synchronous colorectal neoplasms itself may influence the fluctuation in the number of ACF over time. Takayama *et al* reported that both the number and percent of dysplastic ACF increased as one progressed from individuals with normal colons to those with adenomatous polyps and to those with CRC (4). Moreover, another report showed that persons with adenomas have a somewhat larger number of rectal ACF than those without, and that older age is a risk factor for ACF (7). From epidemiological studies so far, it has been established beyond doubt that persons with adenomas or carcinomas are likely to have more rectal ACF than those without. In order to rule out the variations of ACF which may be caused by the presence of colorectal neoplasms, we classified all the subjects into three groups according to whether or not they had present or past colorectal neoplasms (normal group, adenoma group, carcinoma group). Significantly increased ACF were observed at the baseline examination in the carcinoma group compared with those in the normal group, and those in the adenoma group, which is consistent with previous studies. On the other hand, no significant changes in the number of ACF over time were observed regardless of the presence or absence of colorectal neoplasms. A much longer observation period may lead to significant changes in the number of ACF in the carcinoma group, however in this study, no significant changes in the number of ACF were observed in each group at least during the observation period from 6 months to 3 years (mean, 1.1 years).

On the other hand, the factors that promote or inhibit ACF growth remain largely unknown in humans (7). However, it is predictable that some factors associated with the risk of colorectal adenomas and CRC may also affect the ACF growth because patients with colorectal neoplasms tend to have more ACF than those without. The metabolic syndrome (MS), which is an important risk factor for cardiovascular disease and contributes to morbidity and mortality through its main components, including diabetes mellitus, obesity, dyslipidemia and hypertension, has been linked to some malignancies, including CRC. MS is associated with an increased risk of colorectal adenoma, and this risk increases with the number of MS components (20). In addition, Takahashi *et al* demonstrated that obesity, particularly visceral fat accumulation, aging and a low plasma adiponectin level could correlate with colorectal carcinogenesis (22). In our study, we divided the subjects into the increased ACF group and the no change/decreased ACF group, and investigated the correlation between the changes in the number of ACF and known risk factors for CRC, in order to elucidate the factors associated with ACF increase. As a result, TC was significantly increased in the increased ACF group as compared with that in the no change/decreased ACF group (P=0.012). Our results showed that an MS component, but not all components, was associated with ACF increase. In other words, the factors that promoted ACF growth were correlated with the risk factors for colorectal adenomas. In addition, our results also suggest that elevated TC is possible to increase the number of ACF in a relatively short period of

1.1 years average. Although the biological significance of ACF remains controversial, it is certain from previous studies that more ACF are present in patients with colorectal neoplasms than in those without (4,7,23). Our results suggest that the prevention of hypercholesterolemia has the potential to reduce ACF formation, and may lead to reduction of colorectal neoplasms.

In conclusion, the novelty of our study lies in the findings that the number of ACF does not change significantly for at least 3 years (mean 1.1 years) regardless of the presence or absence of colorectal neoplasms, and that TC is the only factor associated with increment in the number of ACF over time. This suggests that ACF might be a reliable surrogate marker for CRC that is not affected by any risk factors for adenomas or CRC except TC, and could therefore be a useful marker in chemopreventive trials. At the same time, the prevention of hypercholesterolemia has the potential to reduce ACF formation and may lead to reduction of colorectal neoplasms.

Acknowledgements

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