Investigation of the prevalence and number of aberrant crypt foci associated with human colorectal neoplasm

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ACF and colorectal carcinogenesis

Key words:

aberrant crypt foci (ACF), colorectal carcinogenesis, high-magnification chromoscopic colonoscopy (HMCC)
Abstract

Background:

Aberrant crypt foci (ACF) are considered to be useful as surrogate biomarker for colorectal cancer (CRC), but the biological significance of ACF remains controversial. We attempted to investigate the relationship between the presence of ACF and human colorectal carcinogenesis using a relatively large sample size.

Methods:

We performed high-magnification chromoscopic colonoscopy to identify ACF in 861 subjects undergoing a diagnostic endoscopy at the Yokohama City University Hospital. The present study compared the prevalence and number of ACF in three subject groups (normal subjects, adenoma cases, and CRC cases). The correlations between the demographic and behavioral characteristics of the subjects and the prevalence of ACF were also assessed.

Results:

The prevalence of ACF was 64%, 88% and 95%, and the mean number of ACF was 3.6, 6.2 and 10.1, in normal subjects, adenoma cases, and CRC cases, respectively. When differences in the prevalence and number of ACF among age- and sex-stratified subject groups were examined, significant stepwise increments from normal subjects to
adenoma cases to CRC cases were apparent ($P<0.001$). Moreover, an age- and sex-adjusted multiple logistic regression analysis revealed that smoking and alcohol habits had a synergistic effect, increasing the prevalence of ACF as well as the risk of CRC ($P<0.001$).

Conclusions:

These results suggested that ACF may serve as a reliable surrogate biomarker for human colorectal carcinogenesis.

Impact:

The use of ACF as an endpoint may enable the size, duration, and cost of CRC chemoprevention studies to be reduced.

(Words 236)
Introduction

Despite recent advances in therapeutic modalities, colorectal cancer (CRC) remains one of the most common causes of cancer-related death in developed countries (1). Currently, chemoprevention for CRC has attracted much attention. The purpose of chemoprevention is to reduce the future mortality of CRC using oral agents that can prevent the occurrence of cancer. Although the occurrence of CRC is the most reliable endpoint, such an endpoint is unsuitable for chemoprevention trials because the occurrence of CRC in the general population is relatively infrequent (1) and such trials would require long-term observation periods. Therefore, to evaluate the efficacy of chemopreventive agents in CRC chemoprevention trials, a more common surrogate biomarker that is robustly associated with CRC is required.

Colorectal carcinogenesis is based on the adenoma-carcinoma sequence, wherein adenomas, spurred by acquired genetic mutations, evolve into CRC. Adenomas have been established as premalignant lesions and are characterized by the presence of genetic and histological changes. Endoscopic screening and the removal of adenomas can reduce the incidence of CRC by as much as 90% (2, 3). Despite retrospective and prospective studies supporting the use of adenomas as a surrogate biomarker of CRC in chemoprevention trials (4), the use of adenomas as a surrogate endpoint biomarker for...
CRC has some limitations. The most obvious limitation is that using adenoma formation as an efficacy endpoint requires hundreds of subjects and a very long observation period. Furthermore, to assess the effects of chemopreventive agents, the regression or loss of adenomas must be evaluated (5); therefore, a total colonoscopy is necessary. Unfortunately, these limitations result in poor compliance and a high frequency of dropouts over time, preventing a reasonable rate of progress for clinical research on CRC prevention. Moreover, large adenomas possibly contain cancer cells; therefore, the assessment of chemopreventive efficacy in patients with large adenomas would involve ethical problems. To overcome these problems, a more useful surrogate biomarker that is reliably correlated with the clinical response, that can be modulated by chemopreventive agents or behavioral characteristics (such as diet changes and smoking cessation) within a short period of time, and that is relatively simple to measure is needed.

Aberrant crypt foci (ACF) were discovered as the earliest microscopic lesions to appear in the colonic mucosa of mice treated with azoxymethane (6). Many studies have shown a dose-response relationship between carcinogens, such as azoxymethane and dimethylhydrazine, and the number of ACF induced (7-11). Moreover, in recent studies, numerous chemopreventive agents have been shown to reduce the number of ACF in
animal models of chemical colonic carcinogenesis. Importantly, many agents that block
ACF growth were also shown to prevent tumor development in these carcinogen-treated
rodent models (11). Thus, in rodent models, ACF have been established as a precursor
of CRC. Shortly after such descriptions in rodent models were made, ACF were
discovered in pathologic specimens of human colonic mucosa (12-14). ACF were
subsequently identified in the colonic mucosa in vivo using high-magnification
chromoscopic colonoscopy (HMCC) with methylene blue staining (15). Although
several previous epidemiological studies have revealed significant associations between
the prevalence and/or number of ACF and the synchronous presence of advanced
neoplasms, including both adenoma and CRC (15-22), most of the sample sizes in these
studies were relatively small. Consequently, the findings were somewhat conflicting. In
addition, these studies had limited data regarding other personal characteristics, such as
smoking habit, alcohol habit, and obesity—all of which are related to an increased risk
of CRC. If ACF are indeed a surrogate biomarker for CRC, the epidemiology of ACF
would likely be similar to that of CRC. Therefore, we attempted to investigate the
relationship between the presence of ACF and colorectal carcinogenesis using a larger
sample size. Here, we compared the prevalence and number of ACF in three subject
groups (normal subjects, adenoma cases, and CRC cases). Moreover, we evaluated the
association between the presence of ACF and the adenoma history. The correlations
between the demographic and behavioral characteristics in relation to colorectal
carcinogenesis and the prevalence and number of ACF were also assessed. Our results
may help to further evaluations of the potential utility of ACF as a surrogate biomarker
for CRC.

Materials and Methods

Subjects

The study protocol was approved by the Yokohama City University Hospital Ethics
Committee. Between 2004 and 2009, we enrolled 861 subjects who underwent
diagnostic endoscopy at the Yokohama City University Hospital, Japan: of the 861
subjects, 383 had no apparent lesions of the colorectum on colonoscopy (normal
subjects), 372 had colorectal adenoma(s), and 106 had CRC. Subjects were excluded if
they had undergone previous surgical or endoscopic excision of colonic adenomas
and/or cancer, or if they had familial adenomatous polyposis, inflammatory bowel
disease, or radiation colitis. Written informed consent was obtained from all the subjects
prior to their participation in the study. Data on the demographic and behavioral
characteristics of the subjects pertaining to the risk of the development of CRC,
including smoking habit, alcohol habit and body mass index (BMI), were obtained from
the subjects prior to the performance of the colonoscopy.

**High-magnification chromoscopic colonoscopy**

A Fujinon EC-490ZW5/M colonoscope was used for the magnifying colonoscopy
(Fujinon Toshiba ES Systems Co., Ltd, Tokyo, Japan). All the subjects were subjected to
bowel preparation using a polyethylene glycol-based solution, and underwent a total
colonoscopy prior to rectal ACF imaging. Any detected adenomas were biopsied and the
histopathological appearance was analyzed. Advanced adenoma was defined as an
adenoma lesion measuring 1 cm or greater in diameter and/or exhibiting a villous
histology and/or high-grade dysplasia. Subsequently, 0.25% methylene blue was applied
to the mucosa using a spray catheter. Based on the results of a previous study, the ACF
were counted in the lower rectal region, from the middle Houston valve to the dentate
line (15). To guard against double counting, the ACF were counted in a sequential
fashion during a single withdrawal of the endoscope. We evaluated the presence of ACF
and the category of the subject (normal subjects, adenoma cases, and CRC cases)
simultaneously.

**Criteria used for the endoscopic diagnosis**

ACF were defined as lesions in which the crypts were larger in diameter and showed a
darker staining with methylene blue than normal crypts, often with oval or slit-like lumens and a thicker epithelial lining (15) (Figure 1).

**Statistical analysis**

Data were expressed as the mean ± SD for continuous variables and as a proportion (%) for categorical variables. The prevalences of ACF among the normal subjects, adenoma cases, and CRC cases were compared using age- and sex-adjusted logistic regression analyses. The numbers of ACF among these three groups were also compared using the Kruskal Wallis test or an age- and sex-adjusted linear regression analysis. The chi-squared test and the Mann-Whitney U test were used to investigate the association between the presence of ACF and the adenoma status as well as the association between the presence of ACF and the location of adenoma(s)/CRC. In addition, univariate and multivariate logistic regression analyses were used to identify variables with significant independent effects on the prevalence of ACF among normal subjects. Univariate and multivariate linear regression analyses were also performed to identify significant variables influencing the number of ACF. The variables entered in the model included age, sex, smoking habit, alcohol habit and BMI. Unless otherwise specified, a $P$ value < 0.05 was considered statistically significant. All the analyses were performed using the SPSS statistical package (version 11.0 for Mac OS X).
Results

Characteristics of the subjects

The characteristics of the subjects according to study group (normal subjects, adenoma cases, and CRC cases) are shown in Table 1. The subjects ranged in age from 19-89 years (62.2 ± 12.4): normal subjects, 19-85 years (59.3 ± 13.8); adenoma cases, 31-88 years (64.2 ± 10.9); and CRC cases, 34-89 years (65.6 ± 9.4). A total of 4737 ACF were visualized endoscopically in 861 subjects: 1382 in normal subjects, 2288 in the adenoma cases, and 1072 in the CRC cases. The prevalence of ACF was 64%, 88% and 95% for the normal subjects, adenoma cases, and CRC cases, respectively. The mean number of ACF was 3.6 ± 5.2, 6.2 ± 7.0 and 10.1 ± 7.9 in the normal subjects, adenoma cases, and CRC cases, respectively.

Prevalence and number of ACF in the three subject groups stratified according to age and sex

The prevalence and number of ACF in the three subject groups according to age and sex are shown in Table 2. The prevalence of ACF was as high as 51-74% even in normal subjects. The prevalence of ACF in the CRC cases was as high as 91-100%, while that in the adenoma cases was intermediate. An age-adjusted logistic regression analysis for
the three subject groups stratified according to sex showed that the differences of the
prevalence of ACF among the three subject groups (normal subjects, adenoma cases,
and CRC cases) were significant ($P<0.001$ and $P<0.001$ for men and women,
respectively). In addition, an age-adjusted linear regression analysis for the three subject
groups stratified according to sex showed that the differences of the number of ACF
among the three subject groups (normal subjects, adenoma cases, and CRC cases) were
significant ($P<0.001$ and $P<0.001$ for men and women, respectively).

**Differences in the presence of ACF between subjects with non-advanced and
advanced adenomas**

The relationship between the presence of ACF and the adenoma history is shown in
Table 3. The prevalence of ACF in subjects with advanced adenoma(s) did not differ
significantly from that of subjects with non-advanced adenoma(s) (89% and 87%,
respectively; $P=0.41$). However, the number of ACF in subjects with advanced
adenoma(s) was larger than that in subjects with non-advanced adenoma(s) (7.8 ± 8.2
and 5.1 ± 6.0, respectively; $P<0.005$).

**Relationship between the presence of ACF and the location of adenoma/CRC**

The relationship between the presence of ACF and the location of adenoma/CRC is
shown in Table 4. Sixty-eight of the 372 adenoma cases (18%) had adenoma(s) only in
the proximal colon. No significant differences were observed between the prevalence and number of ACF and the location of the adenoma(s) \((P=0.86 \text{ and } P=0.73, \text{ respectively})\). Twenty-nine of the 102 CRC cases (28%) had proximal CRC. No significant differences were observed between the prevalence and number of ACF and the location of the CRC \((P=0.52 \text{ and } P=0.26, \text{ respectively})\).

Correlations between the presence of ACF and demographic and behavioral characteristics pertaining to the risk of colorectal carcinogenesis

To investigate the risk factors for the prevalence of ACF, univariate and multivariate logistic regression analyses were performed in normal subjects (Table 5). We defined smoking habit as positive for subjects with more than 10-pack years who were still smoking or who had quit within the past 10 years; alcohol habit was defined as positive for subjects with alcohol consumption in excess of 45 g/day. Both of these factors are reported to associate with an increased risk of adenoma and CRC (23-28). Age- and sex-adjusted multivariate analyses revealed that smoking habit (odds ratio [OR], 1.6; 95% confidence interval [CI], 0.9-3.1) and alcohol habit (OR, 2.0; 95% CI, 0.8-5.0) were not independent risk factor for the prevalence of ACF \((P=0.12 \text{ and } P=0.15, \text{ respectively})\); however these two factors (OR, 5.4; 95% CI, 2.3-13.0) synergistically increased the prevalence of ACF \((P<0.001)\). Obesity was also reported to associate with
an increased risk of adenoma and CRC (28). We defined obesity as positive for subjects with a BMI $\geq 25$. Obesity (OR, 1.5; 95% CI, 0.8-2.6) was also not an independent risk factor for the prevalence of ACF ($P=0.17$). We also performed univariate and age- and sex-adjusted multivariate linear regression analyses to evaluate the correlations between the number of ACF and these factors. Smoking and alcohol habits also synergistically increased the number of ACF, but this trend was only borderline significant ($P=0.06$) (Supplementary Table 1).

**Discussion**

In our study, significant stepwise increments in both the prevalence and the number of ACF were observed from normal subjects to adenoma cases to CRC cases. In addition, the mean number of ACF was significantly higher in the subject group with advanced adenoma than in the subject group with non-advanced adenoma. These results indicate that ACF may serve as a reliable surrogate biomarker of human colorectal carcinogenesis.

Most previous studies (15-18, 20-22) have evaluated ACF in the lower rectal region because HMCC is technically easier to perform at this location, is suitable for use as a follow-up examination and is well tolerated by patients. Therefore, we evaluated the
ACF in the lower rectal region, similar to previous studies. To evaluate whether the rectal ACF reflects the total colonic adenoma/CRC, we examined associations between the presence of rectal ACF and the locations of the adenoma/CRC. In our study, no significant differences were observed between the prevalence and the number of ACF in subjects who had only proximal colonic adenoma/CRC and subjects who had at least one distal colonic adenoma/CRC. This result indicates that rectal ACF examinations may be useful as a biomarker not only for distal colonic neoplasia, but also for proximal colonic neoplasia.

The development of CRC is influenced by several acquired risk factors, including dietary factors and lifestyle factors. If ACF are indeed a surrogate biomarker of CRC, then their epidemiology is likely to be similar to that of CRC. If risk factors influence colorectal carcinogenesis at an early stage, then they may also be associated with the formation of ACF. Therefore, we evaluated whether risk factors which associate with the development of CRC were independently associated with the presence of ACF in normal subjects. In our study, smoking habit and alcohol habit synergistically increased the prevalence of ACF in a significant manner. Interestingly, recent studies have revealed that cigarette smoking and heavy alcohol intake also interact in an additive manner, increasing the risk of CRC, similar to results seen in the aerodigestive tract.
Tobacco contains a large number of carcinogens that may bind to DNA and form adducts, potentially causing irreversible genetic damage to the normal colonic mucosa (31). On the other hand, alcohol is metabolized to acetaldehyde, which binds to DNA and forms carcinogenic adducts (32). Therefore these two factors may share a common pathway in promoting colorectal carcinogenesis at an early stage and initiating ACF formation. On the other hand, obesity was not strongly associated with the prevalence of ACF because only a few patients were regarded as obese in our study. In contrast to our hypothesis, no significant associations were observed between the number of ACF and these factors, although smoking and alcohol habits tended to increase the number of ACF in a synergistic manner. A not insignificant number of subjects exhibited an extremely high density of ACF (as high as 30), even in normal subjects; therefore, the wide variance in the number of ACF might have extinguished the statistical significance (Supplementary Figure 1).

Although most previous epidemiological studies of ACF have shown a significant correlation between the presence of ACF and synchronous advanced neoplasia (15-22), one recent multicenter study raised serious questions about whether ACF can be used as a surrogate biomarker for CRC (33). However, their subject groups were determined 8 years, on average, prior to the actual ACF examination. In addition, they determined the...
subject group based on the results of flexible sigmoidoscopy; thus, proximal adenomas may have been missed. These facts suggest that their control group may have contained a not insignificant number of subjects with adenoma. Therefore, their study may not actually show an association between the presence of ACF and the adenoma status. However, such considerations are inadequate to explain this discrepancy. Differences in participant characteristics, such as race, age and behavioral factors, may be associated with this discrepancy. Variations in the criteria used to detect ACF and the method used to visualize ACF may also affect this discrepancy. A large prospective and cross-sectional study would be useful for resolving this discrepancy.

Recently, several prospective studies have been conducted using the presence of ACF as a surrogate biomarker for CRC in chemoprevention trials in humans (34-36). ACF are considered to be a heterogeneous group of lesions, some, but not all, of which may be robustly associated with the risk of CRC, since the prevalence of ACF was as high as 70% even in normal subjects. Interestingly, our results suggested that even if a very small subset or none of the ACF may progress to CRC, ACF may still be useful as a surrogate biomarker for CRC. In humans, Shpitz et al. showed that the PCNA labeling indices for ACF were significantly higher than those for normal mucosa (14). In addition, we previously demonstrated that metformin, which inhibits the mTOR
pathway through the activation of AMPK, suppresses cellular proliferation and ACF formation (35). These results suggested that ACF may be a marker for epithelial proliferation. Importantly, previous studies have demonstrated that a high proliferative activity in the colon mucosa is associated with an increased risk of CRC (37).

In conclusion, we confirmed that the prevalence and mean number of ACF significantly increased with the stage of the adenoma-carcinoma sequence using age- and sex-adjusted analyses of a relatively large sample. We also showed that smoking and alcohol habits synergistically increased the prevalence of ACF as well as the risk of CRC. These results suggested that ACF may be useful as a reliable surrogate biomarker for human colorectal carcinogenesis.

Disclosure of potential conflict of interest

The authors have no potential conflicts of interest to disclose.

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A. N.
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Metformin suppresses colorectal aberrant crypt foci in a short-term clinical trial. Cancer

Randomized double blind trial of sulindac and etodolac to eradicate aberrant crypt foci
Figure legend

Figure 1.

Typical endoscopic appearance of human ACF. This photograph was obtained using a Fujinon EC-490ZW5/M colonoscope after the rectal mucosa had been stained with 0.2% methylene blue.
Table 1. Characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects</th>
<th>Adenoma cases</th>
<th>CRC cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>383</td>
<td>372</td>
<td>106</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>59.3±13.8</td>
<td>64.2±10.9</td>
<td>65.6±9.4</td>
</tr>
<tr>
<td>Median</td>
<td>62</td>
<td>64.5</td>
<td>66</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>211/172</td>
<td>265/107</td>
<td>73/33</td>
</tr>
<tr>
<td>Number of subjects with</td>
<td>246</td>
<td>326</td>
<td>101</td>
</tr>
<tr>
<td>ACF prevalence (%)</td>
<td>64</td>
<td>88</td>
<td>95</td>
</tr>
<tr>
<td>Total number of ACF</td>
<td>1382</td>
<td>2288</td>
<td>1072</td>
</tr>
<tr>
<td>ACF number (Mean±SD)</td>
<td>3.6±5.2</td>
<td>6.2±7.0</td>
<td>10.1±7.9</td>
</tr>
</tbody>
</table>

NOTE: Normal subjects were defined as subjects with no apparent lesions of the colorectum on total colonoscopy.
Table 2. Prevalence and number of ACF among the three subject groups stratified according to age and sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>&lt;60</td>
<td>60-69</td>
</tr>
<tr>
<td>ACF prevalence*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of subjects</td>
<td>89</td>
<td>62</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td>62</td>
<td>74</td>
</tr>
<tr>
<td>Adenoma cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of subjects</td>
<td>79</td>
<td>92</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td>89</td>
<td>88</td>
</tr>
<tr>
<td>CRC cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of subjects</td>
<td>15</td>
<td>34</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td>93</td>
<td>94</td>
</tr>
<tr>
<td>ACF number (Mean±SD)†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal subjects</td>
<td>2.6±3.2</td>
<td>5.3±7.1</td>
</tr>
<tr>
<td>Adenoma cases</td>
<td>5.0±5.5</td>
<td>6.8±7.9</td>
</tr>
<tr>
<td>CRC cases</td>
<td>8.7±8.4</td>
<td>10.5±7.4</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* P<0.001 and P<0.001 (men and women, respectively), calculated using an age-adjusted logistic regression analysis for the three subject groups stratified according to sex.
† Differences among the age-stratified subject groups (<60, 60-69, and ≥70 years) were analyzed using the Kruskal Wallis test.
‡ An age-adjusted linear regression analysis was performed to evaluate the differences among the three subject groups stratified according to sex.
Table 3. Differences in the presence of ACF between subjects with non-advanced and advanced adenoma(s)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>ACF prevalence (%)</th>
<th>ACF number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-advanced</td>
<td>230</td>
<td>87</td>
<td>5.1±6.0</td>
</tr>
<tr>
<td>Advanced adenoma</td>
<td>142</td>
<td>89</td>
<td>7.8±8.2</td>
</tr>
<tr>
<td>P value</td>
<td>0.41</td>
<td>&lt;0.005</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Advanced adenoma was defined as an adenoma lesion measuring 1 cm or greater in size and/or exhibiting a villous histology and/or high-grade dysplasia.

*P values were calculated using the chi-squared test.
†P values were calculated using the Mann-Whitney U test.
**Table 4.** Relationship between the presence of ACF and the location of adenoma/CRC.

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>ACF Prevalence (%)</th>
<th>ACF number (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoma cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>including distal colon</td>
<td>304</td>
<td>88</td>
<td>6.1±6.9</td>
</tr>
<tr>
<td>only in proximal colon</td>
<td>68</td>
<td>88</td>
<td>6.4±7.6</td>
</tr>
<tr>
<td>P value</td>
<td>0.87</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>CRC cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>distal colon</td>
<td>77</td>
<td>96</td>
<td>10.4±7.6</td>
</tr>
<tr>
<td>proximal colon</td>
<td>29</td>
<td>93</td>
<td>9.3±8.6</td>
</tr>
<tr>
<td>P value</td>
<td>0.52</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: The distal colon was defined as the region colonic lesion from the splenic flexure to the dentate line. The proximal colon was defined as the region colonic lesion.

*P values were calculated using the chi-squared test.
†P values were calculated using the Mann-Whitney U test.
### Table 5. Age- and sex-adjusted multiple logistic regression analysis of behavioral characteristics and the prevalence of ACF in normal subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Proportion (%)</th>
<th>ACF Prevalence</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Univariate</td>
</tr>
<tr>
<td>Smoking (-), Alcohol (-)</td>
<td>63</td>
<td>58</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>Smoking (+), Alcohol</td>
<td>16</td>
<td>67</td>
<td>1.5 (0.8-2.7)</td>
</tr>
<tr>
<td>Smoking (-), Alcohol</td>
<td>7</td>
<td>73</td>
<td>2.0 (0.8-4.9)</td>
</tr>
<tr>
<td>Smoking (+), Alcohol</td>
<td>14</td>
<td>87</td>
<td>4.8 (2.1-11.1)</td>
</tr>
</tbody>
</table>

NOTE: Smoking habit was defined as positive if the subject had more than 10-pack years and was still smoking or had quit within the past 10 years. Alcohol habit was defined as positive if the subject’s alcohol consumption exceeded 45 g/day. The multivariate logistic regression analysis was adjusted for age and sex. OR, odds ratio. CI, confidence interval.
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