now increasingly recognized.

Innovations and breakthroughs

The authors show, for the first time, that the serum levels of tumor necrosis factor-receptor 1 (TNF-R1) may have high diagnostic sensitivity and specificity as a biomarker of colorectal adenoma. TNF-R1 was hardly detected in the normal colorectal mucosa, whereas adenomas showed high expression levels of TNF-R1. In addition, co-expression of phospho-c-Jun N-terminal kinase (p-JNK) with TNF-R1 was observed in adenomas. These results suggest that the TNF-R1/JNK pathway may play an important role in the development/progression of colorectal adenoma.

Applications

TNF-R1 may be a promising biomarker of colorectal adenoma, and further studies may show that TNF-R1 expression can be used to screen for adenomas in patients as an alternative or in addition to hemoccult screening or colonoscopy screening.

Terminology

TNF-R1 belongs to the TNF receptor superfamily. In response to TNF treatment, activation of the transcription factor nuclear factor-κ B and mitogen-activated protein kinase, as well as Extracellular Signal-regulated Kinase, p38, and JNK, has been reported in most types of cells and, in some cases, apoptosis or necrosis was also induced.

Peer review

This is an interesting study investigating the importance of TNF-R1/JNK co-expression in colorectal adenoma. The major finding of the study was that serum levels of TNF-R1 were higher in patients with colorectal adenomas, while immunohistochemistry showed high expression of both TNF-R1 and p-JNK in the adenomatous tissues.

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IL-6 Plays Crucial Roles in Sporadic Colorectal Cancer through the Cytokine Networks including CXCL7

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ABSTRACT

IL-6 is a multifunctional cytokine and involved in variety of carcinogenesis. However, the association between IL-6 and sporadic colorectal cancer has not been fully explained. Here, we investigated the role of IL-6 signaling and the cytokine network in sporadic colorectal cancer. We investigated the serum IL-6 levels in patients with sporadic colorectal adenoma, cancer patients, and normal controls. In addition, the expressions of IL-6, gp130, and the IL-6 receptor subunit were investigated in biopsy specimens collected from these subjects. Furthermore, the expressions of CXCL7 and CXCR2, a chemokine and its receptor involved in IL-6 production, were also investigated. We observed an elevated level of serum IL-6 in colorectal cancer patients and an increased expression of IL-6 in colorectal cancer tissues, compared with the levels in a control group and in patients with adenoma. The phosphorylation of gp130 was also increased in the colorectal cancer tissues, compared with that in control and adenoma tissues. The expressions of CXCL7 and CXCR2 in the colorectal cancer tissues were also higher than those in control and adenoma tissues. IL-6 signaling is involved in sporadic colorectal cancer. In addition, the increased expressions of CXCL7 and CXCR2 might, in turn, increase the expression of IL-6 in colorectal cancer. Further studies are required to elucidate the function of the IL-6 signaling and the cytokine network in sporadic colorectal cancer.

Keywords: IL-6; Colorectal Cancer; gp130; CXCL7

1. Introduction

Colorectal cancer is a major cause of mortality and morbidity worldwide [1], although the mechanism of colorectal carcinogenesis remains unclear. Many studies have reported an association between interleukin-6 (IL-6) and colitis-associated cancer [2-4]. On the other hand, the association between IL-6 signaling and the sporadic colorectal adenoma-carcinoma sequence has not yet been clarified. IL-6 is a multifunctional cytokine important for immune response, cell survival, apoptosis and proliferation. IL-6 binds to soluble or membrane-bound IL-6 receptor (IL-6R) polypeptides that signal by interacting with the membrane-associated gp130 subunit, the engagement of which triggers the activation of Janus kinases (JAK)/STAT3 signaling [5]. Previous study showed that the interaction between mesenchymal stem cells and cancer stem cells is mediated by a positive feedback cytokine loop in which IL6 and CXCL7 play pivotal roles *in vivo* [6]. Chemokines are a family of small molecular weight proteins. CXCL7 is a member of the CXC subfamily of chemokines which can be further subdivided on the basis of the presence of the tripeptide motif glutamate-leucinearginine. The expression of CXCL7 and its receptor CXCR2 has been shown to be increased in breast cells [7]. However, the cytokine network in sporadic colorectal adenoma-carcinoma sequence has not been previously reported. Therefore, in the present study, we investigated IL-6 signaling and the cytokine network in the colorectal cancer patients.

2. Materials and Methods

2.1. Study Population

Thirty patients who were diagnosed as having colorectal adenoma, 30 patients with cancer, and 20 control subjects who were confirmed not to have any colorectal polyps when examined using a colonoscopy were recruited for this study between 2008 and 2009 at Yokohama City University Hospital. The exclusion criteria were subjects with familial adenomatous polyposis, inflammatory bowel disease, radiation colitis, or any malignant disease, as well as subjects with a previous his-

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tory of colectomy, gastrectomy or colorectal polypectomy. Written informed consent was obtained from all the subjects prior to their participation in the study. The study protocol was approved by the Yokohama City University Hospital Ethics Committee.

2.2. Collection and Analysis of Blood Samples for the Determination of IL-6 Levels

Blood samples were obtained in the morning on the day of the colonoscopy after the subjects had fasted overnight. The serum IL-6 levels were measured using an enzyme-linked immunosorbent assay for human IL-6 (SRL Co., Tokyo, Japan).

2.3. Immunohistochemical Analyses

The expressions of IL-6 and gp130 were investigated in normal colorectal, adenoma, and cancer tissues. Samples of normal colorectal, adenoma and cancer tissues were obtained from the study subjects and isolated. Formalin-fixed and paraffin-embedded samples were later deparaffinized and rehydrated. The sections were incubated with primary antibodies for IL-6 (1:50; Leica Biosystems, Mount Waverley, Victoria, Australia) and gp130 (1:100; Santa Cruz Biotechnology, Santa Cruz, California, USA) using an LSAB2 kit (DakoCytomation, Glostrup, Denmark). The sections were then incubated with biotinylated immunoglobulin as the secondary antibody and treated with peroxidase-conjugated streptavidin. The antibody complex was visualized with 3,3'-diaminobenzidine, tetrahydrochloride (Dojindo Laboratories, Kumamoto, Japan).

2.4. Western-Blot Analysis

Samples obtained from normal colorectal, adenoma and cancer tissues were isolated. The extracted protein was separated using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), and the separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Amersham, London, UK). The membranes were probed with primary antibodies specific for phospho-gp130 (p-gp130) and gp130 (Santa Cruz Biotechnology). Horseradish peroxidase-conjugated secondary antibodies and the ECL detection kit (Amersham) were used to detect specific proteins.

2.5. Real-Time RT-PCR

Samples obtained from the normal colorectal, adenoma and cancer tissues were isolated. Total RNA from the colorectal cancer and normal colorectal tissues were extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany). For the real-time reverse-transcriptase polymerase chain reaction, total RNA was reverse-transcribed into cDNA and amplified using a real-time quantitative polymerase chain reaction using the Step One Plus Real Time PCR System (Applied Biosystems, Foster City California, USA). Probes and primer pairs specific for IL-6, CXCL7, CXCR2 and 18S were purchased from Applied Biosystems. The concentrations of the target genes were determined using the competitive computed tomography method, and the values were normalized to the internal control.

2.6. Statistical Analysis

Statistical analyses were performed using the Kruskal-Wallis test and the Fisher PLSD test. All analyses were performed using StatView software (SAS Institute, Cary, NC, USA). A value of $p < 0.05$ was regarded as denoting statistical significance.

3. Results

3.1. Serum IL-6 Levels in Study Subjects and Expression of IL-6 in Normal Colorectal, Adenoma, and Cancer Tissues

The clinical characteristics of the colorectal cancer patients and the control subjects are shown in **Table 1**. No significant differences in age, BMI, or serum CRP levels were observed among these groups. The serum IL-6 level was significantly higher among the colorectal cancer patients than among the normal colorectal subjects or the adenoma patients ($p < 0.05$), although no significant difference was observed between the normal colorectal subjects and the adenoma patients (**Figure 1(a)**). To examine the expression of IL-6 in normal colorectal, adenoma and cancer tissues, immunohistochemical staining and gene expression analyses were performed. The expression of IL-6 was observed in normal colorectal, adenoma, and cancer tissues (**Figures 1(c)-(e)**). The mRNA expression of IL-6 was significantly higher in the colorectal cancer tissues than in the normal colorectal or adenoma tissues ($p < 0.01$). No significant difference was observed between the normal colorectal and adenoma tissues (**Figure 1(b)**).

3.2. Expression and Phosphorylation of gp130 in Colorectal Tissues

IL-6 acts via a receptor complex containing at least one subunit of the signal-transducing protein gp130 [8]. We also investigated the expression of gp130 in normal colorectal, adenoma and cancer tissues using immunohistochemical staining and examined the phosphorylation of gp130 using a western blot analysis. The expression of gp130 was observed in normal colorectal, adenoma, and cancer cells (**Figures 2(a)-(c)**). The phosphorylation of gp130 was significantly higher in the colorectal cancer

Table 1. Characteristics of the study patients.

	Normal	Adenoma	Cancer	*p* value
N	20	30	30	
Age (year)	66.8 ± 11.4	65.2 ± 9.6	68.6 ± 12.0	0.30
Sex m/f	13/7	27/15	15/6	0.34
BMI (kg/m^2)	21.9 ± 3.1	13.4 ± 2.8	22.9 ± 4.2	0.21
CRP (mg/dl)	0.1 ± 0.2	0.3 ± 0.9	1.2 ± 2.4	0.12

Data are shown as mean ± standard deviation (SD).

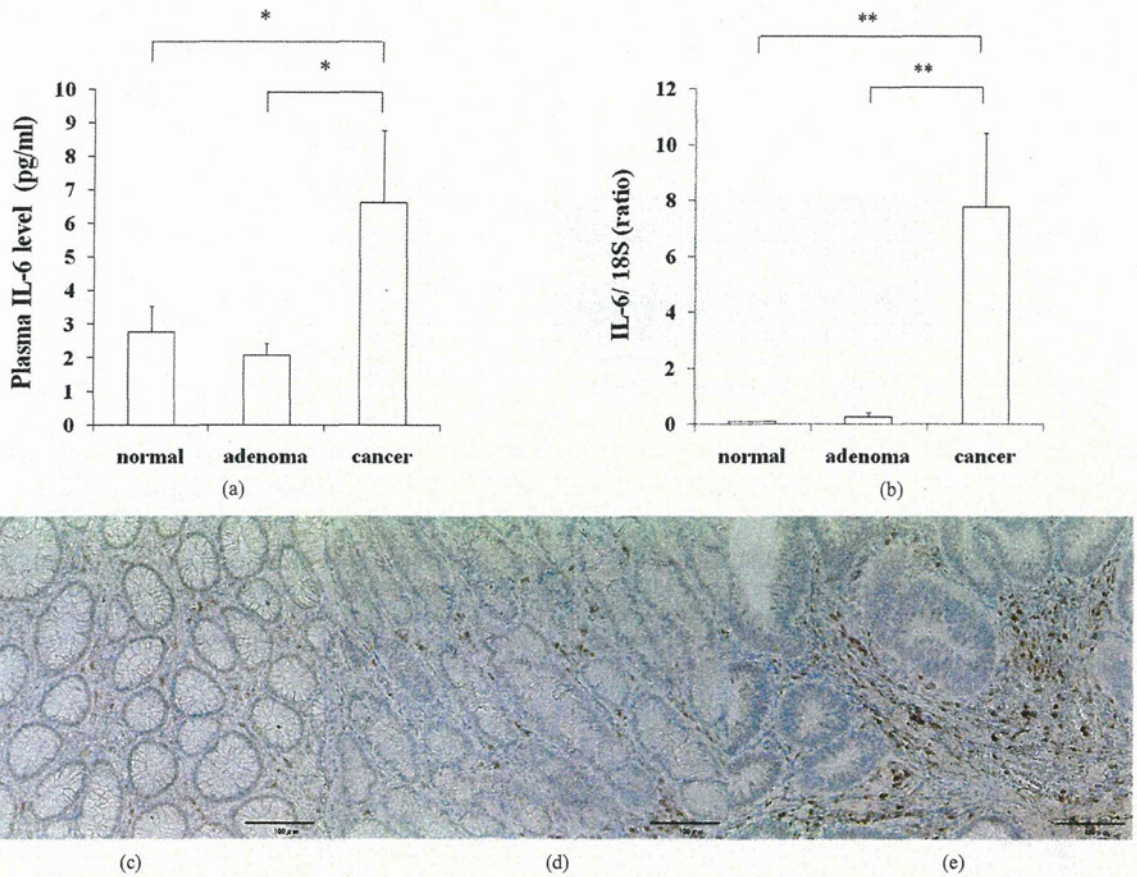


Figure 1. (a) Serum IL-6 levels in normal control, colorecdtal adenoma and cancer patients were expressed. Each column represents the mean ± SEM. $^*p < 0.05$, $^{**}p < 0.01$; (b) The relative mRNA expressions of IL-6 in normal colorectal, adenoma and cancer tissues were expressed as the ratio relative to the expression of 18S. Each column represents the mean ± SEM. $^*p < 0.05$, $^{**}p < 0.01$; ((c)-(e)) Immunohistochemical staining for IL-6. Scale bar: 100 μm; (c) Normal colorectal tissue; (d) Colorectal adenoma tissue; (e) Colorectal cancer tissue.

tissues than in the normal and adenoma tissues (**Figure 2(d)**). No significant difference was observed between the normal colorectal and adenoma tissues.

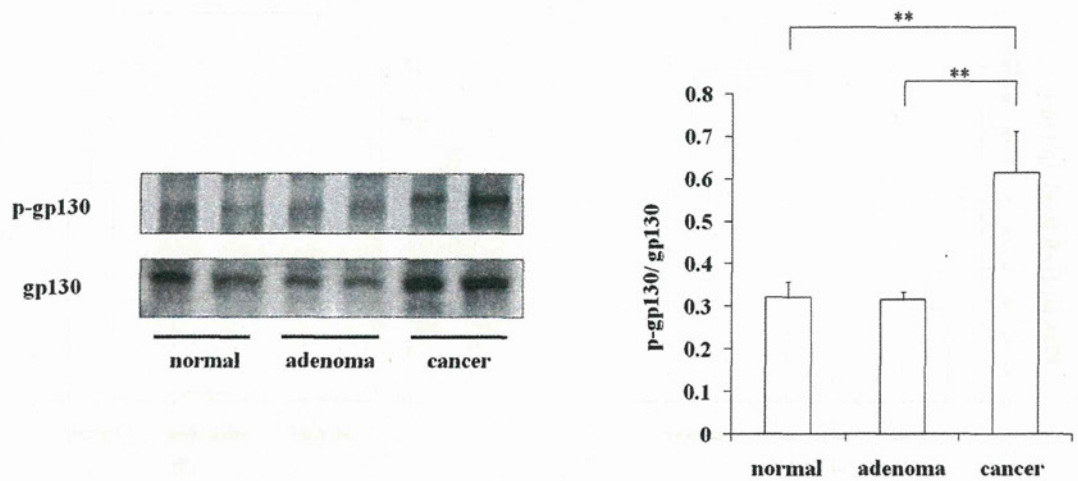
3.3. Expressions of CXCL7 and CXCR2 in Colorectal Tissues

CXCL7 is a member of the CXC subfamily of chemokines, and its receptor is CXCR2. The mRNA expression levels of CXCL7 and CXCR2 were significantly higher in the colorectal cancer tissues than in the normal colorectal or colorectal adenoma tissues (**Figure 3**). No significant difference was observed between the normal colorectal and adenoma tissues.

4. Discussion

Previous studies have reported an association between

(a) (b) (c)



(d)

Figure 2. ((a)-(c)) Immunohistochemical staining for gp130. Scale bar: 100 μm. (a) Normal colorectal tissues; (b) Colorectal adenoma tissues; (c) Colorectal cancer tissues; (d) WESTERN-blot analysis for gp130 and phosphorylated gp130. Left panels: Representative Western blots for phosphorylated and total levels of gp130. Lanes 1, 2: normal colorectal tissue; lanes 3, 4: adenoma tissue; lanes 5, 6: cancer tissue. Right panels: The ratios of the level of the phosphorylated gp130 to the total gp130 level. Each column represents the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$.

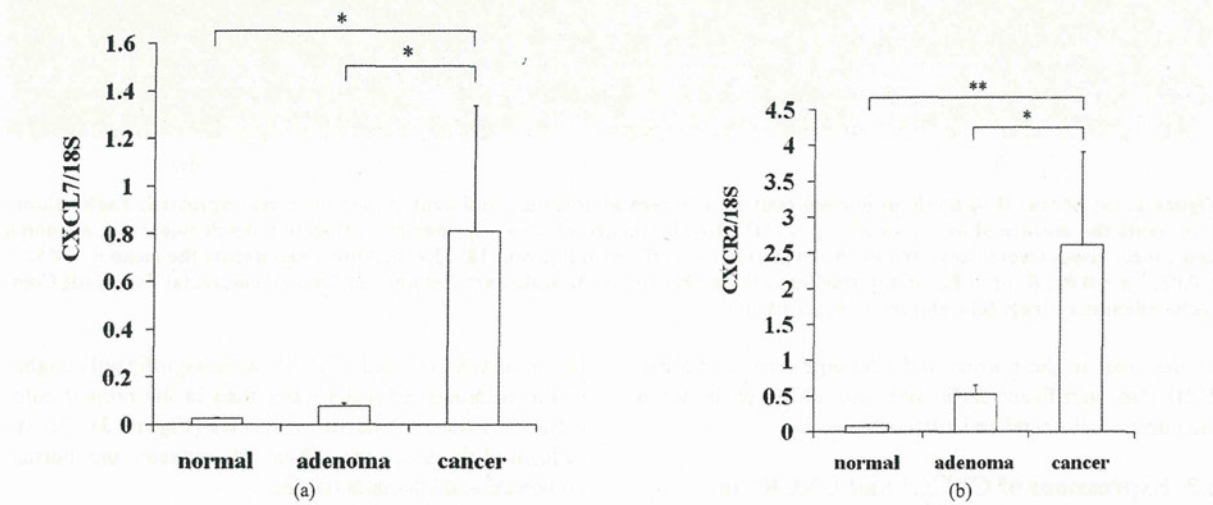


Figure 3. The relative mRNA expressions of (a) CXCL7 and (b) CXCR2 in normal colorectal, colorectal adenoma and cancer tissues are expressed as the ratios relative to the expression level of 18 S. Each column represents the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$.

IL-6 and CAC [2-4], although the relation between IL-6 and the sporadic colorectal adenoma-carcinoma sequence has not been fully explained. In the present study, we investigated IL-6 signaling in the sporadic colorectal adenoma-carcinoma sequence. We observed a significant increase in the serum IL-6 level in cancer patients with colorectal cancer, compared with normal subjects and patients with adenoma. In addition, the level of IL-6 expression was higher in colorectal cancer tissues than in normal or adenoma tissues. Thus, we speculated that IL-6 may play crucial roles in not only CAC, but also sporadic colorectal cancer. No significant difference was observed in the serum CRP levels among the groups. This result indicated that the increase in IL-6 expression was not mediated by systematic inflammation.

We investigated the phosphorylation level of gp130, which is mediated by IL-6 signaling in colorectal tissues. We observed significantly higher gp130 phosphorylation levels in colorectal cancer tissues than in normal or adenoma tissues. To the best of our knowledge, this is the first study that demonstrates the expression of the gp130 phosphorylation in human colon cancer tissue. IL-6 mediates JAK/STAT signaling is involved in cell proliferation [5,9,10]. Previous studies have revealed that the phosphorylation of STAT3 is higher in colorectal cancer tissues than in normal or adenoma tissues [11-14]. Combined with these previous studies, our present results suggested that IL-6 mediates JAK/STAT signaling thorough the phosphorylation of gp130 in sporadic colorectal cancer, but not in adenoma or normal colorectal tissues.

In addition, we observed an increase in CXCL7 and CXCR2 expression in colorectal cancer tissues. Mesenchymal stem cells derived CXCL7, in turn, interacts with cancer cells through CXCR2, inducing the synthesis of a number of cytokines, including IL-6 [15]. Previous studies have reported elevated expression levels of CXCL7 and CXCR2 [7,15], and the formation of a positive feedback loop between increases in IL-6 and CXCL7 has been suggested in breast cancer [6]. However, the expression levels of CXCL7 and CXCR2 have not been previously studied in colorectal cancer. Although we could not show direct evidence of this signaling in human colorectal adenomas, our results were consistent with previous studies in breast cancer; thus, we speculated that IL-6 and CXCL7 may also form a positive feedback loop in sporadic colon cancer. This mechanism would be a novel finding in colon cancer. We propose that this cytokine network may be a novel therapeutic target for the prevention of colorectal cancer.

In conclusion, IL-6 may play a crucial role in the sporadic colorectal cancer. The increased expressions of CXCL7 and CXCR2 might, in turn, increase the expression of IL-6 in colorectal cancer. Further investigations are required to clarify the exact roles of IL-6 signaling in sporadic colorectal cancer.

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An Epidemiologic Survey of Chronic Intestinal Pseudo-Obstruction and Evaluation of the Newly Proposed Diagnostic Criteria

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Key Words
Chronic intestinal pseudo-obstruction · Diagnostic criteria · Algorithm

Abstract
Background and Aims: Chronic intestinal pseudo-obstruction (CIPO) is an intractable disease in which clinical symptoms of intestinal obstruction appear without mechanical cause. No clear diagnostic criteria have been established; therefore, we proposed diagnostic criteria to facilitate the diagnosis of this rare disease and aim to evaluate their usefulness and validity. ***Materials and Methods:*** A questionnaire was sent to 378 institutions belonging to the Japanese Society of Gastroenterology between December 2009 and February 2010. We summarized the returned data and performed a statistical analysis. ***Results:*** A total of 160 cases were included, and 141 cases (88.1%) fulfilled the criterion of disease duration of >6 months, 157 cases (98.1%) the criterion of the clinical symptoms of abdominal pain and/or bloating and 154 cases (96.2%) fulfilled the criterion of imaging findings. Eventually, 138 cases (86.3%) fulfilled all criteria. ***Conclusions:*** The proposed diagnostic criteria were useful, with a high sensitivity of 86.3% for Japanese patients. Improved recognition of CIPO and practical use of the criteria are desired. The criteria should be appropriately modified by additional researchers to make them more practical and internationally applicable.

Introduction

Intestinal pseudo-obstruction (IPO), first reported by Dudley et al. [1] in 1958, is a rare, serious digestive syndrome characterized by failure of the intestinal tract to propel its contents appropriately, resulting in recurrent clinical episodes of intestinal obstruction in the absence of any mechanical cause [1–5]. Acute or chronic abdominal pain and distension are the most common symptoms. Furthermore, nausea, vomiting, constipation and diarrhea are also seen at various frequencies. Based on the pattern of onset, IPO is classified as acute or chronic. The acute type, especially acute pseudo-obstruction of the colorectum, is referred to as Ogilvie syndrome, which encompasses several colonic obstructive syndromes caused

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Table 1. Diagnostic criteria for CIPO proposed by the Research Group of the Ministry of Health, Labour and Welfare

Definition of CIPO
Chronic bowel obstruction not explained by structural abnormalities
Criteria for CIPO
Must include all of the following four points:
1. Onset of one or more symptoms of bowel obstruction[1] at least 6 months prior to the diagnosis
2. One or both of the following for the previous 12 weeks
   a. Abdominal bloating
   b. Abdominal pain
3. Dilatation and/or air-fluid level of the intestine on abdominal X-ray, echo and/or CT imaging
4. No evidence of structural disease (by upper and lower gastrointestinal endoscopy, computed tomography, barium enema, and small-bowel follow-through) that could explain the dilatation and/or air-fluid level of the intestine

Important notice
1. Congenital and/or onset at under 15 years of age must be excluded. Only adult onset is included
2. Surgical history, except surgery for CIPO, within the 6 months prior to the diagnosis must be excluded to rule out Ogilvie syndrome
3. CIPO is defined at two levels: primary CIPO or secondary CIPO. Primary CIPO consists of three types: the myogenic type, neurogenic type and idiopathic type. Secondary CIPO consists of two types: the SSc type and the unclassified type
4. Family accumulation may exist
5. Neuropathy, such as problems with urination, may exist
6. Some psychosocial disorder may be present

[1] Symptoms of bowel obstruction include: abdominal pain, nausea, vomiting, abdominal bloating, abdominal fullness, lack of gas and/or passing gas.

by acute functional transit failure. It is speculated that this syndrome is caused by collapse of the regulation of autonomic nerves distributed in the colorectum. The Ogilvie syndrome is secondary to various diseases and has been mainly reported to occur after abdominal surgery [6].

The chronic type of IPO is the so-called 'CIPO'. Although there are no specific laboratory findings, malabsorption due to bacterial overgrowth, anemia, hypocalcemia, hypolipidemia, folic acid deficiency, iron deficiency and hypoalbuminemia are often observed in CIPO patients due to malnutrition [2–4]. CIPO may affect the entire gut from the esophagus to the rectum in the broad sense, but predominantly, the small intestine is affected. CIPO can be categorized as primary or secondary [7]. Primary CIPO includes the myogenic, neurogenic, mesenchymopathic (arising from the dysfunction of the interstitial cells of Cajal) and the mixed or unclassifiable type (inflammation). Secondary CIPO includes a subtype that is secondary to underlying diseases such as systemic sclerosis (SSc) or mitochondrial encephalomyopathy as well as a subtype that is related to antipsychotic or antidepressant drug use. The subtype of CIPO that is not associated with any apparent underlying disease has been called 'chronic idiopathic intestinal pseudo-obstruction' (CIIP).

At present, the diagnostic criteria for CIPO are not well established. The Research Group for the Survey of the Actual Conditions of Epidemiology, Diagnosis, and Treatment of CIIP in Japan (chief investigator, Atsushi Nakajima), Research Project for Overcoming Intractable Disease, Health Labour Sciences Research Grant in the fiscal year 2009, proposed Japanese diagnostic criteria for CIPO in order to facilitate the diagnosis of this rare disease by the general physician. The criteria are composed of four mandatory requirements and an important note for the diagnosis, as shown in table 1. Recently, Iida et al. [8] investigated the reported data of a total of 121 Japanese CIPO cases between 1983 and 2009 and calculated the sensitivity of the proposed diagnostic criteria, under the assumption that the case reports used contained sufficient information about each patient; therefore, all cases were considered to be correctly diagnosed as having CIPO. However, very little is still known about the pathophysiology of CIPO and the status of CIPO patients in Japan; therefore, we conducted an epidemiologic survey to assess the present status of this rare disease in the Japanese population following the investigation of previous case reports. We investigated the recognition rate of the disease in certified gastroenterology institutions as well as its epidemiology, including the clinical symptoms and

Table 2. Patient questionnaire sent to 378 institutions belonging to the JSGE

I. Patient information									
Sex:	Male	Female							
Age, years:	≤14	15–19	20–29	30–39	40–49	50–59	60–69	70–79	≥80
II. Clinical presentations at first hospital visit									
Abdominal pain for the previous 12 weeks:				Yes			No		
Vomiting for the previous 12 weeks:				Yes			No		
Abdominal bloating for the previous 12 weeks:				Yes			No		
Dilatation of the bowels on radiological imagings:				Yes			No		
Disease duration:				More than 6 months			Within 6 months		
Type of CIPO:				Primary			Secondary		
If secondary CIPO:				Secondary to SSc			Secondary to others		
III. Treatment									
Selected method of treatment:	Diet		Medication		Surgery		Others	No treatment	
Medication drugs: (multiple answers allowed)	Mosapride[1]		Erythromycin		Pantothenic acid		Metoclopramide		Sulpiride
	Domperidone		Daikenchuto[2]		Somatostatin analogue		Kanamycin		Metronidazole
	Polymixin B		Probiotics		Itopride		Calcium polycarbophil		
	Magnesium oxide		Other laxatives		Loperamide		Albumin tannate		
	Dimethicone		PPI		H2RA		Mucosal protective drugs		

JSGE = Japanese Society of Gastroenterology; PPI = proton pump inhibitors.
[1] Mosapride is the 5-HT4 receptor agonist. [2] Daikenchuto is a herbal medicine.

radiological imaging findings; then, we evaluated the validity and usefulness of the diagnostic criteria for CIPO newly proposed by this research group.

Materials and Methods

A questionnaire was sent to 378 institutions belonging to the Japanese Society of Gastroenterology between December 2009 and February 2010. At first, we enquired whether or not each of the participating institutions was aware of CIPO as a disease entity or had encountered patients with CIPO. While enquiring about the institutions' recognition of this disease, CIPO was defined as a disease characterized by recurrent clinical episodes of intestinal obstruction in the absence of mechanical obstruction, as confirmed by clinical examinations, including radiological imaging and gastrointestinal endoscopy. The institutions that had knowledge about the disease entity were asked to fill out the questionnaire, based on the premise that the gastrointestinal specialists in the institutions had certainly performed the aforementioned examinations to exclude mechanical obstruction and made a correct diagnosis of CIPO. The details of the questionnaire are shown in table 2. Here, the term 'dilatation of the bowels on radiological imagings' indicates not only dilatation of the small intestine, but also of the colon. We decided to use the simplistic term 'the bowels' because of the following reasons: (1) our intention in establishing these diagnostic criteria is to facilitate the diagnosis of CIPO by the general physician without any need for complicated or specialized discussions, such as 'which is the dilated bowel, the small intestine or the colon?'; (2) the colon should not be excluded, because special cases such as the colorectal localized type (chronic colonic pseudo-obstruction (CCPO)) sometimes exist.

Table 3. Disease type of a total of 160 CIPO cases

Classification of CIPO	Cases
Primary CIPO	117 (73.1)
Secondary CIPO	41 (25.6)
SSc	23 (56.1)
Non-SSc	18 (43.9)
DM	4 (9.8)
MCTD	3 (7.3)
SjS	1 (2.4)
Amyloidosis	2 (4.9)
Others	8 (19.5)
Unknown	2 (1.3)

Figures in parentheses are percentages. DM = Dermatomyositis; MCTD = mixed-connective tissue disease; SjS = Sjögren syndrome.

Table 4. Clinical presentations at first hospital visit (**a**) and disease duration prior to the diagnosis (**b**) (n = 160)

a Clinical presentation

	Cases		
	Yes	No	Unknown
Clinical symptoms			
Abdominal pain	107 (66.9)	53 (33.1)	0 (0)
Vomiting	81 (50.6)	79 (49.4)	0 (0)
Abdominal bloating	156 (97.5)	4 (2.5)	0 (0)
Abdominal pain and/or bloating	157 (98.1)	3 (1.9)	0 (0)
Radiological imaging findings			
Dilatation and/or air-fluid level of the bowel	154 (96.2)	3 (1.9)	3 (1.9)

b Disease duration

	Cases
Disease duration	
>6 months	141 (88.1)
<6 months	16 (10.0)
Unknown	3 (1.9)

Figures in parentheses are percentages. Of the total CIPO cases, 138 (86.3%) fulfilled all the diagnostic criteria, including abdominal pain and/or bloating, dilatation and/or air-fluid level of the bowel, as well as disease duration >6 months.

The closing date for the receipt of the questionnaire responses was 19 February 2010. We aggregated the data on the type of CIPO (primary or secondary), age at the time of the first hospital visit, clinical symptoms, radiological imaging findings, duration of disease and method of treatment in each patient and conducted a statistical analysis.

Results

Recognition of CIPO and Experience with CIPO at Each Institution

Overall, 216 (57.2%) of the 378 institutions responded to our questionnaire, and of these, 200 (92.6%) were aware of CIPO as a distinct disease entity and 103 (51.5% of those aware of CIPO as a distinct disease entity) had encountered cases of CIPO. None of the institutions that were unaware of CIPO have encountered CIPO cases. The number of cases was 0 in 97 (48.5%), 1 in 52 (26.0%), 2 in 17 (8.5%), 3 in 7 (3.5%), 4 in 1 (0.5%), 5 in 2 (1.0%), 6 in 3 (1.5%), 7 in 2 (1.0%), 8 in 1 (0.5%), 10 in 2 (1.0%), and 27 in 1 (0.5%) of the institutions. A total of 213 patients were accumulated from 103 institutions until 19 February 2010. Of the 213 patients, 53 for whom detailed information (e.g., sex, clinical symptoms) was not available from the questionnaire were excluded. Eventually, the data of a total of 160 patients were included in our study.

Type of CIPO

Data analysis of the 160 cases revealed that 77 (48.1%) were males and 83 (51.9%) were females. The type of CIPO was primary in 117 cases (73.1%), secondary in 41 cases (25.6%) and unknown in 2 cases (1.3%), as shown in table 3. The underlying cause in the cases with secondary CIPO was SSc in 23 cases (56.1%) and non-SSc in 18 cases (43.9%). Collagen diseases were prominent among the non-SSc cases and included dermatomyositis in 4 cases (9.8%), mixed connective tissue disease in 3 cases (7.3%) and Sjögren syndrome in 1 case (2.4%). The other causes of non-SSc CIPO were amyloidosis in 2 cases (4.9%) and 'others' in 8 cases (19.5%).

Age at the Time of First Hospital Visit

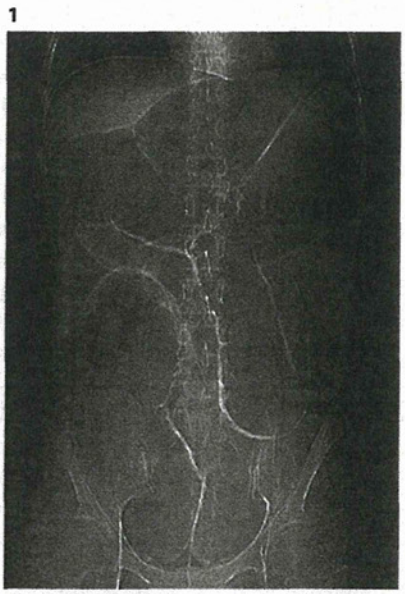
The majority of the patients of both sexes were in their 60s at the time of their first hospital visit (25.7% males, 24.1% females).

Clinical Symptoms

Our evaluation of the clinical symptoms in 160 cases showed that abdominal bloating was the most common symptom, recorded in 156 cases (97.5%), and that abdominal pain and vomiting were relatively common symptoms, recorded in 107 (66.9 %) and 81 cases (50.6%), respectively (table 4). Overall, 157 cases (98.1%) had at least one of these two symptoms, which fulfilled the diagnostic criterion 2.

Radiological Imaging Findings

In this survey, we defined positive imaging findings as the presence of dilatation and/or air-fluid levels of the bowels. Figure 1 shows a typical abdominal radiograph of a CIPO patient with marked distention of the small



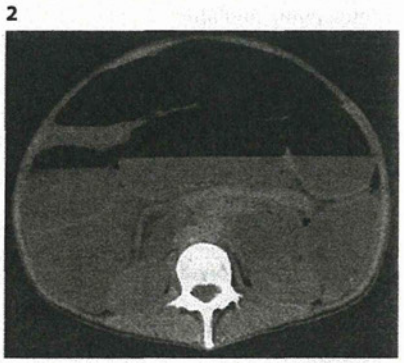


Fig. 1. Abdominal radiograph: marked distention of the intestine filled with a large amount of intestinal gas.
Fig. 2. Abdominal CT: markedly dilated intestinal loops and multiple air-fluid levels are observed. A large amount of small-intestinal gas occupies the greater part of the abdomen.

intestine and a large amount of intestinal gas. Figure 2 shows a typical CT image of a CIPO case. Among the 160 patients, 154 (96.2%) had positive imaging findings and 3 (1.9%) showed no positive findings; the status with regard to this finding was unknown in 3 cases (1.9%) (table 4a). Thus, 154 of the 160 cases (96.2%) showed dilatation of the bowel loops and/or air-fluid levels of the intestine on plain radiographs or CT images of the abdomen, which is included as a positive diagnostic criterion.

Duration of Disease

The number of patients with the criterion of disease duration >6 months was 141 (88.1%), and 16 (10.0%) had a disease duration <6 months; disease duration was unknown in 3 cases (1.9%) (table 4b).

Selected Method of Treatment

Our summarization of the responses to the questionnaire, where multiple answers were allowed, in relation to the selected method of treatment for each patient in the total of 160 cases (table 5) showed that medical conservative (drug) therapy was the most commonly selected treatment: it was selected in 135 cases (84.4%), diet in 107 cases (67.1%) and surgical treatment in 36 cases (22.5%). A total of 46 cases (28.8%) were treated by other methods, including home parenteral nutrition (intravenous hyperalimentation) in 33 cases (20.6%) and endoscopic intestinal decompression, ileus tube placement and enema in a few cases. One case (0.6%) received no treatment. The most commonly used drugs were mosapride citrate (5-HT4 receptor agonist), probiotics, Daikenchuto (herbal medicine), magnesium oxide, and others. Antacids such as proton pump inhibitors and H2 receptor antagonists were sometimes used in the cases treated conservatively (table 6).

Table 5. Selected method of treatment (n = 160)

Treatment	Cases
Medication	135 (84.4)
Diet	107 (67.1)
Surgery	36 (22.5)
Others	46 (28.8)
Home parenteral nutrition	33 (20.6)
Endoscopic decompression	4 (2.5)
Ileus tube placement	2 (1.3)
Enema	1 (0.6)
No treatment	1 (0.6)

Figures in parentheses are percentages.

As a result, 138 patients fulfilled all the diagnostic criteria, and the sensitivity of the proposed criteria for the diagnosis of CIPO in Japanese patients was 86.3%.

Table 6. Drugs used for treatment (n = 160)

Drugs	Cases
Mosapride citrate[1]	101 (63.1)
Daikenchuto[2]	83 (51.9)
Magnesium oxide	69 (43.1)
Probiotics	62 (38.8)
Proton pump inhibitor	45 (28.1)
Erythromycin	41 (25.6)
Pantothenic acid	36 (22.5)
Metoclopramide	34 (21.3)
Mucosal protective drugs	23 (14.4)
Domperidone	20 (12.5)
H2 receptor antagonist	19 (11.9)
Metronidazole	18 (11.3)
Itopride	16 (10.0)
Dimethicone	12 (7.5)
Calcium polycarbophil	11 (6.9)
Kanamycin	10 (6.3)
Somatostatin analogue	7 (4.4)
Loperamide	5 (3.1)
Sulpiride	5 (3.1)
Polymixin B	3 (1.9)
Albumin tannate	3 (1.9)
Other laxatives	45 (28.1)

Figures in parentheses are percentages.
[1] Mosapride is the 5-HT4 receptor agonist.
[2] Daikenchuto is a herbal medicine.

Discussion

CIPO is a serious digestive disease characterized by the disturbance of intestinal propulsive motility, which results in clinical features mimicking mechanical obstruction, in the absence of any mechanical occlusion [1–5]. Long-term outcomes are generally poor, with disabling and potentially life-threatening complications developing at a high frequency over time [9]. The diagnosis of CIPO is difficult and often delayed owing to the lack of biological markers and the symptomatic overlap with several other forms of digestive syndromes associated with similar gut motor dysfunction but different natural histories. The delay of correct diagnosis leads to repeated, useless and potentially dangerous surgical procedures.

Whole-gut transit scintigraphy and antroduodenal manometry are often performed in Western countries to evaluate gastrointestinal motility disorders [2]. In 1999, Di Lorenzo [10] proposed an algorithm for the evaluation of patients presenting with signs and symptoms suggestive of pseudo-obstruction. According to this algorithm, diagnosis of CIPO requires exclusion of mechanical obstruction by an abdominal X-ray series and/or contrast X-rays in patients with chronic signs and symptoms of bowel obstruction, as well as exclusion of potentially underlying causes of pseudo-obstruction. Manometory, scintigraphy and exploratory surgery with full-thickness biopsy are not absolutely necessary but may help confirm the diagnosis. On the other hand, Lacy [11] has proposed yet another diagnostic algorithm. For the diagnosis of CIPO, patients should have had symptoms for at least 6 months, and a stepwise approach is used to make the diagnosis of CIPO, generally including laboratory studies, radiological studies to exclude mechanical obstruction, tests to measure the gastrointestinal transit time and, if necessary, specialized tests of gastrointestinal motility, such as esophageal and antroduodenal manometry. In summary, previous algorithms emphasize that the diagnosis of CIPO requires at least chronic symptoms of bowel obstruction and exclusion of mechanical obstruction and, if necessary, manometry and scintigraphy to confirm the diagnosis.

Full-thickness biopsy of the small bowel should be performed in all patients with severe dysmotility of unknown etiology who are scheduled to undergo surgery for any reason, because of the potential to elucidate the pathophysiology of CIPO. Adoption of this procedure has revealed that neurogenic CIPO can be classified into two major forms, including degenerative neuropathy with hypoganglionosis, characterized by evidence of damage and/or marked reduction in the ganglion cells in the intestinal wall, and inflammatory neuropathy characterized by myenteric infiltration by inflammatory cells, and that myogenic CIPO is characterized by fibrosis or vacuolization of the inner circular muscle and/or the longitudinal muscle of the intestine [12–14]. Although full-thickness biopsy may not be absolutely necessary, it is an important procedure that helps to confirm the diagnosis of CIPO.

As mentioned above, gastrointestinal motility function tests, including whole-gut transit scintigraphy and manometry, and exploratory surgery with full-thickness biopsy of the small bowel are important; however, they are invasive in terms of patient tolerability. This is the reason why we were prompted to develop diagnostic criteria that would not necessitate the use of these special examinations.

Although a few diagnostic algorithms have been reported, no clear diagnostic criteria for CIPO have been established. Iida et al. [8] revealed that it took an average of >7 years from the initial symptoms before a correct

diagnosis of CIPO could be established, and therefore, emphasized the importance of a greater degree of awareness of this disease among physicians and the necessity of diagnostic criteria in order to shorten the period from the initial symptoms to correct diagnosis. Hongo et al. [6], who were co-researchers of the Survey Group, drafted interim diagnostic criteria referring to several textbooks and case reports. In addition, they discussed the usefulness of the interim diagnostic criteria with other collaborators specialized in gastrointestinal motility disorders, soliciting their opinions by e-mail, and laid down the proposed diagnostic criteria as shown in table 1. In our study, we investigated the clinical features of 160 patients and examined the validity of the proposed diagnostic criteria by calculating the diagnostic sensitivity. All the registered patients were diagnosed as CIPO based on the findings on plain abdominal X-ray, CT imaging, gastrointestinal endoscopy and, where necessary, barium enema and small-bowel follow-through. None of the patients underwent manometry, scintigraphy or exploratory surgery with full-thickness biopsy. Of the 160 patients, 138 fulfilled all the diagnostic criteria, and the sensitivity of the proposed criteria for the diagnosis of CIPO in Japanese patients was 86.3%. If the criteria included only 'No evidence of structural disease' (criterion 4) and 'Showing at least one of abdominal pain and abdominal bloating in the previous 12 weeks' (criterion 2), they would have shown higher sensitivity, but lower specificity, because patients with chronic constipation might be included as false-positives. However, most of these false-positives could be excluded based on criterion 1, i.e. 'Onset of one or more symptoms of bowel obstruction at least 6 months prior to the diagnosis', and on criterion 3, i.e. 'Dilatation and/or air-fluid levels of the bowels on plain abdominal X-ray, echo and/or CT images'.

The recognition rate of CIPO is not more than 92%, even in specialized gastroenterology institutes in Japan, which is not optimal. There seems to be an even poorer recognition rate among physicians and surgeons who are not specialized in gastroenterology. The recognition rate of CIPO in foreign countries does not seem to be too satisfactory either, given that no large-scale epidemiological studies have been reported and no clear diagnostic criteria for CIPO have been established. A greater awareness of the clinical features of CIPO among physicians would help limit unnecessary surgical procedures to the minimum.

Both the proposed diagnostic criteria and the previously described diagnostic algorithms have their own advantages and limitations. Previously described diagnostic algorithms are superior in terms of allowing systematic differential diagnosis; however, they are difficult to use for general physicians and need specialized invasive examinations. On the other hand, our proposed diagnostic criteria are superior to the previously described algorithms in terms of the ease of use for the diagnosis of CIPO by the general physician without specific examinations, and also the ease of use in clinical practice; however, they are inferior to the previously described algorithms in that they do not provide a stepwise diagnostic approach or systematic differential diagnosis. New diagnostic algorithms are needed that can complement the shortcomings of the proposed diagnostic criteria and can be used in combination with them.

The main limitation of this study is the lack of a previous gold standard with which to compare the results, and the lack of assessment of fulfillment of the criteria among other gastrointestinal motility disorders. The most important aim of establishing diagnostic criteria is to shorten the interval from the initial symptoms to correct diagnosis and referral to a specialist and to minimize the performance rate of unnecessary surgical procedures. Improved recognition of CIPO and practical use of the diagnostic criteria are urgently desired. In addition, further investigation is required to determine whether or not the proposed diagnostic criteria might also show a high sensitivity for patients in other countries. The proposed diagnostic criteria should be appropriately modified by consultation with additional researchers to make them more practical and internationally applicable.

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Disclosure Statement

There are no conflicts of interest.

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ORIGINAL ARTICLE

Number of aberrant crypt foci in the rectum is a useful surrogate marker of colorectal adenoma recurrence

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Aim: Endoscopic screening and removal of colorectal adenomas can reduce the incidence of colorectal cancer. However, given the possibility of adenoma recurrence, surveillance colonoscopy is currently recommended after the initial screening and removal of colorectal adenomas. Aberrant crypt foci (ACF) have been shown to serve as a reliable surrogate marker of colorectal carcinogenesis. In this study, the relationship between the number of ACF at the initial endoscopic polypectomy and the likelihood of colorectal adenoma recurrence after polypectomy were investigated.

Methods: High-magnification chromoscopic colonoscopy was performed in 82 subjects who underwent endoscopic polypectomy to identify ACF in the lower rectum. Surveillance colonoscopy was then performed 3 years after the baseline polypectomy at Yokohama City University Hospital.

Results: The number of ACF was greater in patients who showed adenoma recurrence (7.88 ± 6.35) than in those who did not (2.19 ± 2.95) ($P < 0.001$). Receiver–operating curve analysis showed that the number of ACF was a highly specific predictor of the risk of adenoma recurrence.

Conclusions: This is the first study conducted to investigate the relationship between the number of ACF after endoscopic polypectomy and the likelihood of recurrence of colorectal adenomas. These results suggest that the number of ACF is a useful predictor of the likelihood of colorectal adenoma recurrence.

Key words: aberrant crypt foci (ACF), colorectal polyp, recurrence.

INTRODUCTION

Colorectal carcinogenesis is based on the adenoma-carcinoma sequence, wherein adenomas, spurred by acquired genetic mutations, can evolve into colorectal cancer (CRC). Adenomas have been established as premalignant lesions that are characterized by the presence of suggestive genetic and histological changes. It has been shown that endoscopic colorectal adenoma screening and removal is an effective strategy for reducing the incidence and mortality of CRC.[1–3] Surveillance colonoscopy is currently recommended after the initial screening and removal of colorectal adenomas, given the high possibility of development of adenoma recurrence.[4,5]

Aberrant crypt foci (ACF) were discovered as the earliest microscopic lesions to appear in the colorectal 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 in the colorectum.[7,8] Moreover, in recent experimental studies conducted on CRC, numerous chemopreventive agents have been shown to reduce the number of ACF.[9,10] Importantly, many agents that block ACF growth have also been shown to prevent adenoma development in these carcinogen-treated rodent models. Thus, in rodent models, ACF have been established as a precursor of CRC. In humans, ACF can be identified in the colorectal mucosa *in vivo* by high-magnification chromoscopic colonoscopy using methylene blue.[11] Some human studies have reported the existence of a strong correlation between the number of ACF and the presence of synchronous adenoma or carcinoma.[11–15] In addition, ACF have been accepted as a surrogate marker for studies on the chemoprevention of CRC.[16–18] However, some other studies have disputed the ACF-adenoma-carcinoma sequence theory or the use of ACF as surrogate marker for recurrent colorectal adenomas.[19,20] These latter studies showed dissociation between the presence of ACF prevalence and the adenoma history,[19] or no significant modulation of ACF by celecoxib.[20] However, the accuracy of the technical aspects of ACF detection in these studies is somewhat controversial because the number of ACF detected was very low compared with that in other reports.[11–13]

In recent years, the incidence of CRC has exploded. As such, there is an urgent need for the development of measures for the chemoprevention of CRC and for the identification of simple surrogate markers for CRC, similar to blood pressure and serum cholesterol for cardiovascular events. Few clinical studies have discussed the validity of using the number of ACF as a surrogate marker of colorectal adenoma recurrence. Therefore, the association between the number of

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ACF at the baseline and the likelihood of adenoma recurrence was investigated by our team. This is the first study to investigate the relationship between the number of ACF after endoscopic polypectomy and the likelihood of recurrence of colorectal adenomas.

METHODS

Study population

In this study, the medical records of 82 colorectal adenoma patients who underwent first endoscopic polypectomy between 2006 and 2007 at the Yokohama City University Hospital (Yokohama, Japan) were reviewed retrospectively. Data of the patients included age, sex, body mass index, characteristics of the adenoma (size, number and histopathology), and the number of ACF at the baseline colonoscopy. Surveillance colonoscopy was performed 3 years after the baseline polypectomy. To be included in the study, the patients had to have undergone complete colonoscopy on both occasions and resection of all adenomas at the baseline polypectomy. A complete colonoscopy was considered to include colonoscopy up to the level of the cecum and good bowel preparation. The exclusion criteria were subjects with familial adenomatous polyposis, inflammatory bowel disease, radiation colitis and a previous history of colectomy.

Written informed consent was obtained from all the subjects prior to their participation in the study. The study protocol was approved by the Yokohama City University Hospital Ethics Committee.

Magnifying endoscopy

A Fujinon EC-490ZW5/M colonoscope was used for the magnifying colonoscopy (Fujinon Toshiba ES Systems Co., Ltd, Tokyo, Japan). All subjects underwent bowel preparation with a polyethylene glycol-based solution, and underwent total colonoscopy and polypectomy prior to rectal ACF imaging. Subsequently, 0.25% methylene blue was applied to the mucosa with a spray catheter. Based on the results of a previous study, ACF were counted in the lower rectal region, from the middle Houston valve to the dentate line.[11] All ACF were imaged on a video and evaluated by two independent endoscopists who were unaware of the subjects' clinical histories.

Criteria used for the endoscopic diagnosis

ACF were defined as lesions in which the crypts were more darkly stained with methylene blue than normal crypts and that had larger diameters, often with oval or slit-like lumens and a thicker epithelial lining (Fig. 1).[11] We diagnosed colorectal adenoma recurrence if any new adenoma was found at the surveillance colonoscopy. The size of the adenoma was measured after resection. The resected adenoma was evaluated histopathologically by a specialist in gastrointestinal pathology. The size and histological type of the adenoma in patients with multiple adenomas were classified according to the largest and the most advanced lesion, respectively.

Statistical analysis

Statistical analysis was performed with SPSS 12.0 (SPSS, Inc., Chicago, IL, USA). As appropriate, the Mann–Whitney

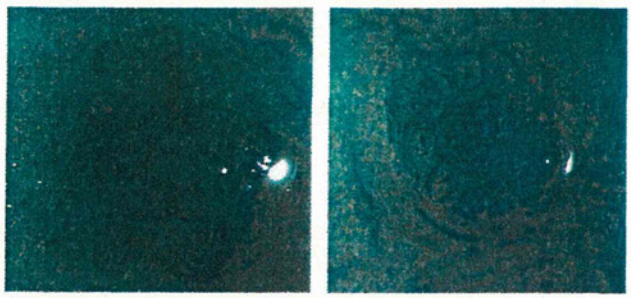

Fig. 1. Typical features of aberrant crypt foci on magnifying colonoscopy after methylene blue staining.

U-test or χ^2 test was used for univariate comparisons between the patient groups. Multivariate analysis was performed by binary logistic regression analysis. *P*-values of <0.05 indicated significance. The predictive performance of the number of ACF for adenoma recurrence was assessed by analysis of receiver–operating curves (ROC). The ROC is a plot of sensitivity versus 1 – specificity for all possible cut-off values. The most commonly used index of accuracy is the area under the ROC, with values close to 1.0 indicating high diagnostic accuracy.

RESULTS

Number of ACF in patients with and without adenoma recurrence

The clinical characteristics of the patients with and without adenoma recurrence are shown in Table 1. Significant differences were found in the size, pathological grade and number of adenomas at the baseline endoscopy between patients with and without adenoma recurrence.

The median number of ACF was 4.0 in the study subjects. No differences in the number of ACF were observed between the polyp locations at the baseline and surveillance colonoscopies.

The number of ACF was greater in patients who developed adenoma recurrence (7.88 ± 6.35) than in those who did not (2.19 ± 2.95) ($P < 0.001$) (Fig. 2).

Multiple regression analysis to identify the demographic factors associated with an elevated risk of adenoma recurrence

Multiple logistic regression analysis was performed with the factors that were found by univariate analysis to be significantly associated with adenoma recurrence. The number of ACF was still identified by multiple logistic regression analysis as a significant factor associated with adenoma recurrence ($P < 0.001$) (Table 2).

ROC for predicting the likelihood of adenoma recurrence based on the number of ACF

To differentiate between patients with a high and low likelihood of developing adenoma recurrence, the area under the ROC for the number of ACF was determined as 0.836 by ROC analysis (Fig. 3). The most appropriate cut-off value for

Table 1. Clinical characteristics of the study participants

Characteristics at the baseline colonoscopy	Adenoma recurrence (+) (*n* (%))	Adenoma recurrence (-) (*n* (%))	*P*-value
Age			
<65 years	16 (44.4)	20 (55.6)	0.487
≥65 years	24 (52.1)	22 (47.9)	
Sex			
Men	34 (85.0)	6 (15.0)	0.053
Women	28 (66.7)	14 (33.3)	
Size of adenoma (mm)			
<10	18 (39.1)	28 (60.9)	0.048*
≥10	22 (61.1)	14 (38.9)	
Pathological grade of adenoma			
Low grade	11 (32.3)	23 (67.7)	0.043*
Middle grade	16 (61.5)	10 (38.5)	
High grade	13 (59.1)	9 (40.9)	
Number of adenoma			
1	13 (36.1)	23 (63.9)	0.002*
2	7 (35.0)	13 (65.0)	
≥3	20 (76.9)	6 (23.1)	
Body mass index			
<20	5 (35.7)	9 (64.3)	0.44
20–25	25 (49.0)	26 (51.0)	
>25	10 (58.8)	7 (41.2)	

χ^2 test was used to determine the statistical significance of associations between patients with and without recurrence of adenoma at the baseline colonoscopy.
*$P < 0.05$.

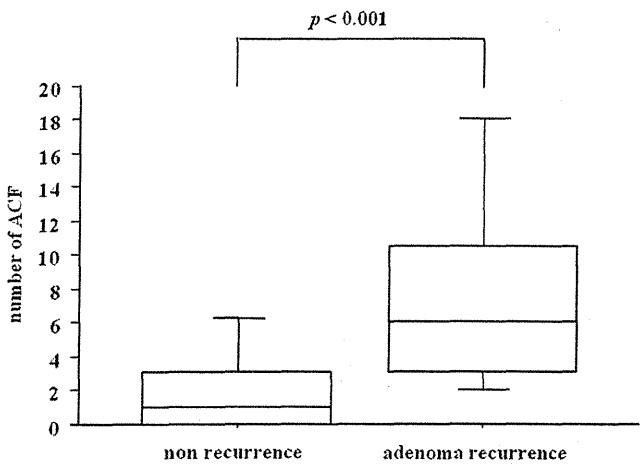

Fig. 2. Number of aberrant crypt foci (ACF) in patients with and without adenoma recurrence. Box plots of the number of ACF showing the interquartile range, median and range. The length of the box represents the interquartile range within which 50% of the values were located. Mann–Whitney *U*-test was used to determine the statistical significance.

predicting the likelihood of adenoma recurrence was also investigated by ROC analysis, and the sensitivity, specificity, positive predictive value and negative predictive value were calculated. The results showed that 6.0 was the optimal cut-off number of ACF predicting the likelihood of adenoma recurrence, and its sensitivity, specificity, positive predictive value and negative predictive value were 55.0%, 90.5%, 84.6% and 67.9%, respectively.

Table 2. Multiple logistic regression analysis to identify factors associated with adenoma recurrence

Factor	Odds ratio	95% CI	*P*-value
Number of ACF	1.456	1.208–1.754	<0.001*
Size of adenoma	1.036	0.244–4.398	0.962
Pathological grade of adenoma	2.129	0.865–5.240	0.1
Number of adenoma	2.938	1.407–6.136	0.004*

Note: Intercept = –35.539, R^2 for entire model = 0.374.
*$P < 0.05$.
ACF, aberrant crypt foci; CI, confidence interval.

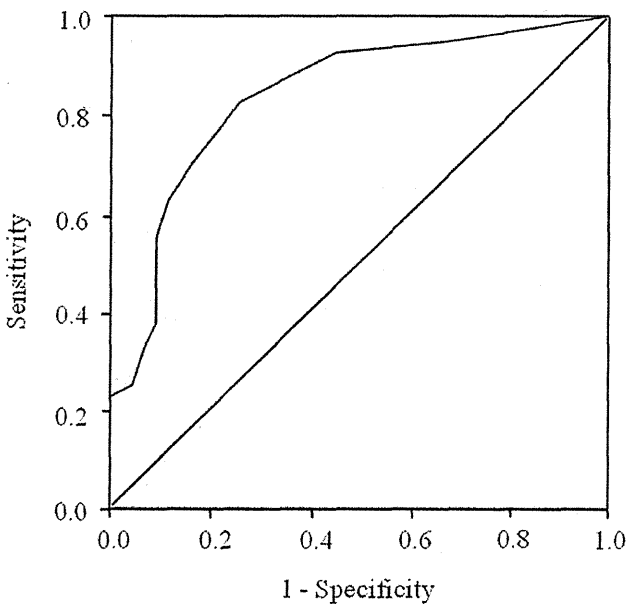

Fig. 3. Receiver–operating curves (ROC) for differentiating between the adenoma groups with and without adenoma recurrence. The ROC is a plot of sensitivity versus 1 – specificity for all possible cut-off values. The most commonly used index of accuracy for predicting the likelihood of adenoma recurrence is the area under the ROC based on the number of ACF in the rectum (area under the curve = 0.836). The optimal cut-off value was determined to be 6.0 ($P = 0.005$; 95% confidence interval, 0.747–0.925).

DISCUSSION

It is estimated that among patients who undergo colorectal adenoma removal by colonoscopy, 20–50% will be found to have a recurrent adenoma on a surveillance colonoscopy performed within 3–5 years.[1,2,21–27] Therefore, numerous clinical studies have attempted to identify predictors of the likelihood of adenoma recurrence. Previous studies have reported on the relationships between adenoma recurrence and adenoma- and patient-related features.[22,24,28–34] The size,

pathological grade and number of adenomas at the baseline colonoscopy have been reported to be associated with the risk of adenoma recurrence.[24,28–30] However, there are few easily and objectively quantifiable predictors of the likelihood of adenoma recurrence. In this study, the association between the number of ACF in the rectum at the baseline polypectomy and the likelihood of detecting recurrent adenomas at a surveillance colonoscopy performed 3 years later were examined; the recurrence rate at 3 years after the baseline polypectomy was 48.8%. At the baseline colonoscopy, a relationship between the risk of adenoma recurrence and the size, pathological grade and number of adenomas was observed. Also, the association between the risk of adenoma recurrence and the number of ACF at the baseline in the rectum was investigated. Some human studies have revealed the presence of a higher number of ACF in subjects with synchronous advanced adenoma.[11–13,35] The median number of ACF in this study was comparable to that in previous studies in which ACF were counted in the same region of the rectum, the lower rectal region.[11] However, another study counted ACF in a larger region (i.e. the distal 20 cm of the colon) and reported a higher number of ACF.[36] Therefore, the measurement protocol for ACF needs to be unified in the future.

A greater number of ACF was observed in patients who eventually developed colorectal adenoma recurrence than in those who did not. This novel finding suggests that the number of ACF could serve as a useful predictor of the likelihood of colorectal adenoma recurrence. These results also indicate that patients with a larger number of ACF in the lower rectum may be at a higher risk of developing colorectal adenoma recurrence, and therefore, close surveillance colonoscopy is needed for these patients. ACF can be easily and safely measured, within a few minutes, after colorectal polypectomy, and their number can be easily and objectively quantified. Therefore, we suggest that the number of ACF in the rectum could serve as a novel predictor of the likelihood of adenoma recurrence.

Our study had some limitations. Firstly, although all the ACF were recorded on video and evaluated by two independent endoscopists who were unaware of the subjects' clinical histories, the operators were not blinded to their clinical histories. Secondly, no pathological confirmation of ACF was undertaken in this study. In addition, we did not perform surveillance colonoscopy for patients without any adenomas at the baseline colonoscopy.

In conclusion, our study showed that the number of ACF in the rectum was correlated with the likelihood of colorectal adenoma recurrence. These results suggest that the number of ACF is a useful factor for predicting the likelihood of adenoma recurrence, and patients with a large number of ACF detected postpolypectomy need close surveillance colonoscopy. Furthermore, our results indicate that ACF may be advantageous as surrogate lesions of colorectal carcinogenesis.

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