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Risk and Management of Intra-Abdominal Abscess in Crohn's Disease Treated with Infliximab

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Key Words
Crohn's disease · Infliximab · Abdominal abscess

Abstract
Background and Aims: Infliximab (IFX) is a monoclonal antibody used to treat patients with Crohn's disease (CD). Intra-abdominal abscess formation is a major complication of CD with negative effects on patient prognosis. We have analyzed risk factors for abscess formation in CD patients treated with IFX. ***Methods:*** CD patients who received IFX between January 2000 and April 2011 at Keio University Hospital were analyzed retrospectively. Risk factors for abscess formation were assessed by univariate and multivariate logistic regression analyses. ***Results:*** Intra-abdominal abscess was seen in 15 of 258 patients. Univariate analyses showed serum C-reactive protein (CRP) concentration at 14 weeks after initiation of IFX ($p = 0.021$), serum albumin concentration at week 0 ($p = 0.022$) and week 14 ($p = 0.004$), the presence of anal lesions ($p = 0.036$), progression of intestine deformation ($p = 0.015$) and early loss of response to IFX ($p < 0.0001$) to be risk factors. Multivariate analysis showed that CRP concentration at 14 weeks [odds ratio (OR) 1.361] and loss of IFX response within 6 months (OR 5.361) were independent risk factors. ***Conclusions:*** Abscess formation should be suspected in patients with symptoms of CD recurrence during IFX therapy. Uncontrolled CRP concentration and early loss of response to IFX are risk factors.

Introduction

Crohn's disease (CD) is a chronic inflammatory disorder of the gut that causes persistent diarrhea, abdominal pain, body weight loss, and fever. CD follows a course of remission and relapse. Although its pathogenesis has not been completely elucidated, multiple genetic and envi-

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ronmental factors have been shown to trigger immunological abnormalities and intestinal inflammation [1, 2]. Among these is the collapse of immunological tolerance caused by inappropriate functioning of innate and/or adaptive immune cells infiltrating the lamina propria, resulting in the production of proinflammatory cytokines such as interferon-γ, interleukin (IL)-6, and tumor necrosis factor (TNF)-α.

Maintaining remission in some CD patients is difficult owing to resistance to conventional therapy, such as 5-aminosalicylates, corticosteroids and immunomodulators (IMs). Treatment with an antibody to TNF is successful [3], leading to the development of a monoclonal mouse/human chimeric anti-TNF antibody, infliximab (IFX).

Some studies have shown that IFX has several ways of preventing intestinal inflammation. IFX has a neutralizing effect on soluble TNF and caspase-mediated apoptosis via membrane-bound TNF in human peripheral monocytes [4]. Other reports indicate that neutralization of TNF signaling protects intestinal epithelial cells from TNF-induced cell apoptosis [5, 6]. IFX downregulates intestinal epithelial apoptosis [7] and this TNF-neutralizing effect can be corroborative evidence that is related to mucosal healing for CD. The current aim of CD treatment is to maintain long-term remission, with several randomized controlled trials showing that IFX infusion every 8 weeks is effective at sustaining remission in patients with CD [8, 9].

In addition to having clinical effects in patients with CD, this anti-TNF therapy is effective in patients with other chronic inflammatory diseases, including ulcerative colitis, rheumatoid arthritis, psoriasis, and psoriatic spondylitis. A systematic review of randomized controlled trials in patients with inflammatory bowel disease (IBD) treated with IFX and other IMs found that these agents increased the risk of infection in patients with ulcerative colitis [10]. Another report suggested that anti-TNF therapy carried a risk of infection in patients with IBD [11], and a report from the Japanese prospective cohort database showed that anti-TNF therapy is an independent risk factor for infection in patients with rheumatoid arthritis [12].

Safe use of anti-TNF therapy in patients with CD requires knowledge of the characteristics of the disease. CD is a progressive disease with a recurrent relapsing and remitting course that causes irreversible damage to the digestive tract, including strictures, fistulae, and intra-abdominal abscesses, which is expressed as the Lémann score [1, 13]. A population-cohort study reported that 18.6% of CD patients experienced digestive tract complications within 90 days of diagnosis [14]. Intra-abdominal abscesses may be difficult to treat and may be exacerbated by immunosuppressive agents including anti-TNF antibodies. The European Crohn's and Colitis Organization articulates that active sepsis, including abscess, is an absolute contraindication when using anti-TNF agents [15]. Sands et al. [9] demonstrated that 54% of IFX responders lost drug efficacy at week 54 on maintenance therapy.

Although the prevalence of CD is thought to be higher in Caucasians than in other ethnic groups, its prevalence has been gradually increasing in Asians. The Japanese Ministry of Health, Labour and Welfare has estimated that >30,000 people are affected by CD, with many treated with anti-TNF agents. Although studies have reported the characteristics and prognostic implications of intra-abdominal abscesses in CD patients treated with conventional therapy [11, 16–21], few have focused on the relationship between abscess formation and IFX. We therefore assessed risk factors for developing intra-abdominal abscess in CD patients treated with IFX.

Methods

Study Design and Patients

Patients diagnosed with CD and treated with IFX at Keio University Hospital, Tokyo, Japan, between January 2000 and April 2011 were eligible. Their medical records were assessed retrospectively, including the results of imaging modalities, including radiography, endoscopic imaging, ultrasound abdominal imaging, computed tomography (CT), and magnetic resonance imaging (MRI). We excluded patients who were administered episodic IFX. IFX administration dose was 5 mg/kg at 0, 2, 6 weeks as induction therapy and 5 mg/kg every 8 weeks as maintained therapy. Our retrospective study received ethical approval from the Keio University Ethics Committee and was performed in accordance with the Declaration of Helsinki.

Definitions

CD was diagnosed based on established criteria, including clinical, imaging, and histological findings. Abscess formation on CT, MRI, or ultrasound was defined as extraintestinal fluid collection and was categorized by location in the peritoneal cavity, abdominal wall, or subphrenic region; abscesses located in the anal region were excluded. Intestinal stenosis was defined as linear formation on double-contrast radiography, CT, or MRI. Loss of IFX response was categorized as shortening of maintenance infusion interval or stopping administration.

Data Collection

Variables recorded included patient age and sex; duration of CD, period from CD onset to start of IFX treatment; CD type (ileal, ileocolonic, or colonic); Montreal classification B (B1, nonstricturing, non-penetrating; B2, structuring; B3, penetrating);

smoking history; concomitant treatments with IFX, including IMs, corticosteroids, antibiotics, seton drainage and/or central venous catheter infusion; anal lesions affected by CD; previous surgical history; duration of IFX maintenance infusion; blood test parameters after initiation of IFX, including white blood cell (WBC) counts and concentrations of C-reactive protein (CRP), total protein (TP), albumin (Alb), total cholesterol (TC), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine transaminase (ALT), and hemoglobin (Hgb). We also assessed the characteristics and outcomes in patients with abscesses, including abscess size and location, intestinal forms, therapeutic regimen for abscess, and whether IFX infusion was continued.

Statistical Analysis

Patients were divided into those with and without abscesses. Risk factors for abscess formation were analyzed by the Mann-Whitney U test, Fisher's exact test, or log-rank test, as appropriate. Categorical variables were analyzed by χ^2 tests and continuous variables by unpaired t tests. Normally distributed results are reported as mean ± SD and non-normally distributed results as median and first (Q1) and third (Q3) quartiles. Factors significant by univariate analyses were assessed by multivariate logistic regression. Statistical analyses were performed using GraphPad Prism version 4.0 software (GraphPad, San Diego, Calif., USA) and SPSS version 20 software (IBM, Chicago, Ill., USA). $p < 0.05$ was defined as statistically significant.

Results

We identified 258 patients administered IFX during the observation period. Five patients received episodic IFX, and 11 could not be shifted to maintenance therapy. Among the latter, 2 had intra-abdominal abscesses, 4 were unresponsive to IFX, 1 had an infusion reaction, 1 had ileus, 1 had opportunistic candidiasis, and 2 refused continuous IFX administration. Of the remaining 242 patients who received IFX maintenance therapy, 13 had intra-abdominal abscesses. Thus, of 258 patients, 15 (5.8%) had intra-abdominal abscesses (fig. 1).

Patient Characteristics

Univariate analyses of patients with and without abscesses (table 1) showed that anal lesions ($p = 0.036$) and progression of intestine deformation for the Montreal classification B ($p = 0.015$) were significantly more prevalent in the abscess group. There were no between-group differences in disease duration, sex distribution, history of smoking, or use of other therapies. Table 2 shows the serological findings before and after IFX treatment in the two groups. CRP concentration at week 14 was significantly higher (0.10 vs. 2.27 mg/dl, $p = 0.021$) and Alb at week 0 (3.7 vs. 3.3 g/dl, $p = 0.022$) and week 14 (4.1 vs. 3.7 g/dl, $p = 0.004$) was significantly lower in the abscess group. CRP at week 0, and TP, TC, ALP, LDH, AST, ALT, WBC, and Hgb at weeks 0 and 14 did not differ between the two groups.

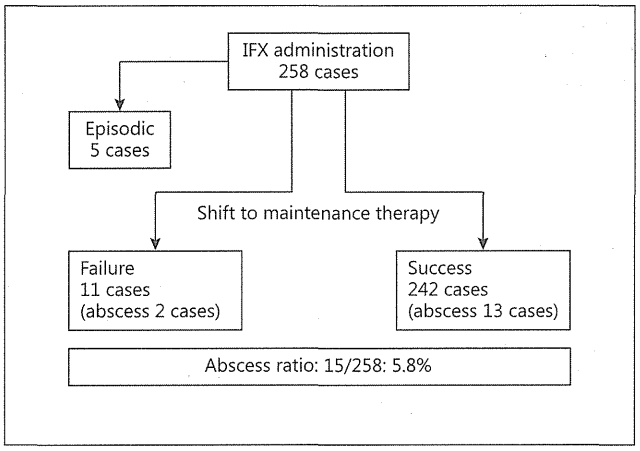

Fig. 1. Outcome of CD patients treated with IFX at the Keio University Hospital, Tokyo, Japan, between January 2000 and April 2011. Of 258 patients, 15 (5.8%) had intra-abdominal abscesses.

IFX Maintenance Treatment Retention Ratio

We investigated whether IFX maintenance therapy retention ratio differed between the abscess and non-abscess groups. We hypothesized that the abscess group would have a shorter maintenance period on IFX resulting from a loss of response to this agent. We found that the abscess group showed loss of IFX response at a median 5 months, which was significantly sooner than in the non-abscess group ($p < 0.0001$; fig. 2).

Multivariate Logistic Regression

Using the factors significant in univariate analyses, we analyzed factors associated with abscess formation by multivariate logistic regression. We found that serum CRP concentration after IFX administration at 14 weeks [odds ratio (OR) 1.361, 95% confidence interval (CI) 1.039–1.783; $p = 0.025$] and loss of response to IFX within 6 months (OR 5.361, 95% CI 1.136–25.293; $p = 0.034$) were significant independent risk factors for intra-abdominal abscess formation (table 3).

Morphological Characteristic of Abscesses

We analyzed the morphological characteristics of abscesses in 15 patients. The most frequent site of abscess development was the peritoneal cavity in 9 patients (60%),

Table 1. Baseline characteristics of CD patients with and without abscesses

	Non-abscess	Abscess	p value
Observation period, months[a]	34 (18–53)	34 (31–59)	0.428
Age at CD onset, years[a]	22 (18–27)	24 (21–26)	0.499
Period after CD onset, months[a]	116 (60–192)	152 (80–272)	0.073
Period from CD onset to IFX, months[a]	68 (18–143)	106 (52–140)	0.109
CD location (ileal:ileocolonic:colonic)[b]	57:151:30	2:12:1	0.568
Montreal classification B (B1:B2:B3)[b]	78:75:85	2:3:10	0.015
Male:female[b]	176:62	12:3	0.766
Smoker:non-smoker[b]	194:44	12:3	0.766
Received IM (yes:no)[c]	137:101	9:6	0.853
Received steroid (yes:no)[b]	33:205	1:14	0.701
Received antibiotics (yes:no)[b]	230:8	13:2	0.112
Central venous catheter (yes:no)[b]	224:14	13:2	0.243
Anal lesion (yes:no)[c]	124:114	12:3	0.036
Seton (yes:no)[b]	237:1	14:1	0.115
Prior single operation (yes:no)[c]	105:133	4:11	0.186
Prior several operations (yes:no)[b]	42:196	1:14	0.478

[a] Mann-Whitney U test. [b] Fisher's exact test. [c] χ^2 test. Montreal classification: B1, non-stricturing, non-penetrating; B2, stricturing; B3, penetrating. Anal lesions (p = 0.036) and progression of intestine deformation (p = 0.015) were significantly more prevalent in the abscess group, but there were no between-group differences in the others.

Table 2. Blood test results in CD patients treated with IFX

Test	Week	Non-abscess median (Q1–Q3)	Abscess median (Q1–Q3)	Mann-Whitney U test p value
CRP, mg/dl	0	1.07 (0.28–3.43)	3.51 (0.69–5.67)	0.130
	14	0.10 (0.04–0.79)	2.27 (0.20–5.07)	0.021
TP, g/dl	0	7.0±0.7[a]	7.1±0.9[a]	0.952[b]
	14	7.3 (6.9–7.7)	7.0 (6.7–8.2)	0.700
Alb, g/dl	0	3.7 (3.2–4.0)	3.3 (2.6–3.7)	0.022
	14	4.1 (3.8–4.4)	3.7 (3.3–3.9)	0.004
TC, mg/dl	0	139±30[a]	130±19[a]	0.347[b]
	14	149 (130–170)	140 (124–151)	0.264
ALP, IU/l	0	240 (194–289)	257 (201–320)	0.301
	14	235 (194–285)	216 (159–282)	0.383
LDH, IU/l	0	134 (117–151)	120 (101–146)	0.151
	14	157 (136–181)	141 (115–174)	0.108
AST, IU/l	0	16 (13–21)	18 (15–22)	0.420
	14	19 (16–25)	14 (12–29)	0.108
ALT, IU/l	0	13 (9–24)	16 (10–26)	0.860
	14	17 (11–25)	10 (7–24)	0.058
WBC,/μl	0	6,100 (4,700–7,800)	5,750 (3,850–7,900)	0.564
	14	5,300 (4,300–6,400)	7,250 (4,450–7,650)	0.079
Hgb, g/dl	0	7.8±10.7[a]	9.4±10.1[a]	0.765[b]
	14	13.1 (11.6–14.0)	12.9 (10.3–13.4)	0.341

[a] Mean ± SD. [b] Unpaired t test. CRP concentration at week 14 was significantly higher and Alb at weeks 0 and 14 were significantly lower in the abscess group.

Table 3. Multivariate logistic regression analysis of intra-abdominal abscess factors

	Partial regression coefficient	p value	OR	95% CI
CRP at week 14 after using IFX	0.308	0.025	1.361	1.039–1.783
Loss of response within 6 months	1.679	0.034	5.361	1.136–25.293
Constant	–3.561	0.000		

χ^2 test p = 0.008; Hosmer-Lemeshow test p = 0.764; discriminative value = 91.8%. Serum CRP concentration at week 14 after IFX and loss of response to IFX within 6 months were significant independent risk factors.

with 7 having abscesses in the middle of the abdomen. Five patients (33.3%) had abscesses in the abdominal wall, and 1 each in the subphrenic and peristomal areas. Thirteen of these patients (86.7%) also had intestinal stenosis associated with abscess location (table 4), with 10 (76.9%) occurring in the colon or terminal ileum. Abscesses were detected soon after the start of IFX infusion, at 0–12 months in 6 (40.0%) patients. Abscesses in the others were detected at 13–24 months in 4 patients (26.7%), 25–36 months in 3 (20.0%), and 37–48 months in 2 (13.3%).

Prognosis

Of the 15 patients with abscesses, 14 were followed up: 11 (78.6%) underwent surgery and the other 3 (21.4%) received conservative therapy including antibiotics. Twelve patients (85.7%) were able to resume maintenance IFX, with IFX retention being higher in the surgery group than in the antibiotic monotherapy group (p = 0.016; fig. 3).

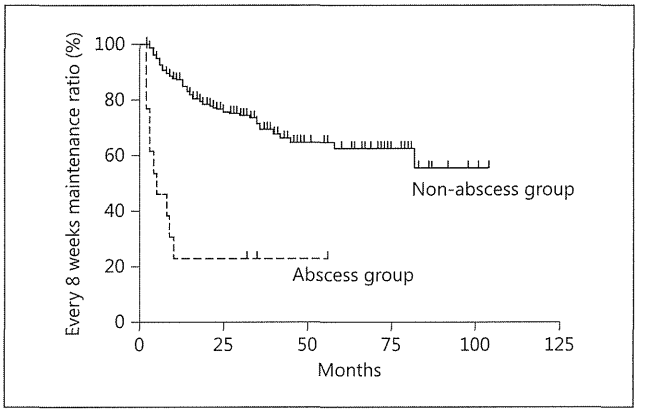

Fig. 2. Kaplan-Meier estimates for time to loss of response to IFX in CD patients with and without intra-abdominal abscesses. The abscess group had loss of IFX response at a median of 5 months, which was significantly shorter than in the non-abscess group (p < 0.0001, log-rank test).

Discussion

Factors affecting intra-abdominal abscess during IFX administration may be caused by insufficiency to control bowel inflammation. There are some possibilities including infusion reaction, bacterial or viral infection potential and imbalance between CD disease activity and IFX concentration for non-responders or loss of IFX response.

Normalization of serum CRP during IFX treatment may be a marker of maintained response [22, 23]. Cohort studies have also shown correlations between CRP concentration and CD disease activity during IFX treatment [24, 25]. As shown in table 2, serum CRP level after IFX administration differed significantly between the abscess and non-abscess groups, while we did not find any difference in CRP level before initiation of IFX in either group. We cannot predict the occurrence of abscess by CRP level before initiation of IFX, but our results suggested that monitoring of CRP is important to find the risk of abdominal abscess. In addition, our univariate analyses showed that Alb concentration during weeks 0 and 14 of IFX infusion was associated with abscess formation. Alb is a representative nutritional marker that is affected by gut condition. Although it did not differ significantly by multivariate logistic regression, Alb concentration may be associated with bowel damage, because it reflects the degree of nutrient absorption and barrier function that protects against gut protein loss.

With univariate analyses the existence of anal lesions was one of the risk factors for intra-abdominal abscess. It may indicate that such anal lesions mean a penetrating

Table 4. Morphological evaluation of abscesses and gut characteristics

	Patients, n (%)	Abscess location			Abscess scale median, cm^3	Intestinal stenosis, n (%)
		right	middle	left		
Peritoneal cavity	9 (60.0)	2	7		12[a]	8/9 (88.9)
Abdominal wall[b]	5 (33.3)	3		2	210	4/5 (80.0)
Subphrenic	1 (6.7)			1	10.6	1/1 (100.0)
Total	15 (100.0)	5	7	3	–	13/15 (86.7)

[a] Q1–Q3: 1.2–30.8. [b] Peristomal 1. The most frequent site of abscess development was the peritoneal cavity in 9 patients (60%), with 7 having abscesses in the middle of the abdomen. Five patients (33.3%) had abscesses in the abdominal wall, and 1 patient each in the subphrenic and peristomal areas. Thirteen of these patients (86.7%) also had intestinal stenosis associated with abscess location.

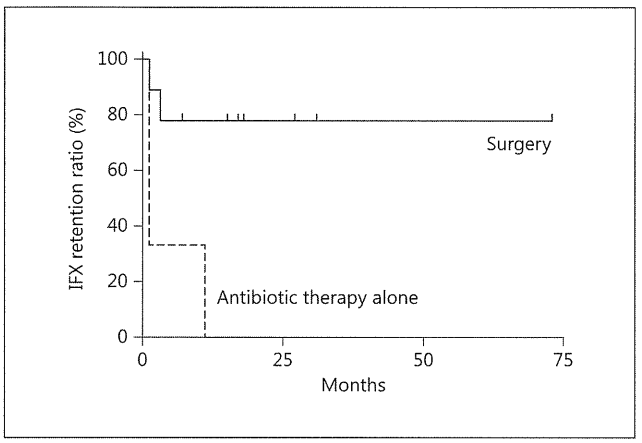

Fig. 3. Kaplan-Meier estimates for time to IFX retention in CD patients treated with or without surgery for intra-abdominal abscesses. Eleven patients who underwent surgery had a larger IFX retention ratio than 3 patients after antibiotic monotherapy (p = 0.016, log-rank test).

type in which deformation can easily cause an intra-abdominal abscess.

Our results showed that the intra-abdominal abscess incidence was 5.8%. Caspersen et al. [11] reported that 34 of 651 IBD patients who received IFX had an abscess (5.2%) in Denmark, 1999–2005, including some parts other than the intra-abdomen. Agrawal et al. [17] informed that 30 of 435 CD patients had an intra-abdominal/pelvic abscess (6.9%) in England, 1996–2003. Yamaguchi et al. [21] analyzed that the cumulative incidence of intra-abdominal abscess in the patients with CD at 10 and 20 years after the cumulative incidence was 9.0 and 25.0%, respectively. Other retrospective research about CD prognosis indicated an incidence of 128/2,236 (5.7%) [18] and 23/188 (16.7%) [19]. There is variability among institutions that may be based on the differences of observation period including therapy transition.

In assessing patients with intra-abdominal abscess, we found that abscess location was associated with bowel stenosis, which tended to occur in the terminal ileum and colon. The terminal ileum is a site of frequent CD inflammation, which can progress to bowel deformation. On the other hand, intra-abdominal abscesses in the colon have a greater microbial population than any other gut lesion that is prone to the development of inflammation. Bowel damage in CD depends on the duration of the disorder and the number of repeated relapse and remission cycles [1, 13, 26]. Although we found that intra-abdominal abscess was not associated with disease duration, along with perforating type, the intensification of inflammatory activity may have easily caused bowel deformation, suggesting that bowel damage may have indirectly induced intra-abdominal abscess.

Our study revealed that treatment with immunosuppressive agents, either corticosteroids or IMs, was not a risk factor for intra-abdominal abscess. A previous study, however, found that intra-abdominal abscess was associated with corticosteroids but not with IMs [17]. The difference may be due to patient selection in that we analyzed patients treated with IFX, whereas the previous study did not. We also did not mention the duration of IM and corticosteroid administration. We cannot deny the possibility that long-term use of these drugs affects the intra-abdominal abscess.

We found that surgical treatment was more effective than antibiotic monotherapy, allowing the resumption of IFX therapy. This finding suggests that treatment of CD with intra-abdominal abscess for IFX resurgence needs to eliminate not only the abscess but also bowel deformation. A previous retrospective cohort study found that surgery was more effective than antibiotic therapy [18], although another retrospective study showed that surgery and non-surgical treatment, including percutaneous aspiration or drainage, were equally effective [20]. In our study, all of our patients requiring drainage underwent surgery. Percutaneous drainage before surgery was shown to improve post-therapeutic complications [19], suggesting that drainage is effective for abscess. Our study indicates that appropriate surgical intervention for CD patients with intra-abdominal abscess can cause IFX re-administration and possibilities of lasting long-term improved prognosis including patients' quality of life.

Insufficient IFX concentration may be due to the presence of an anti-IFX antibody, also called human antichimeric antibody, or to massive inflammation in the gut environment, with the TNF concentration being too high to be controlled by normal IFX doses. In our study, we did not examine serum human antichimeric antibody and IFX concentration. This will be the subject of future investigation.

In summary, we found that abnormally high serum CRP concentrations 14 weeks after the start of IFX therapy and loss of IFX response in the early phase were risk factors for intra-abdominal abscess progression. Our results suggested that we should proactively carry out abscess screening in patients with ineffective IFX therapy.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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Serum microRNA levels in patients with Crohn's disease during induction therapy by infliximab

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Abstract

Background and Aim: microRNAs (miRNAs) have been suggested to be candidates for biomarkers in various diseases including Crohn's disease (CD). To identify possible biomarkers predictive of the therapeutic effect of infliximab in CD, we investigated serum miRNA levels during the induction therapy by the medication.

Methods: Nineteen CD patients who were applied to the induction therapy by infliximab were enrolled. Serum samples for miRNA analyses were obtained at weeks 0 and 6, and the therapeutic efficacy by infliximab was assessed according to the Crohn's disease activity index value at week 14. Exploratory miRNA profiling by low-density array was initially performed in three patients. The levels of candidate miRNA were subsequently determined by real-time polymerase chain reaction (PCR) assays in the remaining 16 patients. The miRNA levels during the induction therapy were compared between the two groups classified by the clinical response to infliximab at week 14.

Results: Low-density array analysis identified 14 miRNAs that showed twofold or more altered expression during the induction therapy by infliximab. Subsequent analysis by real-time PCR demonstrated significantly increased levels of five miRNAs (let-7d, let-7e, miR-28-5p, miR-221, and miR-224) at week 6 when compared with those at week 0 ($P < 0.05$ each). In addition, miRNA levels of let-7d and let-7e were significantly increased in the group of patients who achieved clinical remission by infliximab ($P = 0.001$ and $P = 0.002$, respectively).

Conclusion: let-7d and let-7e might be possible therapeutic biomarkers in patients with CD, who are treated by infliximab.

Introduction

Crohn's disease (CD) is a chronic inflammatory disease characterized by transmural inflammation involving any part of the gastrointestinal tract. If not promptly or adequately treated, the disease may cause various intestinal complications, such as stricture, perforation, fistula, and abscess, which may require surgical interventions. However, the advent of infliximab (IFX) has dramatically improved medical management in patients with CD, and the biological therapy has now become the mainstay for the induction and the maintenance therapy for the disease.[1] However, IFX has been reported to be ineffective for the induction of remission in one third of the patients.[2] Furthermore, loss of response to IFX occurs in up to 50% of CD patients during the maintenance therapy.[3] Hence, factors associated with less therapeutic effect of IFX have been rigorously investigated from the clinical,[4,5] immunological,[6,7] and genetic viewpoints.[8,9]

MicroRNAs (miRNAs) are endogenous small noncoding RNAs that lead to translational repression and degradation of target genes,[10] and they have been considered to play crucial roles in the pathophysiology of various diseases through multiple biological processes, such as cell differentiation, growth, and apoptosis.[11] Since miRNAs have also been detected as a stable form in human circulating blood,[12–15] circulating miRNAs have been noted as possible noninvasive biomarkers in various diseases such as cancer,[16,17] cardiovascular disease,[18] and autoimmune diseases.[19,20] As for inflammatory bowel diseases (IBDs), distinct miRNAs expression has been demonstrated in intestinal tissues of ulcerative colitis (UC) and CD patients,[21–24] with an emphasis of the association between miR-192 and the colonic cell derived chemokine MIP-2α (CXCL2).[21] Although Zahm *et al.* previously investigated the diagnostic potential of serum miRNAs in pediatric CD patients,[25] the pathophysiological role of serum miRNAs with a reference to a specific treatment has not been analyzed in adult CD patients.

In the present study, we investigated serum miRNA levels during the induction therapy by IFX in CD patients in order to identify possible biomarkers, which are predictive of the short-term therapeutic effect of IFX.

Methods

Patients and study protocol. This was a prospective single-center study conducted at our institution from February 2009 to March 2012. CD patients who were applied to induction therapy by IFX were the subjects of the present investigation. At the enrollment, patients who had a prior history of IFX and/or other biologics administration were excluded. Patients receiving concurrent treatment of immunosuppressive drugs or corticosteroid were included in the present study when the dosage had been stable for at least 8 weeks before IFX administration. The diagnosis of CD was based on clinical, radiological, endoscopic, and pathological findings using established criteria.[26]

The induction therapy was composed of the administration of IFX (5 mg/kg) at weeks 0, 2, and 6. All of the patients received a single dose of methylprednisolone (125 mg intravenously), acetaminophen (400 mg orally), and diphenhydramine (40 mg orally) before the administration of IFX so that the risk of an immediate infusion reaction could be minimized. Patients were followed for 14 weeks after the first administration of IFX, and the therapeutic efficacy of the induction therapy was assessed at the end of the follow up. Patients who could achieve a Crohn's disease activity index (CDAI) value less than 150 at week 14 were classified into the remission group (R-group). Patients who had CDAI value of 150 or more at week 14, or who required a rescue therapy before week 14, were classified into no-remission group (N-group). Blood samples for routine laboratory tests were obtained at each clinical visit, and those for miRNAs analyses were obtained at weeks 0 and 6.

The study protocol was approved by the ethical committee at Kyushu University Hospital, and the study was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all the enrolled subjects.

Preparation of serum samples and total RNA extraction. After stabilizing at room temperature for at least 30 min, blood samples were centrifuged at 1600× g for 15 min at 4°C. Isolated sera were aliquoted into microcentrifuge tubes and stored at −80°C until further procedure.

Two hundred fifty microliter of each serum was thawed on ice and mixed thoroughly with 750 μL of Isogen-LS (Nippongene, Toyama, Japan). After incubation at room temperature for 5 min to deactivate the RNase function completely, 250 amol (total volume of 5 μL) of synthetic *Caenorhabditis elegans* miRNA cel-miR-39 (synthesized by Hokkaido System Science, Sapporo, Japan) were added to each denatured sample as the spiked-in control.[13] Two hundred microliter of chloroform were added and tubes were shaken vigorously, then samples were centrifuged at 12 000× g for 15 min at 4°C. Thereafter, about 500 μL of aqueous phase were carefully transferred into new tubes and mixed with 1.5 volumes of 100% ethanol. RNA purification was performed using miRNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and the final elution volume was 50 μL.

Serum miRNAs profiling by low-density array platform. Three microliter of total RNA was mixed with Taqman microRNA Reverse Transcription Kit and Megaplex RT primers Human pool A v2.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's recommendation. For reverse transcription, mixture was set on GeneAmp PCR system 9700 (Applied Biosystems) as follows: 40 cycles of 16°C for 2 min, 42°C for 1 min, and 50°C for 1 s, then incubation at 85°C for 5 min. Generated complementary DNA was pre-amplified by Megaplex PreAmp primer, Human Pool A, and PreAmp Master Mix (Applied Biosystems) according to the manufacturer's instructions. The pre-amplification reaction was performed by the incubation of samples, consisted of 25-μL mixture, at 95°C for 10 min, 55°C for 2 min, 72°C for 2 min, followed by 12 cycles of 95°C for 15 s and 60°C for 4 min. After the pre-amplification cycle, samples were heated at 99.9°C for 10 min to deactivate the enzyme function, then held at 4°C. Pre-amplified products were diluted by 0.1 × Tris-EDTA (pH 8.0) to a final volume of 100 μL.

The expression profile of miRNAs was determined by Taqman Array Human MicroRNA A Card v2.0 (Applied Biosystems), which is capable of detecting 377 mature human miRNAs based on real-time polymerase chain reaction (PCR) technique. Reaction solution consisted of 450 μL of TaqMan Universal Master Mix II No UNG (Applied Biosystems), 9 μL of diluted pre-amplified product and 441 μL of nuclease-free water. Reaction was carried out on 7900HT Fast Real-Time PCR System (Applied Biosystems) with manufacturer's recommended program.

Validation by real-time PCR. Taqman microRNA Reverse Transcription Kit and Taqman miRNA Assays (Applied Biosystems) were used for individual miRNAs tests. Reverse transcription and real-time PCR were performed in the scaled-down condition. In brief, 2.5 μL of total RNAs were mixed with 0.75 μL of 10 × reverse transcription buffer, 0.095 μL of RNase inhibitor, 0.075 μL of dNTPs with dTTP, 0.5 μL of Multiscribe reverse transcriptase, 1.5 μL of miRNA-specific stem-loop RT primer, and 2.08 μL of nuclease-free water. The mixture was incubated at 16°C for 30 min, 42°C for 30 min, and 85°C for 5 min. After preserving at −30°C for several days, 0.67 μL of generated complementary DNA solution was mixed with 5.0 μL Taqman Universal Master Mix II No UNG, 0.5 μL of primer and probe set, and 3.83 μL of nuclease-free water. Real-time PCR was performed on 7500 Real-Time PCR System (Applied Biosystems) at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Each reaction was performed in triplicate. The cycle threshold (C_T) values were calculated with SDS 2.0.5 software and each miRNA level was normalized to the level of cel-miR-39.

Statistical analysis. Clinical characteristics were compared using Fisher's exact test, chi-square test, or Mann–Whitney *U*-test where appropriate. Paired samples within the same subjects were compared by Wilcoxon signed-rank test, while unpaired samples were compared by Mann–Whitney *U*-test. Spearman's rank correlation coefficient was used for the assessment of correlation between the continuous variables. A P value less than 0.05 was regarded as statistically significant for each test.

Results

Clinical characteristics of CD patients. We recruited 21 Japanese CD patients for the present study. However, two patients were excluded, one for acute pancreatitis after the second infusion of IFX, and the other for severe hemolysis of the collected sample. Thus, the remaining 19 patients were the subjects of the present analyses. Among these subjects, we chose three newly diagnosed CD patients for exploratory array test, who responded to the induction therapy by IFX alone. In the patients, the mean CDAI value decreased from 186.5 ± 10.8 at baseline to 61.1 ± 14.8 at week 6. The mean C-reactive protein (CRP) levels decreased from 0.56 ± 0.24 at baseline to 0.11 ± 0.03 at week 6. The other 16 patients were allocated to the validation experiment by real-time PCR.

Table 1 summarizes baseline characteristics of the validation cohort. Of the 16 patients, the induction therapy by IFX resulted in clinical remission in 11 patients (R-group), while the remaining five patients failed to achieve clinical remission by IFX (N-group). Although there were no significant difference of CDAI values and serum CRP levels at baseline between two groups, the time after initial diagnosis of CD was significantly longer in N-group than in R-group ($P = 0.005$). In addition, patients having history of intestinal resection were more frequent in N-group than in R-group ($P = 0.013$). There was a trend toward a higher frequency of antibiotics therapy in N-group; however, no significant difference was observed between the two groups concerning other medical treatment.

Table 2 shows CDAI values and serum CRP levels of R-group and N-group at each time of assessment. Although serum CRP levels at weeks 2 and 6 temporarily decreased in N-group ($0.05 < P < 0.1$), the value regained at week 14. CDAI values in N-group were not different at each assessment period.

Screening for candidate miRNAs. Exploratory miRNAs profiling by low-density array platform was initially performed using six samples obtained from three subjects (at weeks 0 and 6). When C_T values < 33 were regarded as positive, the profiled data identified 126 miRNAs at least in four samples. We

Table 1 Patients' characteristics of the validation cohort

	R-group ($n = 11$)	N-group ($n = 5$)	P value
Gender, male/female	11/0	3/2	0.083
Age (years)	24 (17–38)	29 (23–69)	0.053
Time after initial diagnosis of CD (years)	0.5 (0.2–8)	12 (4–39)	0.005
Involved site			0.14
Ileum	7	1	
Ileum and colon	3	4	
Colon	1	0	
History of intestinal resection	1	4	0.013
Fistula	0	1	0.31
Perianal disease	7	3	1.0
Current smoking	3	1	1.0
Treatment			
5-aminosalicylates	11	4	0.31
Immunosuppressants	3	2	1.0
Antibiotics	1	3	0.063
Nutrition therapy	7	3	1.0
CDAI	183.9 (83.2–341.7)	197.7 (153.7–350)	0.61
CRP (mg/dL)	0.36 (0.07–8.18)	3.63 (0.02–5.75)	1.0

Parametric data are expressed as median (range).
CDAI, Crohn's disease activity index; CRP, C-reactive protein; N-group, no-remission group; R-group, remission group.

Table 2 Clinical course during the induction therapy by infliximab

	Week 0 (baseline)	Week 2	Week 6	Week 14
CDAI				
R-Group	183.9 (83.2–341.7)	73.3 (55.5–144)***	60.1 (29.1–97)***	59.4 (14.8–110.7)***
N-Group	197.7 (153.7–350)	155.7 (127.2–406.2)	165.4 (111–386.4)	195.2 (154.6–291.1)
CRP (mg/dL)				
R-Group	0.36 (0.07–8.18)	0.05 (0.01–0.45)**	0.04 (0.01–0.37)***	0.05 (0.01–2.6)*
N-Group	3.63 (0.02–5.75)	0.18 (0.01–0.65)	0.11 (0.01–5.03)	0.7 (0.01–3.87)

Data are expressed as median (range). Values are compared to those at week 0 (baseline) using Wilcoxon signed-rank test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$.
CDAI, Crohn's disease activity index; CRP, C-reactive protein; IFX, infliximab; N-group, no-remission group; R-group, remission group.

subsequently identified 14 miRNAs that showed altered signals by twofold or more when compared between the samples of weeks 0 and 6. The relative values of 14 miRNAs at week 6 are listed in Table 3.

These miRNA levels were additionally measured by real-time PCR using the same samples in order to compare the values of array profiling. As shown in Figure 1, the values determined by two different methods showed an excellent agreement ($\rho = 0.838$, $P < 0.0001$).

Table 3 miRNAs showing altered expression during the induction therapy by infliximab

Increased miRNAs		Decreased miRNAs	
miRNA	Fold change	miRNA	Fold change
miR-361-5p	4.49	miR-324-3p	0.46
miR-127-3p	4.00	miR-486-3p	0.48
miR-28-5p	3.46		
miR-539	2.79		
miR-224	2.77		
let-7e	2.64		
miR-221	2.56		
miR-125a-5p	2.28		
miR-485-3p	2.27		
let-7d	2.23		
miR-199a-3p	2.11		
miR-223	2.02		

Data are shown as average fold change of miRNA levels of week 6 relative to those of week 0 ($n = 3$).

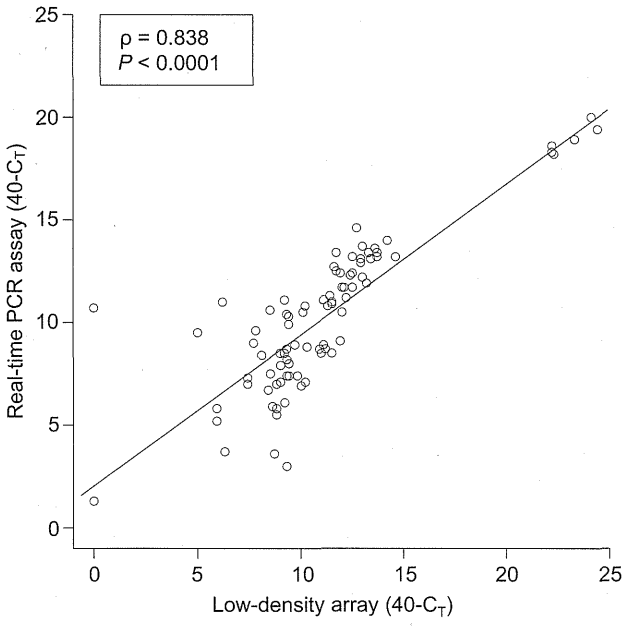

Figure 1 Comparison of cycle threshold (C_T) values by low-density array and real-time polymerase chain reaction (PCR). Fourteen candidate microRNAs (miRNAs) elicited by low-density array were additionally measured by real-time PCR using the same samples. Significantly positive correlation was found by Spearman's rank correlation coefficient.

Assessment of candidate miRNAs by real-time PCR. The 14 candidate miRNA levels were further analyzed by real-time PCR using the samples of the other 16 CD patients. When C_T values < 35 were regarded as positive, detection rates of miR-485-3p and miR-539 were low (72% and 28%, respectively). We thus excluded these two miRNAs from the following validation analysis. As shown in Figure 2, the relative values of five miRNAs (let-7d, let-7e, miR-28-5p, miR-221, and miR-224) at week 6 were significantly higher than those at week 0 ($P < 0.05$). There was a trend toward a higher value of miR-125a-5p at week 6 when compared with the value at week 0. However, the difference did not reach a statistical significance ($P = 0.051$). No obvious difference was observed between the values at weeks 0 and 6 in the remaining six miRNAs.

Correlation of miRNAs expression with inflammatory parameters at baseline. In order to ascertain possible correlation of miRNA levels with inflammatory parameters, we compared miRNA levels with CDAI values, serum CRP levels, white blood cell counts, and platelet counts at week 0. As shown in Table 4, neither CDAI values nor serum CRP levels showed significant correlation with miRNA levels at week 0. Other inflammatory parameters, including white blood cell counts and platelet counts, did not show any correlation with miRNA levels (data not shown).

Correlation of miRNA expression with therapeutic efficacy by IFX. We subsequently analyzed the incremental ratio of five miRNAs according to the therapeutic efficacy by IFX. As indicated in Figure 3A, the incremental ratio of let-7e was statistically significant in R-group when compared with that in N-group (median fold change: 1.68 *vs* 0.68, $P = 0.031$). The incremental ratio of let-7d tended to be higher in R-group than in N-group, whereas the difference did not reach a statistical significance (median fold change: 1.57 *vs* 0.83, $P = 0.089$).

Figure 3B shows the individual expression levels of let-7d and let-7e at weeks 0 and 6. Although significantly increased expression of let-7d and let-7e at week 6 were observed in R-group ($P = 0.001$ and 0.002, respectively), such a trend was not observed in N-group.

Discussion

Several factors associated with therapeutic effect of IFX have been rigorously investigated from clinical, immunological, and genetic viewpoints.[4–9] Consequently, some clinical biomarkers, including CRP level,[6] have been suggested to be possible candidates. However, because clinical biomarkers do not show similar trends in serial changes, it remains unclear whether they are inevitably reliable. In this regard, we investigated the association of miRNAs with therapeutic effect of IFX in CD. Considering miRNAs to have potential pathophysiological roles in various diseases, they are provisionally plausible targets for this kind of analysis.

In the present study, we investigated serum miRNA levels in CD patients, and detected five miRNAs (let-7d, let-7e, miR-28-5p, miR-221, and miR-224) that demonstrated significant increases during the induction therapy by IFX. Among them, two miRNAs (let-7d and let-7e) were shown to have a similar expression pattern

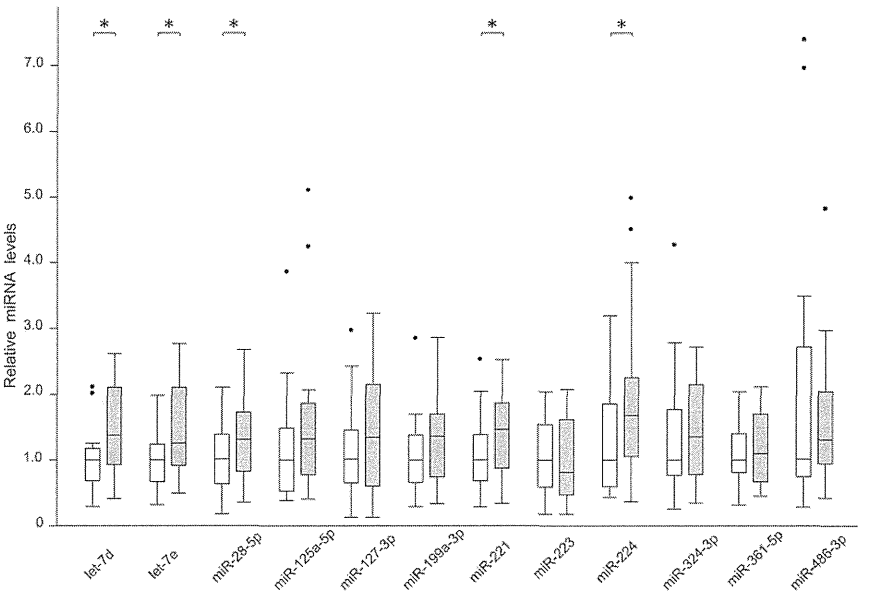

Figure 2 Relative microRNAs (miRNA) levels of week 0 and week 6 in validation cohort ($n = 16$). Relative expression levels of each miRNA are shown as ratio to the median value of week 0. Boxes indicate the interval between the 25th and 75th percentiles, and horizontal bars inside boxes indicate median. Whiskers indicate the interval of data within 1.5 × interquartile ranges (IQR). Closed circles indicate data points outside 1.5 × IQR. miRNA expression levels of week 0 (white boxes) and week 6 (gray boxes) were compared by Wilcoxon signed-rank test. $*P < 0.05$.

Table 4 Correlation between miRNAs and clinical parameters at baseline

miRNA	CDAI		CRP	
	ρ	*P* value	ρ	*P* value
let-7d	0.17	0.53	0.37	0.16
let-7e	0.12	0.65	0.39	0.13
miR-28-5p	0.08	0.77	0.19	0.49
miR-221	0.22	0.42	0.14	0.61
miR-224	0.17	0.53	0.02	0.94

Correlation is assessed by Spearman's rank correlation coefficient. CDAI, Crohn's disease activity index; CRP, C-reactive protein.

according to the therapeutic effect of IFX. Because miRNA levels at baseline did not show any correlation with inflammatory parameters including CDAI and CRP, it can be possible that let-7d and let-7e are novel biomarkers closely associated with therapeutic efficacy of induction therapy by IFX.

Let-7d and let-7e are the members of the let-7 family that contain several miRNAs with high sequence homology.[27] Although biological functions of let-7 miRNAs have been reported to range widely,[28,29] recent studies have demonstrated a significant role of let-7 miRNAs in the regulation of apoptosis through the inhibition of Fas[30,31] and Bcl-xL.[32] It has been demonstrated that a decrease in Bax expression with concurrent higher Bcl-xL/Bax ratio occurs in the lamina propria T lymphocytes from CD patients, and that such imbalances of pro-apoptotic and anti-apoptotic Bcl-2 protein family contribute to resistance to apoptosis and to chronicity of inflammation.[33,34] Because the anti-inflammatory effect of IFX is partly attributed to the induction of apoptosis of lamina propria T lymphocytes[35–37] and monocytes,[38] and because IFX induces apoptosis of such effector immune cells through mediating the expressions of Bcl-2 protein family,[37–39] let-7 miRNAs seem to play a certain role via controlling apoptosis of effector immune cells. Considering their function for the inhibition of Bcl-xL, let-7 miRNAs may assist pro-apoptotic effect of IFX with the restoration of the balance of Bcl-2 protein family in inflammatory cells, thus resulting in positive association with therapeutic efficacy by IFX. In addition, 3′-untranslated region of IL-23R gene has been recently disclosed to induce loss of let-7e/f regulation and enhanced protein production in IBD patients.[40] Because a close association of IL-23R in the pathogenesis of CD has been demonstrated from genetic[41] and immunological aspects,[42] an association between IL-23R and let-7e/f further imply the contribution of let-7 miRNAs in CD.

miR-28-5p, miR-221, and miR-224 increased significantly during the induction therapy by IFX. However, we failed to demonstrate positive association of those miRNAs with the therapeutic efficacy by IFX. Among these miRNAs, the upregulation of miR-221 has been reported to have an association with liver fibrosis. Furthermore, possible contribution of miRNAs to intestinal fibrosis of CD has been recently reported.[43] Since the induction therapy by IFX can cause dramatic mucosal healing of inflamed intestine, altered expression of miR-221 during the induction therapy by IFX may be representative of the regenerative process of luminal CD. In addition, an increase in the expression of miR-28-5p has been shown in blood samples from UC patients.[44,45] It thus can be possible that these miRNAs are subclinical biomarkers predictive of the therapeutic efficacy of IFX.

In addition to the identification of 11 miRNAs as possible diagnostic biomarkers in pediatric CD patients, Zahm *et al.* also showed decreases in the expression of several miRNAs after medical treatments for 6 months.[25] However, we failed to show any association of those miRNAs with the efficacy of IFX as the induction therapy. Such contradictory results may be caused by the following two reasons. First, the subjects recruited by Zahm *et al.* were pediatric CD patients, who generally manifest widespread involvement and rapid disease progression when compared with adult CD patients.[46,47] Furthermore, growth failure may have altered the pattern of miRNA expressions in such pediatric

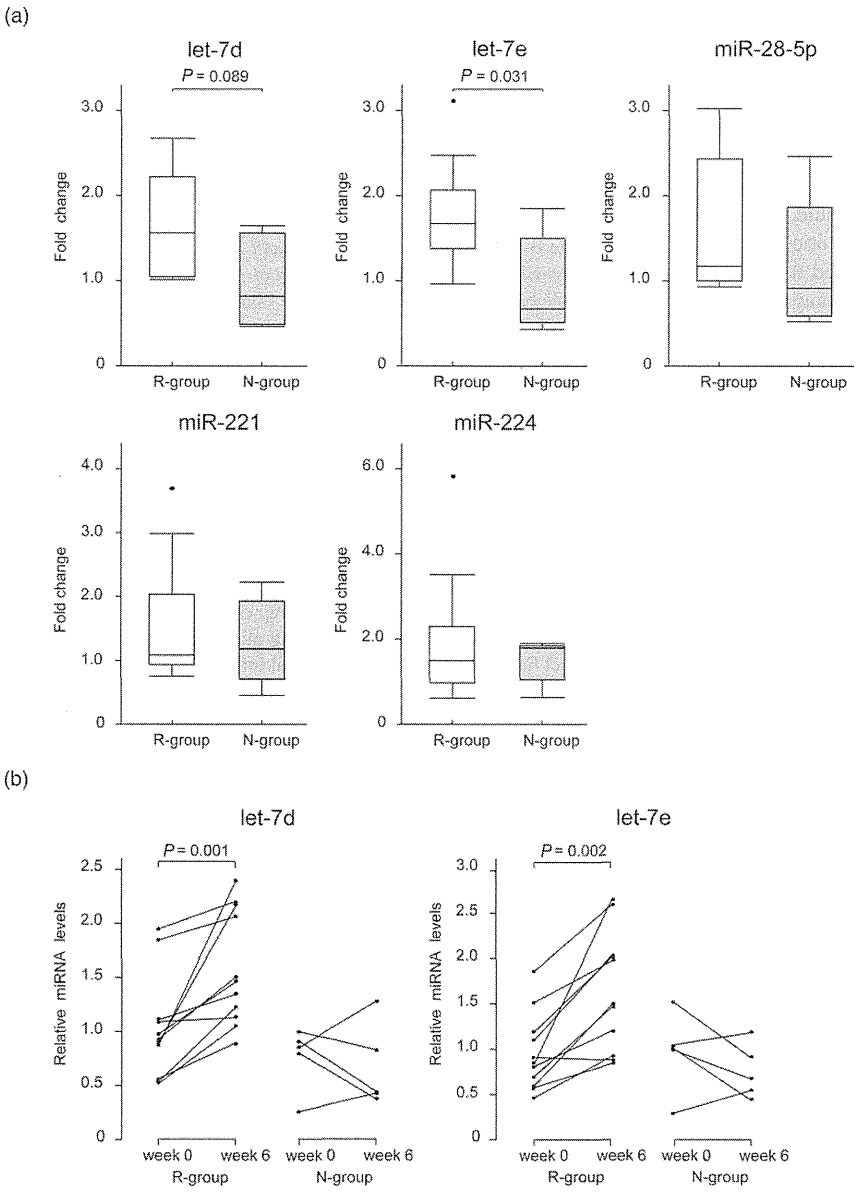

Figure 3 Relative change of microRNAs (miRNA) levels according to therapeutic efficacy by infliximab (IFX). (a) Comparison of relative change of miRNA levels between R-group and N-group. Data are shown as ratio of miRNA levels of week 6 relative to those of week 0. Boxes indicate the interval between the 25th and 75th percentiles, and horizontal bars inside boxes indicate median. Whiskers indicate the interval of data within 1.5 × interquartile ranges (IQR). Closed circles indicate data points outside 1.5 × IQR. Mann–Whitney *U*-test identified significantly greater change of let-7e in R-group than in N-group. (b) Comparison of let-7d and let-7e levels of week 0 and week 6 in R-group and N-group. Wilcoxon singed-rank test showed significantly higher levels of let-7d and let-7e of week 6 when compared with the values of week 0 in R-group.

CD patients. Second, since we aimed to identify miRNAs in association with therapeutic efficacy of IFX, the nomination of candidate miRNAs was methodologically different from that of the previous investigation. It thus seems likely that miRNAs identified in the present study are distinctive with respect to clinical implication and they are specific to the therapeutic efficacy of IFX in adult CD patients.

The present study has several limitations. First, we failed to validate twofold or more altered expression of nine miRNAs (miR-125a-5p, miR-127-3p, miR-199a-3p, miR-223, miR-324-3p, miR-361-5p, miR-485-3p, miR-486-3p, and miR-539) that were initially elicited by low-density array. A possible explanation of the inconsistent results would be the small number of subjects allocated for both analyses. Another source of the inconsistency might be a relatively small amount of extracted RNAs especially for low-abundance targets, although miRNA levels determined by low-density array and real-time PCR showed excellent agreements (Fig. 1). On the other hand, since let-7d and let-7e demonstrated sufficient levels of signal intensity (range of C_T values: from 25 to 29), we believe that the significant association of these miRNAs with therapeutic efficacy of IFX is reliable. Second, the number of enrolled patients was small so that some of the patients' demographics were significantly different between our R-group and N-group. Because our present study was a pilot one, a well-designed study with a large sample size is mandatory to explore valid biomarkers predictive of therapeutic effect of IFX. Third, we did not measure miRNA levels in healthy subjects. Accordingly, the increased let-7d/e levels in R-group do not necessarily indicate the augmentation of those miRNA functions. However, considering the significant increase in let-7d/e levels in R-group compared

with those in N-group, it seems plausible that the increased levels of those miRNAs can be a possible therapeutic biomarker for the induction therapy of IFX. Finally, we did not specify the source of serum miRNAs. Since miRNAs from different cell types seem to have different biological consequences for the development of CD,[22,44] simultaneous analyses of miRNAs expression levels using intestinal tissues and peripheral blood cells ought to be necessary. However, we believe that the present study could demonstrate important implications with regard to the therapeutic mechanisms of IFX because this is the first study that showed the distinctive expression patterns of the serum miRNAs according to the therapeutic efficacy by IFX.

In conclusion, our study demonstrated differences in serum expression of let-7d and let-7e according to the therapeutic efficacy by IFX. Although the other three miRNAs (miR-28-5p, miR-221, and miR-224) also showed increases in their expression during the induction therapy, the biological implication of those miRNAs remains unclear. The accumulation of studies focusing on biological and clinical roles of miRNAs may reveal further pathophysiological mechanisms in CD.

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Clinicopathologic features of chronic nonspecific multiple ulcers of the small intestine

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Abstract

Chronic nonspecific multiple ulcers of the small intestine is a rare but distinct clinical condition, characterized by multiple small intestinal ulcers of nonspecific histology and chronic, persistent gastrointestinal bleeding without nonsteroidal anti-inflammatory drug use. However, because of the term "nonspecific" in its nomenclature, some gastroenterologists have misinterpreted the disease as the condition with small intestinal ulcers caused by undetermined etiologies without considering clinical features. Such misinterpretation led to the heterogeneity of clinicopathologic features of the disease, as well as to the ambiguity of possible genetic contribution. It thus seems necessary to recognize clinical entity of the disease precisely to avoid misinterpretation. In this review, we describe clinicopathologic features, differential diagnosis, and possible genetic contribution of the disease.

Introduction

The clinical application of capsule endoscopy (CE) and balloon-assisted enteroscopy (BAE) has led to a chance of encountering various small intestinal pathologies [1-3]. Among conditions manifesting small intestinal ulcers, Crohn's disease, intestinal tuberculosis, Behçet's disease, and nonsteroidal anti-inflammatory drugs (NSAIDs) enteropathy are the conditions predisposing to chronic or recurrent small intestinal ulcers, while there still remains cases with unknown etiologies.

We previously reported on a peculiar form of enteropathy of an unknown etiology, referred to as chronic nonspecific multiple ulcers of the small intestine (CNSU), which is characterized by chronic blood and protein loss through persistent small intestinal ulcers [4-6]. Although this rare but distinct clinicopathologic condition was initially reported in the Japanese literature by Okabe and Sakimura in 1968 [7], the term "nonspecific" in its nomenclature which is based on nonspecific histology of the ulcers occasionally led to the misinterpretation of the disease as the condition with small intestinal ulcers of undetermined etiologies. In Western countries, while clinicopathologic features seem different from CNSU, Perlemuter et al. [8] reported a similar enteropathy, termed "cryptogenic multifocal ulcerous steno sing enteritis (CMUSE)". In addition, a recent review handled CNSU to be the identical to CMUSE [9]. Thus, precise recognition of clinical entity of CNSU is mandatory to avoid misinterpretation. In this review, we describe clinicopathologic features, differential diagnosis, and possible genetic contribution of the disease.

Clinicopathologic Features

Demographics, clinical symptoms, and laboratory data

Table 1 summarizes clinical features of 16 cases who were determined to have CNSU. The disease predominantly occurs in females, and the ages of disease onset ranged from 7 to 53 years. Clinical symptoms of CNSU are mainly attributed to chronic and persistent blood loss from small intestinal ulcers. Patients usually manifest fatigue, edema, or abdominal pain, while they rarely complain diarrhea, hematochezia, or fever. Although most patients had histories of long-term anemia, patients visit gastroenterologists long after the disease onset because of their uncertain abdominal symptoms, resulting in the delay of the diagnosis of CNSU [6,7,10].

Laboratory data also reflect persistent blood loss from the ulcers. Fecal occult blood tests are continuously positive and peripheral blood test shows hypochromatic and microcytic anemia. Most patients also manifest hypoproteinemia and hypoalbuminemia. However, acute inflammatory reactions, including C-reactive protein, α 1- and α 2- globulins, are usually within normal ranges or slightly increased [6,7]. Leukocyte and platelet counts also are within normal ranges.

Small intestinal findings

The small intestinal ulcers in CNSU occur predominantly in the ileum, while the terminal ileum is usually intact [7,10]. The ulcers occur in multiple (usually >20), and each lesion manifests shallow and flat ulcer bed surrounded by a discrete margin.

The configuration of each ulcer is usually linear or tall triangle, and the ulcer is aligned circularly or obliquely. The ulcers occasionally fuse, thus showing geographic configuration. Such small intestinal lesions are radiographically depicted as multiple rigidity or eccentric deformities (Fig 1a,b). Sharply demarcated barium flecks can also be depicted using compression study or double-contrast barium study, however, BAE is more suitable for the