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Original Article

Non-invasive Monitoring of Pouchitis After Total Proctocolectomy Using Fecal Calprotectin Levels

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Summary: ***Background*:** Fecal calprotectin (FC) is the most widely used marker for evaluating the disease activity of ulcerative colitis (UC). However, studies on FC in pouchitis after total proctocolectomy are scarce. We aimed to examine the correlations between the FC level and clinical findings and Pouchitis Disease Activity Index (PDAI) in UC patients who underwent total proctocolectomy (TP) with ileal pouch-anal canal anastomosis (IPAA) or ileal pouch-anal canal anastomosis (IACA).

***Methods*:** Between April 2008 and March 2018, 15 patients, consisting of 8 males and 7 females, with an average age at operation of 46.5 years, participated in this study. The average observation period was 68.3 months. The subjects underwent FC level measurements and endoscopic examinations.

***Results*:** The mean FC level was 418.69 μg/g (range: 10-1650 μg/g). Pouchitis was found in one (6.6%) patient, as detected by endoscopy. Among the 15 cases, FC levels were positively correlated with white blood cell count as well as albumin and C-reactive protein levels. There was a significant positive correlation between the PDAI score and FC levels ($p<0.05$). The median FC level was 111 mg/g in those with pouchitis, which was significantly higher than the 16 mg/g in those without pouchitis ($p<0.05$). Moreover, a significant positive correlation was found between the endoscopic findings of inflammation and FC levels ($p<0.00005$).

***Conclusion*:** FC levels were correlated with the PDAI score, blood testing data, and endoscopic findings, suggesting that the FC level could be a useful index of postoperative pouchitis and ileal pouch condition in patients undergoing TP with IPAA as UC treatment.

Keywords calprotectin, feces, PDAI, pouchitis, ulcerative colitis

INTRODUCTION

Total proctocolectomy with ileal pouch-anal anastomosis (IPAA) or ileal pouch-anal canal anastomosis (IACA) is the surgery of first choice for ulcerative colitis (UC)[1]. IPAA is currently accepted as the standard method for restoring continence after total proctocolectomy for medically refractory UC and familial adenomatous polyposis [2]. The operation is associated with considerable morbidity, with leakage and pelvic sepsis being the most serious complications, which occur in 6%–25% of patients [3,4]. Pouchitis is the most common late complication, with at least one episode experienced within 10 years after the surgery by 25%–48% of patients, and pouch failure requiring permanent ileostomy was observed in 3%–17% of the patients [5-7].

Pouchitis is a non-specific inflammatory condition

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Abbreviations: Alb, albumin; CRP, C-reactive protein; FC, fecal calprotectin; IACA, ileal pouch-anal canal anastomosis; IPAA, ileal pouch-anal anastomosis; PDAI, pouchitis disease activity index; WBC, white blood cell.

in the ileal pouch reservoir [8], with an unknown etiology. Some evidence suggests that inflammation could be caused by abnormal mucosal immune responses to the altered microflora in the ileal pouch. However, inflammatory cytokines, including interleukin-6 and tumor necrosis factor-α, are known to be released in the inflamed mucosa [9] and considered thus to be secondary to the development of pouchitis.

There is an increasing use of non-invasive biomarkers for the diagnosis and management of inflammatory bowel disease (IBD). One such biomarker is calprotectin, which is a neutrophil cytosolic protein with antimicrobial properties. It is presented at an increased concentration in feces during bowel inflammation [10]. This fecal calprotectin (FC) level is a highly sensitive and specific biomarker for detecting intestinal inflammation [11]. FC has shown a close correlation with endoscopic findings of inflammation in patients with IBD [12,13]. Additionally, elevated FC levels have been associated with a subsequent clinical relapse in patients with IBD [14] and may, therefore, be useful in predicting a clinical relapse [15,16]. However, studies on FC in pouchitis after total proctocolectomy are scarce [17,18]. In this study, we examined the correlations between the FC level and clinical symptoms, blood inflammatory parameters, and the Pouchitis Disease Activity Index (PDAI), including clinicopathological findings [19], in UC patients who underwent total proctocolectomy with ileostomy closure and IACA or IPAA.

PATIENTS AND METHODS

Study design

This prospective, single-center study was conducted at Kurume University Hospital in accordance with good clinical practice and the Helsinki Declaration. Written informed consent was obtained from all patients, and the study was approved by the Research Ethics Committee for Human Subjects at Kurume University School of Medicine (Approval No. 09315).

Patients

A total of 29 patients who were histologically diagnosed with UC and had undergone total proctocolectomy with ICAA or IPAA during the period from April 2008 to March 2018 at Kurume University Hospital were included in this study. At 3 months after restorative proctocolectomy, ileostomy closure was performed. These patients had no pouch-related complications (such as anastomotic leakage, pelvic abscess, pouch fistula, or others). Among the 29 patients, 15 provided informed consent to participate in this study and underwent FC measurements and endoscopic examinations. They were also asked to answer survey questionnaires, consisting of subjective questions on their clinical symptoms and postoperative conditions.

Clinical follow-up

All patients were evaluated in our hospital every 2 months after the ileostomy closure to monitor their clinical symptoms, such as stool frequency, rectal bleeding, abdominal pain, fever, and fecal urgency. We also measured blood inflammatory parameters, including white blood cell (WBC) and platelet counts as well as hemoglobin, C-reactive protein (CRP), and albumin levels.

Collection of stool samples

The stool samples were collected on the day of colonoscopy, or a few days prior to colonoscopy and stored in a refrigerator until the day of the colonoscopy. Upon receipt in the laboratory, all stool samples were registered and stored at -20 degrees Celsius.

Measurement of FC level

The FC level was measured by using a quantitative enzyme-linked immunosorbent assay (fCAL™ ELISA; Bühlmann Laboratories AG) according to the manufacturer's protocol.

Endoscopic examination

All patients underwent follow-up endoscopy at 12 months after ileostomy closure. Moreover, an endoscopic examination was performed immediately whenever patients presented any clinical signs suggestive of pouchitis, such as increased stool frequency, urgency, tenesmus, incontinence, abdominal pain, and nocturnal seepage. During endoscopy, biopsy specimens were taken from the ileal pouch. Then, the endoscopic and histological findings were assessed according to the clinicopathological factors of the PDAI [19]. Pouchitis was diagnosed if the PDAI score was ≥ 7.

Statistical analysis

Comparisons of frequencies were analyzed using Spearman's correlation. Differences between median values were compared using the Mann-Whitney U test. The change in median values with time was evaluated using the Wilcoxon signed rank test. Correlations were calculated by using Spearman's rank correlation coefficient (ρ) and $p<0.05$ was considered to be statistically significant.

RESULTS

Patients

A total of 15 patients, including 8 male and 7 female, with an average age at operation of 46.5 years (range: 23-98 years) were included in the study. The average observation period was 68.3 months (range: 2-567 months). The indication for surgery was internal medical treatment resistance in seven patients, colitis-associated cancer in six patients, general severe deterioration in one patient, and an emergency in one patient (Table 1).

Measurement of FC level and endoscopic findings

Patients were closely monitored using FC measurements and endoscopic examinations. The mean FC level was 418.69 μg/g (range: 10-1650 μg/g) (Table 2) (Figure 1). In the 15 patients, the FC levels were positively correlated with WBC count and albumin and CRP levels. There was a significant positive correlation between FC level and PDAI score ($p<0.0000001$)

Endoscopic examinations during follow-up revealed that one patient (7%) had developed pouchitis (PDAI score of 8). In this case, the interval from the ileostomy closure until the diagnosis of pouchitis was 2 months. Two patients (14%) further developed some clinical symptoms suggestive of pouchitis, but the endoscopic examination did not find any apparent inflammation in the ileal pouch, and, in each of them, the PDAI score was <7. In the other 12 patients, the median PDAI score was 3 (range: 0-6). No patient developed Crohn's disease in the ileal pouch during the observation period. Among the 15 patients, endoscopic examinations showed no abnormal findings in five patients, mild inflammation and superficial erosion in eight patients, and marked inflammation and small ulcerations in two patients (Table 2) (Figure 1).

TABLE 1.
Clinical and background characteristics of patients enrolled in this study

Male/female	8 / 7
Average age at operation	46.5 years
Duration of UC before IPAA	68.3 months
Operative indications	
Resistant to conventional medical management	7
Dysplasia and/or cancer	6
Severe	1
Fulminant	1
Average follow-up duration after stoma closure	66 months
Endoscopic findings of the pouch after operation	
No definite abnormal findings	5
Erosion	8
Small ulcer	2
Endoscopic and histological findings (PDAI)	
PDAI score > 7	1

PDAI: Pouchitis Disease Activity Index, IPAA: ileal pouch-anal anastomosis; UC, ulcerative colitis

Laboratory markers for the prediction of pouchitis

In patients with and without pouchitis, significant positive correlations were found between blood inflammatory parameters, including WBC and platelet counts, and albumin and CRP levels, and the FC level (Table 2) (Figure 1). There was no correlation found between the FC level and the interval from ileostomy closure until the diagnosis of pouchitis (data not shown).

Correlation between pouchitis and the FC level

We examined whether there was an association between the FC level and the blood test data. The FC levels were positively correlated with WBC count, and CRP and albumin levels (Figure 1A-C). There was a significant positive correlation between the PDAI score and the FC levels ($p < 0.05$) (Figure 1D).

In Figure 1 B, one patient shows a very high CRP level with low FC level. This patient had suffered from side effects of taking steroids for many years. The patient is being treated for uveitis and chronic respiratory disorders but has not experienced pouchitis.

This suggests that FC values may be an excellent criteria for pouch evaluation, even in patients with systemic inflammation where CRP is high and FC is low.

At the time of endoscopy, the median FC was significantly higher in patients with pouchitis than in those without pouchitis (111 vs. 16 mg/g, $p < 0.05$). Similarly, there was a significant positive correlation between the endoscopic findings of inflammation and histological findings and FC level (Figure 2A and B).

In the endoscopic examinations, mild erosion was seen in eight patients and small ulcerations in two cases, whereas the other five patients did not have inflammatory findings. Among the 10 patients with erosion or small ulcerations, four showed active UC-like findings on the biopsy specimens, and the FC level was

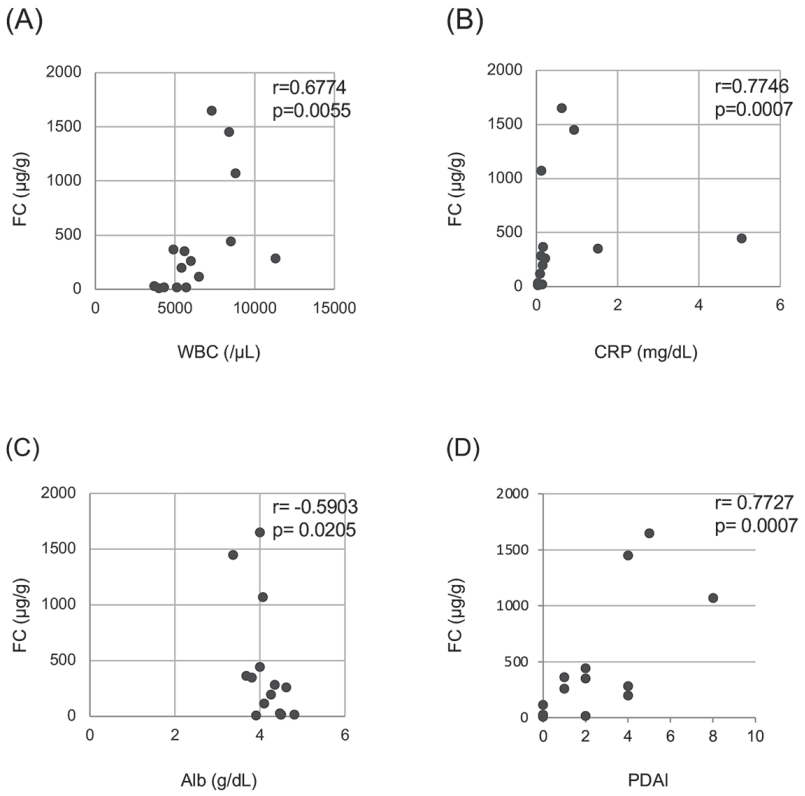

Fig. 1. Correlation between the fecal calprotectin (FC) level and blood inflammatory parameters [white blood cell (WBC, A), C-reactive protein (CRP, B), albumin (Alb, C)], and Pouchitis Disease Activity Index (PDAI, D)].

TABLE 2.
Correlation between the FC level and blood data and PDAI

Pt. no.	FC (μg/g)	WBC	CRP	Alb	Endoscopic findings	Histological findings	PDAI
1	260	6000	0.21	4.61	Aphtha	Ileitis, UC-like	1
2	365	4900	0.16	3.67	Erosion	Ileitis	1
3	443	8500	5.05	3.99	Erosion	Ileitis	2
4	1450	8400	0.93	3.36	Small ulcer	Ileitis	4
5	17.7	5700	0.14	4.48	Erosion	Ileitis, UC-like	2
6	116	6500	0.09	4.09	No inflammation	–	0
7	<10	4000	<0.04	3.9	No inflammation	–	0
8	351	5600	1.51	3.8	Erosion	Ileitis	2
9	1650	7300	0.62	3.99	Erosion	Ileitis	5
10	285	11300	0.11	4.34	Erosion	Ileitis	4
11	198	5400	0.15	4.25	Erosion	Ileitis	4
12	17.7	4300	0.04	4.49	No inflammation	–	0
13	29.4	3700	0.04	4.46	No inflammation	–	0
14	17.6	5100	0.04	4.8	No inflammation	–	0
15	1070	8800	0.12	4.06	Small ulcer	Ileitis, cryptitis	8

FC: fecal calprotectin, WBC: white blood cell, CRP: C-reactive protein, Alb: albumin
PDAI: Pouchitis Disease Activity Index

high in all 10 of these patients (average: 1153.25 μg/g) (Table 2) (Figure 1).

Serial measurements of FC

We illustrated the progress of case 15 in Figure 3. At 2 weeks after the colostomy closure, an endoscopic biopsy specimen confirmed pouchitis, and the patient's FC level increased. With conservative treatment, the pouchitis improved and healed. During this period of conservative treatment, the FC level gradually decreased to within the normal range. Not only the FC level but also blood inflammatory parameters (WBC and albumin level) and endoscopic findings were all associated with the state of pouchitis. The FC levels reflected the pouchitis status more clearly than the other blood test data (WBC count and albumin level).

DISCUSSION

In this study, the FC levels were correlated with PDAI, blood test data, and endoscopic findings. Moreover, we investigated whether FC was a predictive index of postoperative pouchitis and/or other pouch conditions.

The risk factors for pouchitis include primary sclerosing cholangitis [20], backwash ileitis [20,21], extraintestinal complications [22,23], and being female [24]. The symptoms of pouchitis include diarrhea, increased frequency of defecation, abdominal pain, bleeding, and fever. The frequency of defecation is usually high in patients after total colectomy, and macroscopic changes depend generally on the content of the meal. In other words, there are no characteristic symptoms of pouchitis, and it is difficult to diagnose the condition using only clinical symptoms.

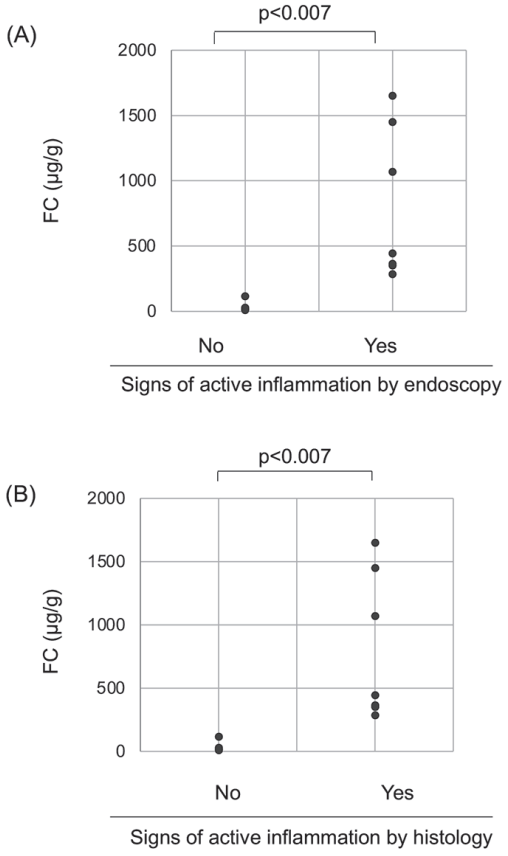

Fig. 2. Comparison between the fecal calprotectin (FC) level and endoscopic findings with or without active signs of inflammation that include aphtha, erosion, or ulceration (A), and between the FC levels and histological findings with or without active signs of inflammation (B).

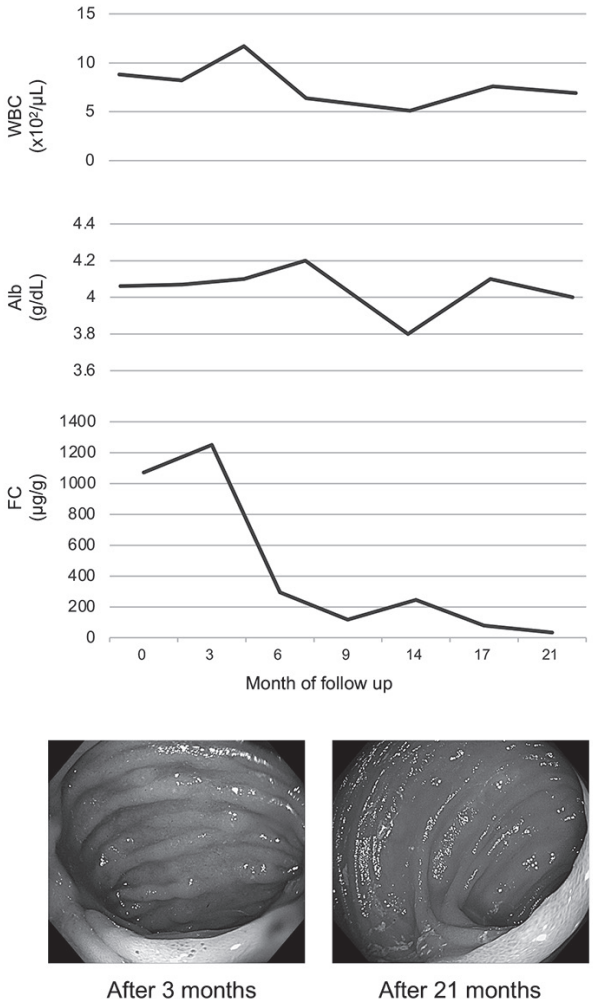

Fig. 3. Time-courses of fecal calprotectin (FC) level, blood inflammatory parameters [white blood cell (WBC), albumin (Alb)], and endoscopic images of one patient (case 15).

A few studies [25-28] have investigated the FC levels in patients with pouchitis after restorative proctocolectomy for UC. In those studies, the FC level was determined by only one test using a single stool sample. Therefore, any correlation between FC and course progression after restorative proctocolectomy remains unknown. In the present study, the FC level significantly increased in patients with pouchitis, and significantly increased in patients with pouch inflammation who were otherwise asymptomatic. In contrast, FC remained at constant low levels in all other patients without inflammation. These results suggested that FC was a significant predictor of pouch inflammation. Continuous monitoring of FC may therefore be useful for the early diagnosis of any inflammation in the pouch. It seems likely that the measurements of FC level reveal an ongoing escalating inflammatory process in the ileal pouch that produces clinical symptoms when sufficiently severe.

This prospective study was designed to evaluate the significance of FC in the early diagnosis and prediction of pouchitis after restorative proctocolectomy in UC patients. The calprotectin level is stable in feces for several days at room temperature [10,29,30]. FC is convenient to measure at a low cost and may be used as a noninvasive approach to monitor mucosal inflammation and to predict a subsequent relapse [11-16] in patients with IBD.

The limitation of this study was that it was a single-center design and included only a limited number of patients owing to the very strict admission criteria used to obtain a homogeneous population. Moreover, the diagnosis of pouchitis was defined using PDAI, which was considered as the gold standard for the diagnosis. In patients with IPAA, FC was shown to be correlated with the histologic subscore [27], suggesting that the measurement of calprotectin may represent a potentially useful substitute for histology in the assessment of pouchitis.

In the future, the number of patients with UC is expected to increase, and the number of these patients undergoing total colectomy and developing pouchitis is also expected to increase. When performing follow-up monitoring of patients after total colectomy, the progression to pouchitis is of high concern, and using FC levels as a predictive biomarker could be useful.

CONCLUSION

In conclusion, postoperative endoscopy is essential for the accurate evaluation of the pouch, and routine endoscopic examinations are required during the follow-up period. For non-invasive screening, the FC level was found to be effective as a predictor of pouchitis in patients undergoing proctocolectomy with IPAA or IACA as UC treatment.

CONFLICT OF INTEREST: The authors declare that they have no competing interests.

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