Inflammatory Intestinal Diseases

Research Article

Inflamm Intest Dis
DOI: 10.1159/000531654

Received: April 3, 2023
Accepted: June 17, 2023
Published online: July 12, 2023

Associations between Pouchitis and Fecal Calprotectin after Restorative Proctocolectomy in Patients with Ulcerative Colitis

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Abstract

Introduction: Recently, fecal calprotectin has been identified and used as an assessment tool for the confirmation of disease activity in ulcerative colitis. Although a meta-analysis suggested the usefulness of fecal calprotectin for the assessment of pouchitis, the number of participants was still insufficient. Therefore, we prospectively measured fecal calprotectin levels during pouchoscopy and analyzed their associations with pouchitis. ***Methods:*** Patients who underwent pouchoscopy after total proctocolectomy and ileal pouch-anal anastomosis for ulcerative colitis were included. Fecal samples were collected for the measurement of calprotectin during pouchoscopy. Patients either with or without suspicious pouchitis were included. Pouchitis was defined as a modified pouchitis disease activity index (m-PDAI) score of ≥5. The associations between the development of pouchitis and the m-PDAI score and fecal calprotectin and serum markers, including C-related protein, albumin, and white blood cells, were assessed. ***Results:*** A total of 170 patients were included. Seventy-two patients were diagnosed with pouchitis with an m-PDAI score of 7.3 ± 1.5. The values of fecal calprotectin were 1,500 ± 1,544 μg/g in patients with pouchitis and 259 ± 402 μg/g in patients without pouchitis ($p < 0.01$). The correlation coefficient between calprotectin and the m-PDAI score was significant ($r^2 = 0.279$, $p < 0.001$). The cutoff value of fecal calprotectin in receiver operating characteristic analysis was 246 μg/g (area under curve 0.85, sensitivity 83.9%, specificity 71.0%). Fecal samples were able to be collected from 6 patients. The levels of fecal calprotectin significantly decreased from 2,101.3 ± 880.3 μg/g to 284.2 ± 96.9 μg/g in response to the treatment. ***Conclusions:*** Elevated fecal calprotectin appeared to be significantly correlated with pouchitis. We should consider the alteration of this marker during treatments in further studies.

Introduction

Although novel treatments for ulcerative colitis (UC) have been developed and the number of surgeries for refractory UC is now decreasing, surgical treatment is still needed for patients with refractory disease, severe/fulminant disease, or cancer/dysplasia [1, 2]. The standard surgical procedure for UC is restorative proctocolectomy (RPC), which includes total proctocolectomy with ileal-pouch anal anastomosis (IPAA). However, a major problem after surgery is the development of pouchitis [3].

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It is known that pouchitis can develop in 20–50% of patients who undergo IPAA [4–7]. Although the etiology of pouchitis has not been established even today, pouchitis may be caused by immune disorders, such as UC, and it is diagnosed with endoscopic clinical findings with or without histological findings, such as the pouchitis disease activity index (PDAI) [8, 9].

Recently, it has been shown that fecal calprotectin is useful for the diagnosis of disease severity not only for UC but also for pouchitis [10]. However, there are few previous studies of pouchitis, and it is unclear whether fecal calprotectin can be useful monitoring tool. Therefore, we evaluated the association between pouchitis and fecal calprotectin after IPAA in our institution to prove this association in more patients who underwent IPAA.

Methods

Patients

We conducted a prospective study between April 2018 and August 2022. Patients who underwent a pouchoscopy following a total proctocolectomy and IPAA for UC were analyzed regardless of the presence of symptoms that were suspicious of pouchitis. We routinely examined the patients by pouchoscopy 1 year after RPC regardless of symptoms if they agreed to the examination. In particular, we recommend examination by pouchoscopy for patients whose symptoms are suspicious of pouchitis. Fecal samples were collected for measurement of calprotectin at the time of the pouchoscopy. The characteristics of the patients, such as sex, age at pouchoscopy, extent of colitis, disease severity, and preoperative characteristics, were collected before surgery. Treatments before colectomy include all previous treatments for UC in regardless of their timings and durations.

Inclusion Criteria

Patients who were 18 years of age or older and scheduled to undergo pouchoscopy regardless of the presence of symptoms that were suspicious of pouchitis after RPC were included.

Exclusion Criteria

This study was limited to patients who underwent pouchoscopy and were examined for each biomarker. Patients who were suspected of having or were diagnosed with Crohn's disease by histological findings, such as epithelioid granulomas, discontinuous crypt distortion, and discontinuous inflammation, and patients with transmural inflammation based on a review of colectomy specimens or endoscopic biopsy specimens were not included in this study. Patients were also excluded if they had secondary pouchitis triggered by cytomegalovirus or *Clostridium difficile* infection, radiation, any other form of infectious enteritis, pelvic sepsis, including an abscess or anal fistula, or obstruction due to anastomotic stricture.

Diagnosis and Treatment of Pouchitis

Diagnoses of pouchitis were based on clinical symptoms (increased stool frequency, bleeding, abdominal cramping, urgency, and fever) and endoscopic findings (edema, granularity, friability, loss of vascular pattern, mucous exudates, and ulceration). Additionally, the diagnosis was confirmed using the modified pouchitis disease activity index (m-PDAI), a commonly used instrument for measuring disease severity [9]. An m-PDAI score ≥5 suggests a diagnosis of pouchitis. All patients who were diagnosed with pouchitis were treated by the following antibiotics. The typical first-line therapy for pouchitis is 2 weeks of oral metronidazole (15 mg/kg/day) or ciprofloxacin (12 mg/kg/day) at our institution. Additional antibiotics, concomitant 5-aminosalicylate, corticosteroids, or biologics/molecular targeted agents primarily applied as second-line therapy for chronic pouchitis were not included in this study. Other treatments, such as rifaximin, budesonide, or VSL#3, were either unavailable or were not sufficiently covered by insurance in Japan at the time of this study.

Concurrent Medications

Concomitant administration of other immunosuppressive therapies, including corticosteroids, immunomodulators, 5-aminosalicylate, biologics/molecular targeted agents, or antibiotics, was not employed during the initial antibiotic treatment for pouchitis. These treatments could be selected for chronic pouchitis (including antibiotic-resistant or dependent pouchitis) after the initial treatments for acute pouchitis. Only concomitant use of *Bifidobacterium bifidum*, which is routinely administered soon after RPC, was performed in all patients regardless of pouchitis.

Pouchoscopy and Fecal Sample Collection

Fecal samples were collected during pouchoscopy to decrease the time difference between sample collection and endoscopic diagnosis. Any bowel preparation before pouchoscopy was not performed because the bowel preparation could affect the results obtained from the fecal sample. The fecal sample collected by pouchoscopy was not solid feces because it was collected from the ileum after colectomy. Therefore, the fecal samples could be aspirated via an endoscope because the fecal character was type 5, type 6, or type 7, which indicated diarrhea according to the Bristol stool scale [10]. Fecal samples were collected by using a suction tube with a trapping container which could directly collect the aspirated sample soon after initial insertion of the endoscope. The fecal samples could be collected in volumes ranging from 5 mL to 50 mL with no bowel preparation, and these samples were submitted to the hospital laboratory soon after endoscopic examination.

Measurements of Biomarkers

Inflammation-related values from various blood tests, including C-reactive protein level, white blood cell count, serum albumin, and fecal calprotectin, were examined at the time of pouchoscopy. A clinical laboratory technician measured these biomarkers. Fecal calprotectin was measured by using a fluoroenzyme immunoassay (Calprotectin kit [EliA Calprotectin 2, Thermo Fisher Scientific K.K.]). The upper limit of normal is 50 μg/g.

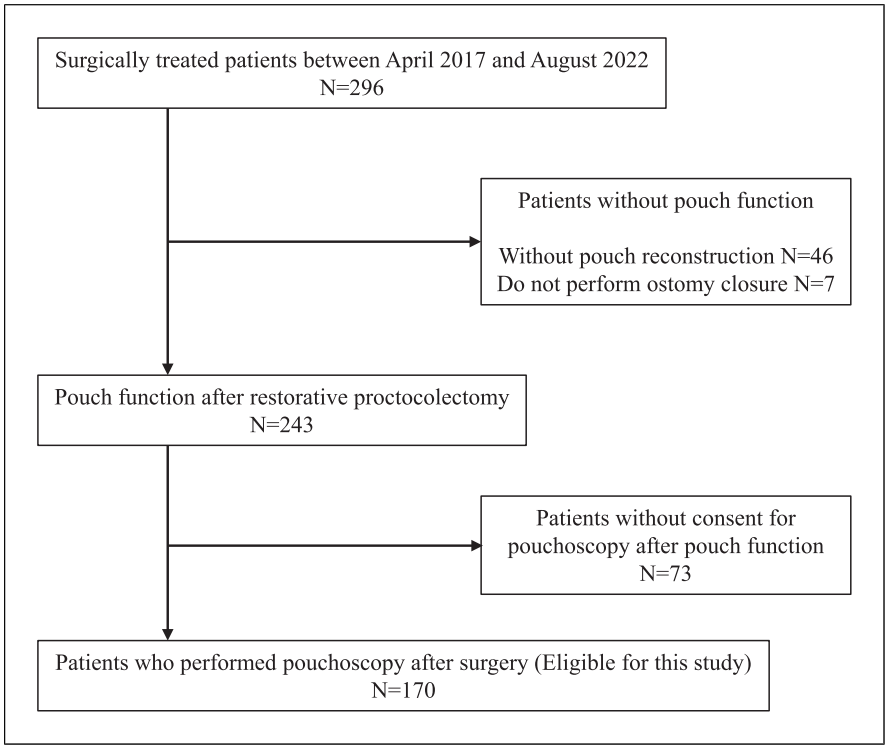

Fig. 1. Flowchart of patient's eligibility.

Evaluation of Treatment Efficacy

We evaluated the correlation between fecal calprotectin and the efficacy of antibiotic treatment for pouchitis. We also assessed fecal calprotectin after 2 weeks of treatment. These fecal samples were collected at the patient's home on the outpatient clinic day within 2 weeks after the completion of antibiotic treatment. Treatment responders were defined as those whose m-PDAI score decreased below 5.

Statistical Analysis

Categorical valuables are indicated as numbers with percentages in parentheses. Continuous variables are indicated as the mean and standard deviation. The Wilcoxon matched pairs test was used to calculate the significance of m-PDAI scores before and after treatment in patients with pouchitis. The Mann-Whitney U test was used to evaluate the association between the fecal calprotectin level and the existence of pouchitis. A two-sided p value of less than 0.05 was considered statistically significant. The cutoff values for each blood test were determined based on the closest left upper values in the receiver operating characteristic curve analysis.

Results

Patient Characteristics

The patient flow in this study is shown in Figure 1. A total of 296 patients were administered surgical treatment for UC from April 2017 to August 2021. Endoscopic examination and prospective fecal collection were started from April 1, 2018, to August 2022. Forty-six patients did not receive pouch reconstruction, and 7 patients had not yet undergone ostomy closure.

A total of 170 patients (100 males and 70 females) who underwent pouchoscopy were eligible for this study. The patient characteristics and backgrounds are shown in Table 1. The mean age at initial surgery and pouchoscopy was younger in patients with pouchitis. The duration from ostomy closure to pouchoscopy was 1.5 ± 1.2 years. Although the disease severity was similar between patients with or without pouchitis, the surgical indications were significantly different. A surgical indication of refractory disease was most common in patients with pouchitis; however, the indication of cancer/dysplasia was mainly present in patients without pouchitis. According to these differences in the surgical indications, the use of immunosuppressive agents or biologics was more common in patients with pouchitis. No patients were treated with other biologics/molecular targeted agents before the initial surgery.

Pouchitis

Pouchitis developed in 72 (42.4%) patients with an m-PDAI score of 7.3 ± 1.5. The comparisons of fecal calprotectin values between patients with or without pouchitis are shown in Figure 2. The values of fecal

Table 1. Patients' backgrounds

	Patients, *n* with pouchitis (*n* = 72)	Patients, *n* without pouchitis (*n* = 98)	*p* value
Age at initial surgery, years	41.4±17.7	45.4±16.7	0.01
Age at pouchoscopy, years	43.2±12.6	48.2±14.1	0.02
Gender (male:female)	45:27	55:43	0.51
Before colectomy			
Extent of colitis (pan colitis:left side colitis)	51:21	62:36	0.30
Disease severity (mild-moderate:severe:fulminant)	17:25:29:1	37:29:32:0	0.41
Timing of surgery (urgent, emergent)	17 (23.6)	31 (31.6)	0.25
Treatments before colectomy			
PSL administration	22 (30.6)	36 (36.7)	0.40
Total given PSL dose, mg	4,681.7±6,777.8	3,354.5±7,860.8	0.25
Pre-operative PSL, mg/day	8.0±16.2	11.2±20.2	0.27
Immunosuppressant administration			
AZA/6-MP	45 (62.5)	35 (35.7)	<0.01
CNI	35 (48.6)	17 (17.3)	<0.01
Anti-TNF-α antibody therapy	55 (76.4)	30 (30.6)	<0.01
Surgical indication(refractory: cancer/dysplasia: TMC/perforation)	47:18:7	33:46:19	<0.01

Continuous variables are indicated as mean and standard deviation. Categorical data are numbers with percentages in parentheses. TMC, toxic megacolon; PSL, prednisolone; AZA, azathioprine; 6-MP, 6-mercaptopurine; CNI, calcineurin inhibitor; TNF, tumor necrosis factor.

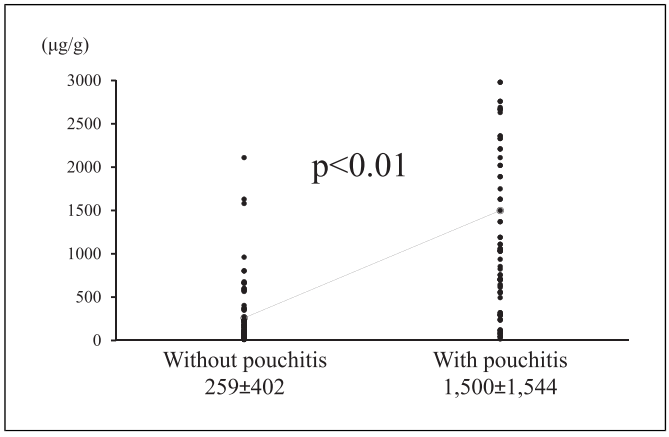

Fig. 2. Comparisons of fecal calprotectin values between patients with or without pouchitis. The values of fecal calprotectin were 259 ± 402 μg/g in patients without pouchitis and 1,500 ± 1,544 μg/g in patients with pouchitis. These values were significantly different ($p < 0.01$).

calprotectin with or without pouchitis were 259 ± 402 (μg/g) or 1,500 ± 1,544 (μg/g), respectively, which were significantly higher in patients with pouchitis than in those without pouchitis.

Correlation Coefficients between the Biomarkers and Pouchitis

Figure 3 presents the correlation coefficients between albumin, C-reactive protein, white blood cell count, and pouchitis. There were no correlations between these markers. Figure 4 shows that the correlation coefficient between calprotectin and the m-PDAI score was statistically significant ($r^2 = 0.279$, $p < 0.001$).

Cutoff Value for Pouchitis

As shown in Figure 5, the cutoff value of fecal calprotectin in receiver operating characteristic analysis was 246 μg/g (area under curve 0.85, sensitivity 83.9%, specificity 71.0%).

Alteration of Fecal Calprotectin Levels during Treatment for Pouchitis

Fecal samples were collected from 6 patients who were treated with antibiotics for pouchitis. All patients responded to antibiotic treatment; the m-PDAI score decreased below 5, and the levels of fecal calprotectin significantly decreased from 2,101.3 ± 880.3 (μg/g) to 284.2 ± 96.9 (μg/g) after treatment (Fig. 6).

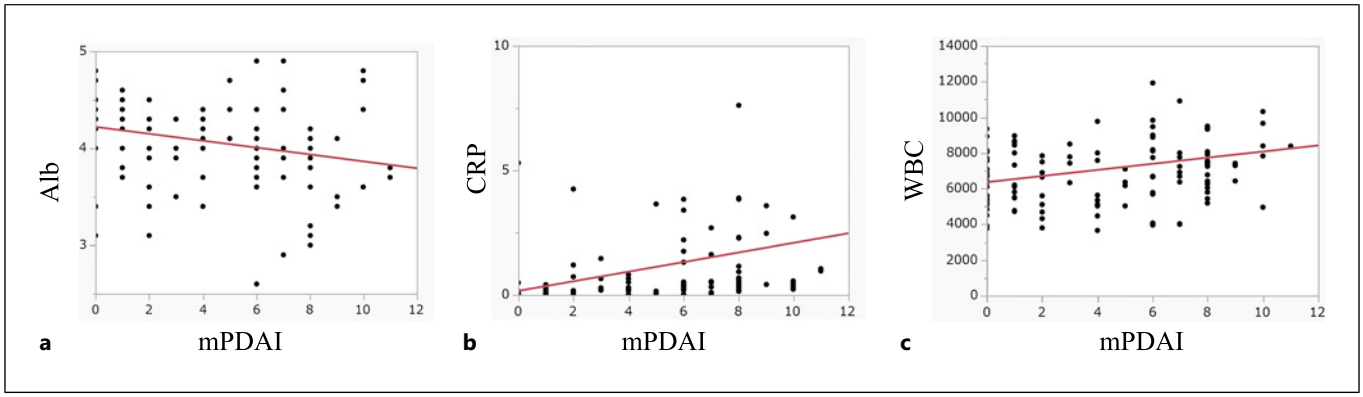

Fig. 3. The correlation coefficients between the biomarkers and pouchitis. The values of r^2 and p were (**a**) Alb (r^2 = 0.017, p = 0.153), (**b**) CRP (r^2 = 0.0008, p = 0.747), and (**c**) WBC (r^2 = 0.072, p = 0.28). Alb, albumin; CRP, C-reactive protein; WBC, white blood cell.

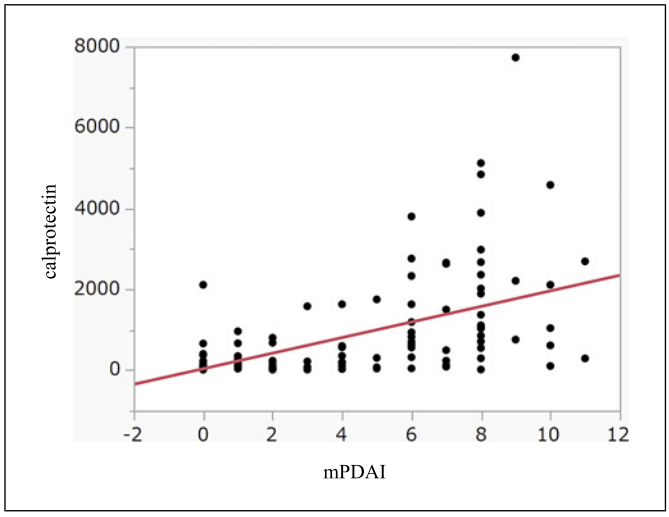

Fig. 4. The correlation coefficient between fecal calprotectin and m-PDAI score. The values were r^2 = 0.279, p < 0.001.

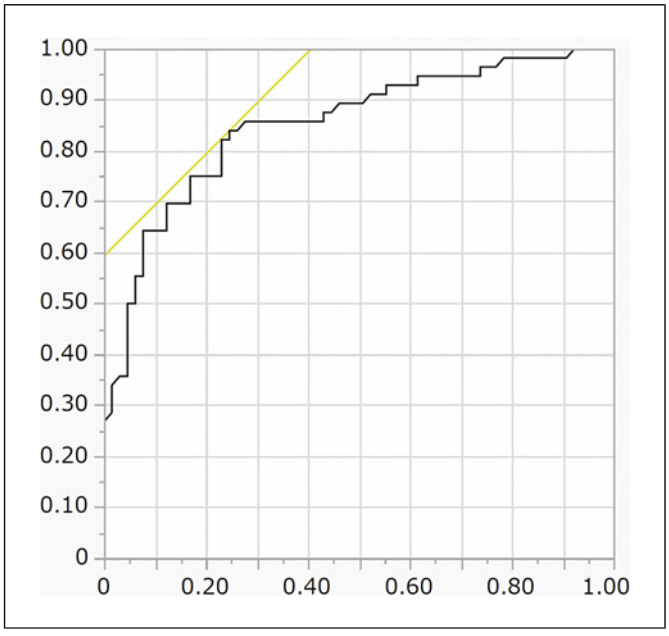

Fig. 5. Cutoff value of fecal calprotectin for pouchitis. The cutoff value of fecal calprotectin in ROC analysis was 246 μg/g (area under curve 0.85, sensitivity 83.9%, specificity 71.0%).

Discussion

Similar to previous studies, the usefulness of fecal calprotectin for the diagnosis and treatment of pouchitis was also evaluated in this study. The value of fecal calprotectin at diagnosis was significantly higher in patients with pouchitis and was significantly decreased after antibiotic treatment. However, the cutoff value of fecal calprotectin was 246 μg/g in this study, which differed from some previous studies [11]. The different assay methods and value ranges might have affected the results.

The diagnosis of pouchitis was first evaluated with the PDAI score in 1994, which includes endoscopic, clinical, and histological scores [8]. However, the results of histological scoring take several days after the biopsies, and delaying diagnosis is a major problem for clinical practice. Therefore, the modified PDAI, which is composed of clinical and endoscopic findings, is the usual diagnostic score due to its convenience [9]. In the future, biomarkers such as fecal calprotectin and clinical symptoms may be used as diagnostic indices.

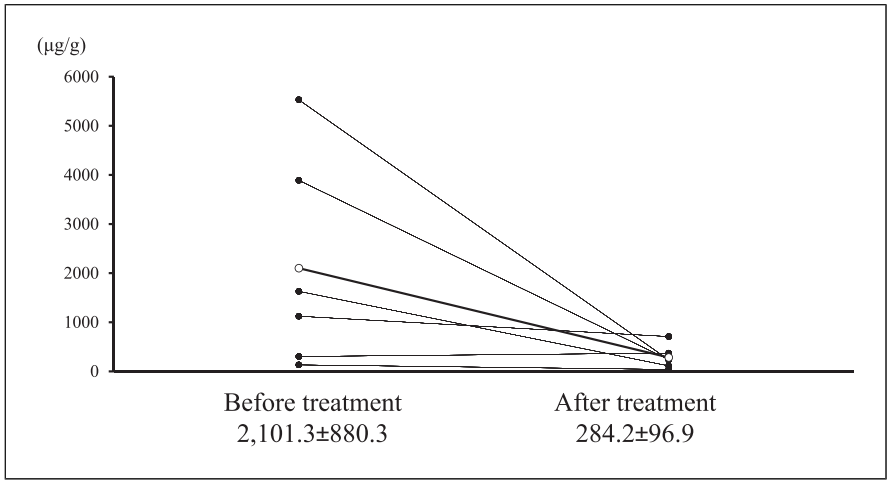

Fig. 6. Alteration of fecal calprotectin levels during treatment for pouchitis. The levels of fecal calprotectin significantly decreased from 2,101.3 ± 880.3 μg/g to 284.2 ± 96.9 μg/g after treatment ($p < 0.01$).

The first-line treatment for pouchitis is oral antibiotics, which include ciprofloxacin and/or metronidazole [3, 12]. These antibiotic treatments can achieve a high rate of remission for acute pouchitis; however, antibiotic-resistant or antibiotic-dependent chronic pouchitis can develop as a secondary problem. We need to evaluate whether fecal calprotectin is also useful during the clinical course of chronic pouchitis in the future.

In real clinical situations, we might frequently choose treatment first instead of beginning with an examination [13]. Antibiotic treatment might be started before endoscopic examination to avoid delaying treatment, to decrease medical costs without endoscopy, and to reduce the use of antibiotics. Therefore, biomarkers such as fecal calprotectin can be the first tool for diagnosis in clinical situations.

Some patients had a high fecal calprotectin level without pouchitis. Moreover, there were significant variations in the values of calprotectin among patients with pouchitis. Although there was a significant association between the m-PDAI score and the fecal calprotectin level, it could not explain or suggest why a high calprotectin value could be found in patients without pouchitis. Moreover, it could not prove whether high levels of calprotectin are associated with disease severity or can be a predictor for the development of pouchitis. We need to evaluate this factor in a further longitudinal study.

The limitations of this study are indicated. First, this was a single-center prospective study with few participants. Second, only patients who underwent endoscopic examination after IPAA were included in this study. Some patients without any clinical symptoms could be missing. Third, although the data from clinical and endoscopic evaluations and from each biomarker were collected prospectively, the study design seems to be a retrospective review of data that were collected only from patients whose consent was obtained. Moreover, comparisons before and after treatment were performed. However, this is not a genuine interventional study because the treatment was performed only in patients with pouchitis, and its choice was limited to first-line therapy. Fourth, fecal samples were collected from few patients after treatment for pouchitis. Most patients who respond well to antibiotic therapy tend to not return to the outpatient clinic 2 weeks later, even if consent is acquired before treatment. Fifth, we did not consider the severity of pouchitis. We need to establish an index for the severity score for pouchitis and perform further evaluations. Sixth, the methods of fecal sample collection differed between the initial diagnosis of pouchitis during the endoscopic examination and the evaluation of the treatment efficacy. This difference may have affected the results; however, the method of collection mostly complied with international consensus statements, which included the following: "the feces should be collected in a dedicated clean container without additives to avoid any accidental contamination," "the most appropriate timing for stool sampling is unclear," and "the analysis of a single stool sample is usually sufficient for measurement" [14]. Whether there was a difference is not certain; however, this is also limitation of the present study because the different methods of fecal collection have not been proven to be equivalent.

In conclusion, fecal calprotectin is useful for monitoring and rapidly diagnosing pouchitis. We should further evaluate these results with more participants and prove the association or comparison with other new biomarkers for intestinal inflammation.

Statement of Ethics

Written informed consent was obtained from all participants before eligibility for this study. All study protocols were approved by the Institutional Review Board at Hyogo College of Medicine (No. 2344). Informed consent and approval for the use of patient data were obtained before examinations. This study followed the ethical guidelines of human subjects based on the Helsinki Declaration.

Conflict of Interest Statement

Motoi Uchino declares lecture fees from Tanabe Pharmaceuticals, AbbVie, Takeda Pharmaceuticals, EA Pharmaceuticals, and Kyorin Pharmaceuticals. None of the other authors have conflicts of interest related to this study to declare.

Funding Sources

This publication has no funding support.

Author Contributions

Motoi Uchino designed the study, analyzed the data, and wrote the manuscript. Yuki Horio, Ryuichi Kuwahara, Kurando Kusunoki, and Kentaro Nagano collected clinical data and samples. Hiroki Ikeuchi revised the manuscript and drafted the final manuscript. All authors read and approved the final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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